EXPLORING THE USE OF CARBOHYDRATE RESOURCES FOR THE PRODUCTION OF WATERBORNE POLYMERS

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CONTENTS

Chapter I- General

Introduction

I- 1. INTRODUCTION

The Industrial Revolution was the transition to new manufacturing processes in the period from about 1760 to 1840. This transition included the large scale production of chemicals as an important development. Industry became heavily dependent on fossil fuels (crude oil, coal and natural gas) both as a feedstock for commodity chemicals and as a primary energy source. An additional major turning point in history was marked by the emergence of synthetic polymers. In 1907, Backeland developed a phenol/formaldehyde resin for wood,[1] which was later on designated a National Historic Chemical Landmark by the ACS as the world's first synthetic plastic. Not long after, in 1931[2] the first patent describing the process that turns methyl methacrylate into poly(methyl methacrylate) was published. Since then, a broad range of macromolecular materials appeared, changing considerably the lifestyle of mankind. Nowadays our contemporary society is built on fossil resources.

However, this mentioned feedstock needs millions of years to be formed and current status of resources availability is creating increasing concerns. The progressive decline of fossil resources and their resulting price fluctuations, promoted an essential debate on the need of alternatives.

Exploring alternatives requires parallel investigation of new chemicals prepared directly from biofeedstocks, as well as improvements in the production of current well-known monomers, eventually from synthetic biology, catalytic and thermal conversion.[3] Both options open the window to sustainable developments.[4] However, sustainability is a complex and ambitious concept that includes the consideration of environmental, social and economic aspects.[5] This is the reason why it appears essential for the scientific community to provide

with more intensive and systematic efforts in the search for simple routes to sustainable monomers and processes to use them thereof.

Although it is not necessarily the case, sustainable developments very often refers to renewable resources, e.g. any natural product which can be exploited without endangering its survival and which is renewed by biological (short term) phenomena instead of geochemical (very long term) activities.[6,7] The terrestrial biomass offers an array of low and high molecular weight species, exemplified by sugars, proteins and fats (Figure 0-1).[8] Lignocellulosic biomass is an example of a very abundant natural resource consisting of 20 % of the biomass. Its conversion into exploitable small molecular weight molecules is not easy and requires various treatment methods, sometimes critical to handle.[9] Fats, terpenoids, nucleic acids, etc. represent only 5 % of the biomass. They are generally low to medium molecular weight structures, and exhibit high hydrophobicity which can be problematic when dealing with solvent-free environmentally friendly processes. In contrast, carbohydrates represent a rich class of natural products in terms of availability, functionality and molecular weights, existing as mono, di, oligo or poly saccharide species.

Various strategies are being employed to explore the use of renewable resources while decreasing our consumption of fossil feedstock. Lately, industries have been deeply investigating the possibilities of producing plastic materials from biomass or waste.[10] Aside from lactic acid which is nowadays one of the most used bio-based building block for the production of commodity plastics, well-known monomers leading to polyamides, polyesters, polyurethanes and vinyl or acrylics polymers are also under investigation.[11] Recently, several alternative processes, mostly build on sugar fermentation, have emerged.[12,13]

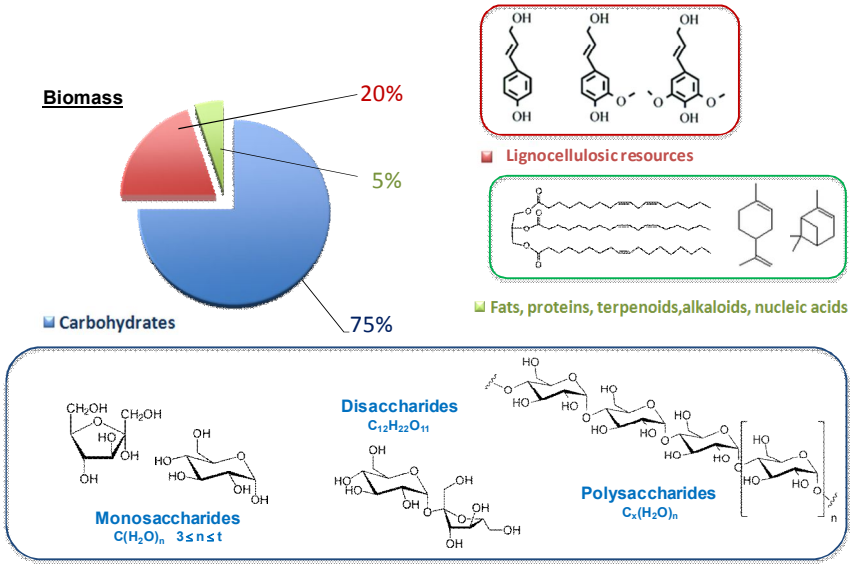


Figure 0-1. Distribution of type of natural products in the biomass

In this work, the potential use of carbohydrates as raw material for the production of bio-sourced monomers and polymers will be explored. In fact, sugars are receiving a great interest as green raw material for the chemical industry.[8] It is noteworthy that large amounts of carbohydrates are commercially available in the food industry but a substantial part of it is regrettably wasted as a surplus of their agricultural production or through domestic food wastage. Valorization of these food wastes appears more relevant than ever.[14–17]

In the past decades, many works have been published on sugar-based polymers, describing all kind of application, such as chromatographic support, water absorbents, medical devices, tissue engineering or even cell recognition systems.[18] Various strategies are being employed to prepare sugar-based polymers, also very often referred as glycopolymers.

(i) As for instance, glycopolymers can have the sugar unit incorporated into the main chain. A recent review from Galbis et al[19] reports the use of carbohydrate-based monomers for the production of polyesters, polycarbonates, polyamides and polyurethanes, mostly by means of polycondensation reactions.

(ii) Another approach consists of having sugar units pendant from the main backbone. Indeed, thanks to their multifunctionalities, sugars can be easily grafted onto conventional synthetic polymers. Hence, carbohydrates with protected as well as unprotected groups could be grafted onto functionalized polymeric backbone using either amide linkage,[20,21] click chemistry[22,23] or non-click approaches.[24] Difficulties are found though when grafting large monomeric molecules quantitatively onto polymers[25] and controlling their randomness along the chains for low degree of substitution is also an issue. Nevertheless, glucose was successfully grafted onto poly(ethylene glycol) as well as poly(acrylic acid).[26] Binding of mono-, di-, and oligosaccharides by amide linkage to polymers carrying carboxylic or amino groups was also reported, studying the effect of number or length of attached carbohydrates on the resulting polyelectrolyte properties.[25] The grafting of both protected and unprotected monosaccharides onto poly(vinyl alcohol) was studied by Kraska et al. for biomedical applications.[27] More recently carbohydrates grafting was extended to surface modifications such as liposomes for biomedical research,[28] pol(ε-caprolactone) substrate for tissue engineering,[29] PET surfaces to control wettability,[30] or metals for anticorrosion applications.[31] A widely used commodity polymer, namely styrene–butadiene–styrene (SBS) was also successfully functionalyzed by sugar units, aiming at improving its biodegradability.[32]

(iii) Besides, the preparation of pendant sugar-carrying polymers can also be achieved by the actual polymerization of reactive monomers synthesized from sugars. Those polymers are

often referred as poly(vinylsaccharide)s. Indeed, in literature a great proportion of sugar polymerization studies is dedicated to the production of well-defined polymers obtained by controlled radical polymerization, including NMP,[33] ATRP[34] and RAFT[35] polymerization techniques. For instance, acetylated (meth)acryloyl galactose-based monomers can exhibit good reactivities to be copolymerized and result in well-defined block to gradient copolymers,[36,37] to achieve stimuli responsive systems. Because of their apparent biocompatibility and biodegradability, most of these developed glycopolymers are described as great tool for the preparation of drug-delivery carriers,[38] protein conjugates, etc, as stated in many reviews.[39–46]

However, to our knowledge, conventional free radical polymerization as simple tool to produce sugar-based polymers has not been much exploited. Yet, the preparation of high molecular weight random copolymers from sugars can find interest industrially. Indeed now more than ever both customers and manufacturers are willing to achieve more renewability. Therefore, in addition to biomedical purposes, sugar-based polymers could be considered for commodity plastics applications. Given that most of the commercial polymers produced by free radical polymerization involve the use of vinylic or acrylate monomers, the preparation of reactive species carrying double bonds from sugar turns out to be essential.

A brief review on the preparation of low molecular weight carbohydrate monomers will be given here. It is difficult to cover all published work in this field given the great attention it has received during the past decades. Consequently, monosaccharides carrying reactive groups able to polymerize through free radical polymerization will be the focus of the next section.

I- 2. MONOSACCHARIDE-BASED MONOMERS WITH FREE RADICALLY POLYMERIZABLE GROUP

Using sugar-based species as monomer for commodity plastics is rather challenging. The first drawback lies in sugar multiple functionality. Indeed, monosaccharides are carrying several hydroxyl groups resulting in difficulties to achieve selective modifications. Still, in order to obtain linear polymers, it is essential that the sugar-based monomer contains one single reactive group. Consequently, controlled protection-deprotection is usually required in the preparation of accurate sugar-based monomers. Hopefully advanced organic chemistry together with innovative enzymatic strategies have offered possibilities to attain such objective. Here below are given examples of pathways available to prepare reactive monosaccharides.

(i) Enzymatic functionalization from unprotected saccharides

(ii) Conversion of sugars to their corresponding lactone or amino-equivalent, to obtain mono-functionalized hydrophilic monomers

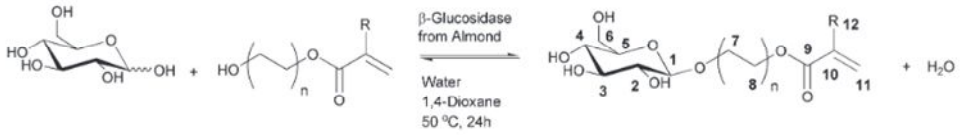
(iii) Selective hydroxyl group protection to achieve mono-substituted hydrophobic monomers

I- 2.1. Enzymatic selective functionalization

Enzymes are highly stereoselective catalysts and thanks to that feature, they have been used in the production of poly(vinylsaccharide)s wherein no protection of the hydroxyl groups of the sugar was required.[47] In 1992, Martin et al[48] described the lipase-catalyzed preparation of

acrylates from various monosaccharides. Their procedure used anhydrous pyridine as solvent and last 40 hours. More recently, Kloosterman et al selectively functionalized glucose in aqueous media by enzymatic action to obtain glucoside-acrylate monomers within a more reasonable time of 24 hours (Scheme 0-1).[49]

Scheme 0-1. Enzymatic synthesis approach of glucoside-acrylates catalyzed by β-glucosidase from almonds.

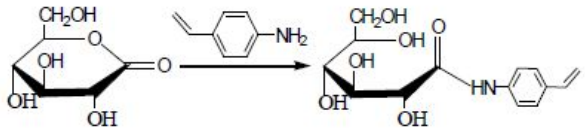


The resulting monomer was able to further polymerize in water or DMF. The same authors also succeeded the obtention of disaccharide acrylate monomers by the amylase catalyzed transglycosidation of starch.[50] Enzymatic processes are very powerful strategies, but their main limitation remains the very slow rate of reaction and scale-up difficulties, resulting in expensive prices thereby making the process less valuable.

I- 2.2. Selective functionalization of aminosugars and lactones

Another strategy to monofunctionalize saccharides is the synthesis of unsaturated amides from aminosugars[17] or sugar-lactones.[18] Here, the reactivity difference between primary and secondary amines or lactones versus hydroxyl groups allows selective functionalization of most common sugars.[53,54]

Scheme 0-2. Selective functionalization of hydrophilic lactone

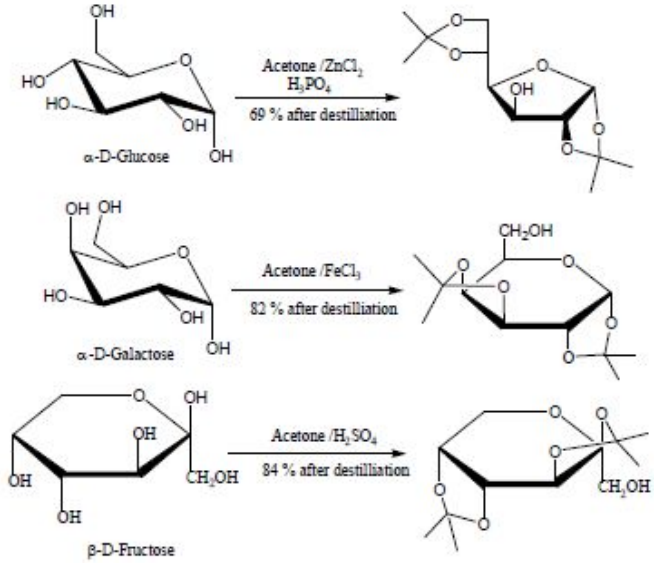


Matsuda *et al* described the synthesis of N-acryloyl-D-glucosamine and polymerized it in water at 80 °C to obtain low molecular weight (7700 g/mol) water soluble glycopolymers.[55] The same monomer was later on shown to be able to copolymerize with N-isopropyl acrylamide (NIPAAm) by RAFT polymerization.[36] Starting from lactones, styrene derivative monomers can be produced (Scheme 0-2).

I- 2.3. Selective hydroxyl protection and mono-functionalization

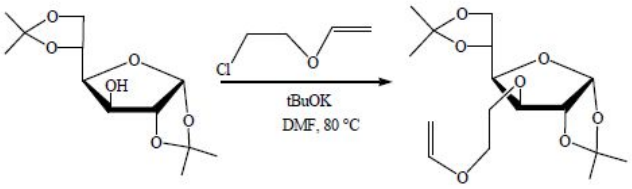
Mono-substitution of carbohydrates can also be achieved by simultaneous protection and functionalization reactions. There are many ways to prepare protected carbohydrate compounds.[56] Among them, the generation of isopropylidene derivatives, also called diacetonides, is the cheapest method to be used. It can be done according to several techniques, basically using acetone in combination with catalysts. This type of acetonation was patented in 1955 by Lawrence Glen[57] wherein diacetone glucose (DAG) was obtained by using a mild condensing agent consisting of anhydrous $ZnCl_2$ and a small amount of H_3PO_4 (Scheme 0-3). Nowadays many isopropylidene derivatives (prepared from glucose, galactose, fructose, etc.) are commercially available.

Scheme 0-3. Diacetonide protection of glucose, galactose and fructose



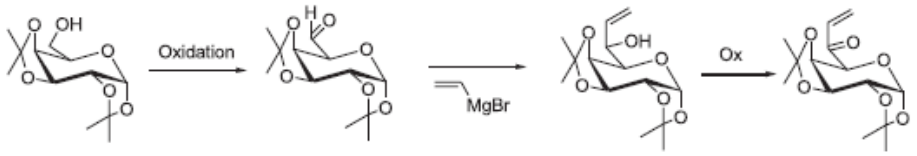
Vinyl derivatives can be produced from protected sugars. Cramail et al[58] reported the synthesis of a glycosidic vinyl ether monomer (Scheme 0-4) and its copolymerization with styrene. Amphiphilic block copolymers were obtained by deprotection of the sugar moieties after polymerization.

Scheme 0-4. Synthesis of a hydrophobic vinyl ether monosaccharide monomer



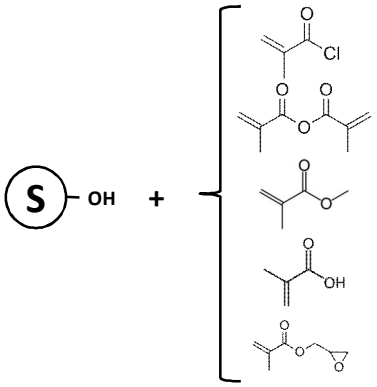
Another strategy to synthesize vinyl containing compounds consist of using Grignard type reactions, as shown in Scheme 0-5.[59] However, organomagnesian compounds are not easy to handle with.

Scheme 0-5. Synthesis of a hydrophobic vinyl monosaccharide through Grignard reaction



(Meth)acrylates from diacetonide sugars can also be produced. A common and instinctive route would be their esterification with eventually a wide range of functionalizing agent as represented in Scheme 0-6.

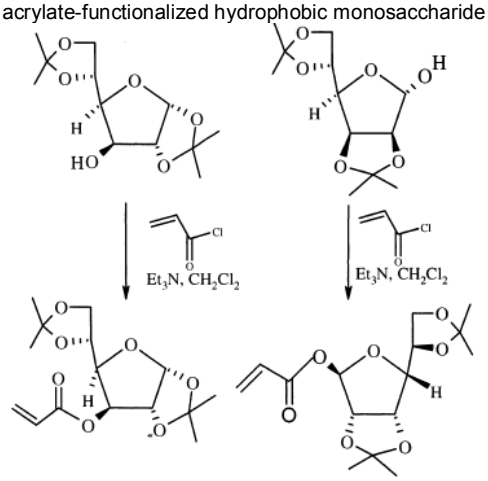
Scheme 0-6. Possible functionalizing agent to be used with protected sugars, carrying one hydroxyl group



In fact, Koβmehl et al extensively explored the synthesis of protected sugar acrylate and methacrylates in the 80's, including sorbose, fructose and glucose (Scheme 0-7), using

different synthesis strategies.[60] But the very beginning of sugar based methacrylate monomers dates back the mid 60's, when Black and coworkers investigated glucose and galactose acrylate based monomers both in their unprotected[61,62] and protected forms.[63,64] Polymerization with free radicals as well as cationic catalysts were achieved and copolymerizations with common monomers such as styrene and vinyl acetate were reported, enhancing the high potential of such molecules for the preparation of novel materials.[65]

Scheme 0-7. Synthesis of an acrylate-functionalized hydrophobic monosaccharide



Another very important piece of work was done by Klein and coworkers, who published a serie of articles describing the preparation of poly(vinylsaccharide)s by synthetizing various reactive species carrying vinyl or (meth)acryloyl carbohydrates for the production of amide-carrying monomers,[66,67] or structures where the sugar scaffold is attached to the vinyl function by an urea linkage.[68] The same group also investigated the preparation of galactose[69] and glucose[70] derivatives, they showed very good reactivity of methacrylate functionalyzed sugars

while monomers carrying allyl functionality failed to give homopolymers of high molecular weights. Their work included also the preparation of sugar-carrying polymer surfactants[71] as well as anionic poly(vinylsaccharides).[72]

Very recently, Salman et al[73] prepared methacrylates based on fructose, galactose, manose and glucose through a three step process to end up with hydrophobic sugar structures carrying spacer between sugar scaffold and functional group. Homopolymerization of the resulting monomers was achieved in DMF and good thermal properties was highlighted by TGA.

I- 3. EMULSION POLYMERIZATION OF SACCHARIDE MONOMERS

Interestingly, polymerization in dispersed media was inspired by nature. Natural rubber latex from the para rubber tree or others is a sticky, milky colloid recovered by making incisions and collected as such directly from trees. Natural rubber, that is to say natural poly(isoprene) is produced at room temperature in dispersed particles stabilized by polymers. The idea of using an emulsified monomer in an aqueous continuous phase to mimic this natural process raised up in the early 1910's.[74–76] A century later, emulsion polymerization is being widely used in large scales for a broad range of application and the theory behind it has been deeply investigated.[77–80]

In this process hydrophobic monomers are dispersed in water by means of surfactants, used to achieve stable colloidal dispersions, in concentrations usually above the critical micellar concentration. The first hypothesis of the mechanism of emulsion polymerization was proposed by Harkins in 1947.[81] As polymerization is initiated, particles will start nucleating by

radical entry into micelles (heterogeneous nucleation), by precipitation of growing oligoradicals in the aqueous phase (homogeneous nucleation) or eventually by radical entry in monomer droplets. Since the surface area of micelles is much greater than that of monomer droplets, the probability for a radical to enter a monomer droplet is very low. Nucleation occurs thus by homogeneous or heterogeneous nucleation.

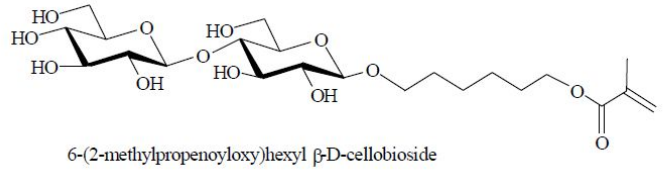
Nucleated particles grow by polymerization. For that, the monomer must diffuse from the monomer droplets to the polymer particles through the aqueous phase. Thus monomer characteristic is a crucial aspect and it can represent a severe limitation in the case of very hydrophobic monomers, as diffusion is precluded. The need of monomer transport through the aqueous phase would be significantly reduced if nucleation could occur directly within monomer droplets. Yet, predominant droplet nucleation can only take place if the surface area of monomer droplets is larger compared to that of micelles, and this requires submicron droplet sizes. Energy can be applied, by means of sonication or high-pressure homogenization to break down monomer droplets. In addition, coalescence should be prevented using both a surfactant and a co-stabilizer. These are the principles of the particular case of miniemulsion polymerization.[82–84]

The use of renewable feedstock to achieve sustainability is being greatly improved by the use of environmentally friendly technologies, such as low VOC (Volatile Organic Compound) techniques. Given this, polymerization in dispersed media appears of high importance to develop more sustainable products.

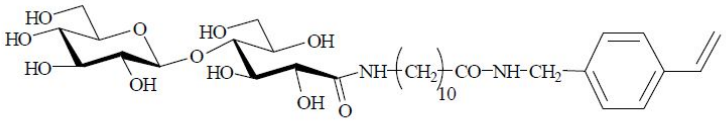
In the 1990's, Pichot at al[85,86] reported the use of reactive amphiphilic sugar-based structures for producing latex particles with covalently attached sugar residues. The incorporation of surface active disaccharide based monomers whose structures are given in

Scheme 0-8 allowed the emulsifier-free seeded emulsion polymerization of either styrene or methyl methacrylate.

Scheme 0-8. Disaccharide-based amphiphilic monomers



6-(2-methylpropenoyloxy)hexyl β-D-cellobioside



11-(N-p-vinylbenzyl) amido undecanoyl maltobioamide

In contrast, Klein et al,[70] in the 80's investigated the proper emulsion polymerization of a sugar-based monomer 3-MDG (protected methacryloyl glucose) at solids content up to 15 wt%. Using non-ionic emulsifiers, they stated conversions up to 92 %. In fact in the current context of environmental concerns, their studies represent a pioneering work.

Indeed and surprisingly, the use of hydrophobic sugar monomers in emulsion polymerization has not received much attention since then. Only few examples can be cited, on one hand Forcada et al[87,88] studied the emulsion copolymerization of 3-MDG for the production of microgels for biomedical applications. Takasu et al investigated the seeded emulsion copolymerization of VAc with vinyl sugars and focused their investigations on the resulting accelerated rate of biodegradation of the material. On another hand, Yaacoub and coworkers widely investigated 3-MDG homopolymerization in emulsion using sodium lauryl sulfate as emulsifier at low solids content from 5 to 20 wt%. High conversions were achieved ranging

from 85 to 99 wt%.[89] The same group studied the batch[90] emulsion copolymerization of 3-MDG with butyl acrylate at 10 wt% solids content and subsequently succeeded increasing solids content up to 50 wt% with reasonable coagulum amounts by using semicontinuous processes.[91–93] These experiments open the window towards a new type of waterborne binder for coating application[94] but paint performance were not reported. They also extended their investigations to free radical[95] and RAFT[96] miniemulsion copolymerization.

I- 4. MAIN MOTIVATION AND OBJECTIVES

It has been fairly demonstrated that nowadays the main ambition of the scientific community as well as society is to go towards more sustainability. However renewing our contemporary chemical industry is a multifaceted challenge, referring not only to the chemical processes themselves but also to social and economic concerns. As a consequence, this initiative requires in-depth long-term investigations involving chemists, polymer scientists, (micro)biologists and more; all committed to provide with more knowledge within a variety of field of expertise.

In this context, the exploration of the use of carbohydrates for the preparation of waterborne polymeric binders came out as relevant proposal. Aiming at contributing to the global effort for the development of more sustainable alternatives, this project offers a broad range of possibilities. Within this study, several aspects were approached, including organic synthesis, polymer engineering, product applications and biodegradability.

Since carbohydrates can be recovered from agricultural and domestic wastes and can be considered as renewable feedstock, one of the objectives was to use them as starting material

for the preparation of valuable monomer units. The latest were tested in solution polymerization as well as emulsion process to obtain sugar-based homopolymers with well-characterized properties. Copolymerization of the sugar-based monomer with others in dispersed media was the second step of the project, aiming at achieving high solids content stable copolymer latexes. Potential industrial application could be thus evaluated, giving an overview of the performance of the obtained bio-based polymers.

A parallel objective was the study of the biodegradability features of the produced materials. Many polymers based on renewable resources are claimed to be biodegradable but in fact very little is known about the consequence of natural products modification on microbial attack in the environment. Indeed being bio-sourced does not necessarily mean being biodegradable. In this sense, it appears important to start paying more attention to this aspect at the very early stage of such materials development, and proceed microbial degradation assays.

I- 5. THESIS OUTLINE

This thesis will be divided into 6 parts referred as chapters, whose description is given below.

Chapter I gives a general introduction in relation with the thesis topic.

Chapter II describes the different strategies employed to produce methacrylate sugar-based monomers. Their homopolymerization in solution and in emulsion is also presented, together with the thermal properties of the resulting polymeric materials.

Chapter III presents the copolymerization in emulsion or miniemulsion of the selected methacrylate sugar-based monomers. Polymerization kinetics as well as polymer characteristics and film properties are given.

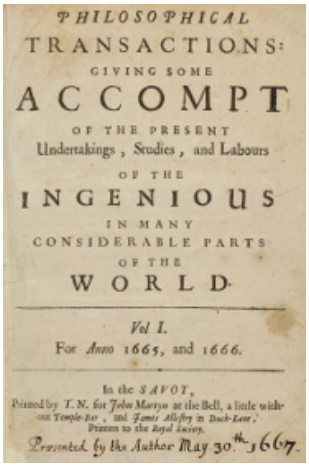
Chapter IV reports the study carried out at Allnex company, The Netherlands regarding the potential use of sugar-based latexes as waterborne binder for decorative application. Great attention was given to paint formulation and paint quality evaluation .

Chapter V describes the use of methacrylate sugar-based monomers for the preparation of alkali soluble resins (ASR) and their use as stabilizers in the emulsion polymerization of common monomers. The resulting waterborne polymers were formulated and paints prepared thereof were tested.

Chapter VI consists of a microbial attack assessment of some sugar-based polymers, aiming at extrapolating their potential biodegradability features.

In Chapter VII the most relevant conclusions of this thesis are presented.

Finally in Chapter VIII, a perspective regarding the feasibility of obtaining fully renewable polymers is given.


PHILOSOPHICAL
TRANSACTIONS:
GIVING SOME
ACCOMPT
OF THE PRESENT
Undertakings, Studies, and Labours
OF THE
INGENIOUS
IN MANY
CONSIDERABLE PARTS
OF THE
WORLD.
Vol I.
For Anno 1665, and 1666.
In the SAVOY,
Printed by T. N. for John Martyn at the Bell, a little without Temple-Bar, and James Allestry in Duck-Lane, Printers to the Royal Society.
Presented by the Author May 30th 1667.

In 1665 the first volume issue of the world's first scientific journal was edited. Untitled "Philosophical Transactions", this journal was meant to inform the Fellows of the Society and other interested readers of the latest scientific discoveries. As such, the journal Philosophical Transactions established the important principles of scientific priority and peer review, which have become the central foundations of the scientific community ever since.

It is the combination of efforts from a whole community that allows fascinating developments. As a result, this work is meant to modestly contribute to the beautiful power of science…

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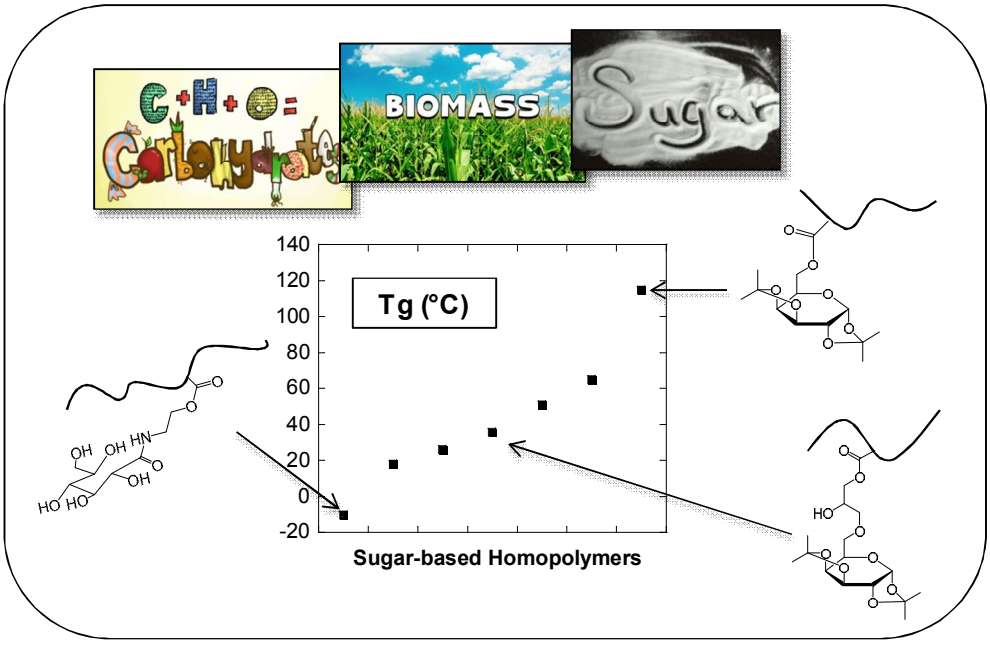
Chapter II- Synthesis, Characterization and Homopolymerization of Functional Monomers from Carbohydrates



II- 1. INTRODUCTION

The current status of petroleum feedstock depletion together with our incontestable society polymer dependence are directing modern research towards more sustainable materials and processes. This phenomenon is illustrated by the impressive increase in the number of publications, reviews, books and scientific symposia dealing with this topic, demonstrating the rising involvement of both the public and the industrial sectors. Two main trends can be distinguished, namely the bio-production of well-known monomers traditionally produced from fossil resources and the exploration of the extremely rich and varied array of molecules offered by renewable feedstock. Gandini et al. regularly publish reviews providing recent concise assessment of the state of the art related to this novel polymer discipline.[1–4]

Renewable feedstock versatility definitely provides the possibility of preparing a variety of remarkable molecules and macromolecules. As for instance natural essences, such as terpenes or terpenoids, as well as vegetable oils can be converted into building blocks for polymerization and resulting materials cover a quite broad range of applications. On the other hand, sugars are more of a particular type of natural sources since they consist of an assortment of small to very high molecular weight species. Polysaccharides can be used as macromolecule in blends or can also be converted into a variety of monomers or precursors to them. In fact, recent progresses are promising, but broad possibilities are still available to further explore the perspectives of renewable feedstock smart usage.[4]

The purpose of the work is the utilization of low molecular weight carbohydrates as building blocks in the production of polymers. Polymerization in aqueous media will be used herein as a valuable environmentally friendly process.

As mentioned above, monosaccharides are abundant and attractive molecules, however their raw structure, mostly consisting of alcoholic functions do not allow their direct use as monomer in conventional radical polymerization. The presence of a reactive vinyl or (meth)acrylic group in the monomer is required. Moreover, the molecule has to be hydrophobic enough to be polymerized in the aqueous dispersed media. Therefore, and aiming at designing several sugar-based monomers that would yield polymers with different application properties, two types of monosaccharides were selected: ones with cyclic structure and others in an open form.

With respect to the cyclic compounds, a galactose and a fructose based monosaccharides, bearing acetonide protecting groups and commercially available, were envisaged as valuable precursors. These structures were selected because they both present a primary alcohol, which allows a high selectivity in terms of functionalization as well as high reactivity towards common nucleophile. The alcohol group is situated in the anomeric position for the fructose-based carbohydrate and in the 6^{th} position for the galactose. The effect of such position difference in synthesis, reactivity and final applications will be part of the present study. The raw materials are shown in Figure II-1.

On the other hand, gluconic acid and lactobionic acid were chosen as potential raw materials for the preparation of linear methacrylate sugars. Indeed, gluconic acid is obtained from agro-industrial residues with high content of sugars, by partial oxidation of glucose.[6] Lactobionic acid is a cheap high value-added natural product commercially available with numerous applications in food, cosmetics or chemical industries. Its use has been recently extended to its conversion into potentially biocompatible and biodegradable systems, mostly used in pharmaceutical and biomedicine fields.[7] Nevertheless, in this context lactobionic acid

has prompted the development of novel system for its biotechnological production that are both sustainable and efficient.[8]

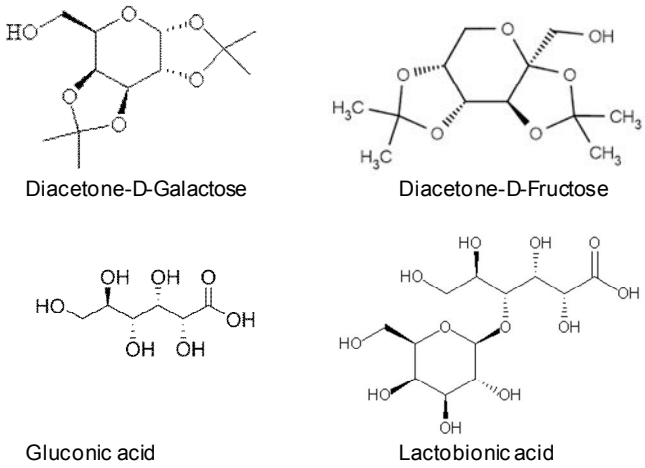


Figure II-1. Raw materials for the preparation of methacrylate sugar-based monomers

In which follows, the synthesis and characterization of several functional monomers derived from the above mentioned carbohydrates will be presented first. Next, in order to compare the different properties induced by such different structures, the monomers will be homopolymerized in solution and eventually in emulsion using conventional radical polymerization. Based on these results, a selection of the most suitable processes and structures in terms of synthetic routes, scale-up capabilities and properties will be assessed.

II- 2. EXPERIMENTAL

Carbohydrate-based compounds 1,2:3,4-Di-O-isopropylidene-D-galactopyranose, also named diacetone-D-galactose (DAGA), 2,3:4,5-Di-O-isopropylidene-b-D-fructopyranose also called diacetone-D-fructose (DAF), gluconic acid, lactobionic acid, were supplied by Carbosynth, UK. AMC-2, which is a mixture of 50% trivalent organic chromium complexes and 50% phthalate esters, was purchased from AMPAC Fine Chemicals. Solvents were purchased from Acros-Organic and Sigma-Aldrich. The rest of reagents were purchased from Aldrich and used without prior purification.

Organic syntheses were performed in round-bottom glass flasks from 50 mL to 2 L capacities. Temperature was controlled by an oil bath equipped with thermometer and magnetic stirring. Nitrogen was used to purge reactions. Chromatography columns from 3 to 15 cm of diameter were used to purify molecules. Thin layer chromatography (TLC) was carried out on aluminium precoated plates (silica gel 40-60Å 400mesh, F_{254}, Aldrich) using hexane/ethyl acetate (EtOAc) 7:3 (v:v) as eluent for hydrophobic compounds and using 1-propanol/$NH_4OH_{(28\%aq)}$/water 11:8:2 (v:v) for hydrophilic molecules. In all cases, compounds were highlighted by spraying TLC plates with ethanol/H_2SO_4 9:1 (v:v) stain and heat.

Proton and carbon NMR were recorded on a Bruker 400 MHz equipment.

Ultra Performance Liquid Chromatography (UPLC-QTOF) was performed using a chromatograph from Acquity, equipped with a diode array detector and coupled with a mass spectrometer (Waters, model SYNAPTTM G2 HDMSTM) with ESI ionization sources. Analyses were conducted in positive ionization mode.

The average particle diameter (dp) was determined by dynamic light scattering, using a Zeta-Sizer instrument from Malvern. Latexes were diluted with deionized water before measurements.

The glass transition temperature (Tg) of the sugar-based polymers was measured by means of a differential scanning calorimeter, series DSC Q1000 (TA Instruments). The measurements were carried out in a range from -70 to 200°C at a heating rate of 10 °C/min.

Polymers molecular weights were determined via gel permeation chromatography, using an Agilent technologies GPC system, equipped with 3 Shodex columns, refractive index and UV detectors. Tetrahydrofuran was used as solvent. Precipitated polymers were solubilised in concentrations ranging from 0.1 to 5.0 mg/mL and passed through 0.45 µm pore size filters before being injected. The analysis was carried out at 1.0 mL/min flow rate. Relative molecular weights were determined by means of a conventional calibration obtained with polystyrene narrow standards.

II- 3. SYNTHESIS OF GALACTOSE-BASED METHACRYLATE MONOMERS

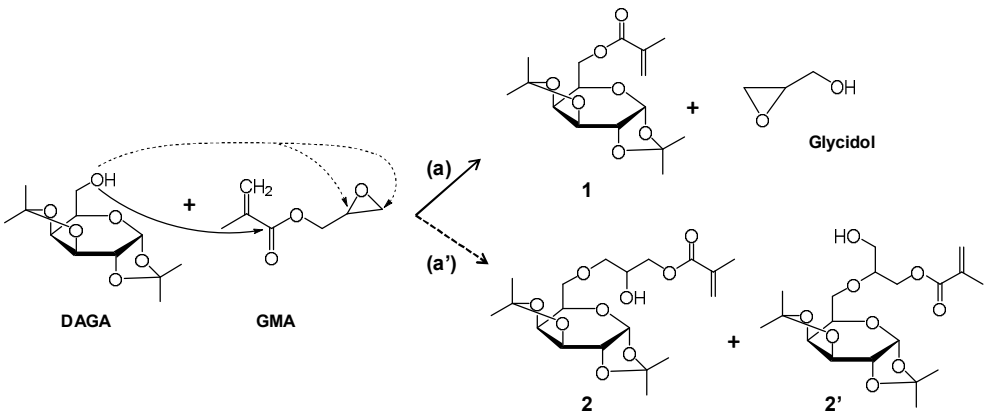
A commercial galactose based monosaccharide carrying acetonide protecting groups was selected as hydrophobic precursor: diacetone-D-galactose (DAGA). The protection step of galactose is quite simple and yield efficient; it involves the use of acetone as reagent/solvent with a catalytic amount of zinc chloride. This type of acetonation was patented in 1955 by Lawrence Glen et al[9].

(Meth)acrylates are of particular interest since they are the main components of waterborne coatings and adhesives. Therefore, in this part of the work the synthesis of different

methacrylate galactose monomers will be investigated. There are many strategies that allow the incorporation of methacrylic groups. Herein, the use of different functional agents will be explored, namely glycidyl methacrylate (GMA), methacrylic acid (MAA), methyl methacrylate (MMA) and methacrylic anhydride (MA) reagents. It is noteworthy that the first two are both coming from the biggest biodiesel production side product: glycerol.[10,11]

Glycidyl methacrylate has been widely used as functionalizing agent for the production of reactive structures derived from dextran,[12–15] sucrose,[16,17] and other polysaccharides,[18,19] because it shows advantages such as reactivity against nucleophiles on milder synthetic conditions. The molecule is carrying two functional sites available for reaction: the methacrylate group and the epoxy group, which can lead to different products, namely from the transesterification of the methacrylic ester and/or from the epoxide ring opening (Scheme II-1, pathway (a) or (a') respectively).

Scheme II-1. Possible reaction pathways for the functionalization of DAGA with GMA



In the literature, a variety of different mechanisms are reported for the reaction between an alcohol and GMA.[12–19] In organic solvents, even if there are some exceptions in recent articles,[13,20] the predominant mechanism appears to be the transesterification (pathway a). The predominance of this mechanism is due to the extreme stability of the epoxy-enolate moiety detached as leaving group during the reaction. This idea was demonstrated by Van Dijk-Wolthuis et al.[21] In aqueous media, the reaction of glycidyl methacrylate with alcohol groups is pH dependant and could lead to both products.[22,23]

These observations are of highest importance here, since the structure of the resulting monomer is very different whether the reaction pathway is transesterification or ring opening. The main difference lies in the presence of a spacer between the sugar ring and the methacrylate group. This spacer may have a significant impact on the properties of the final polymer, thus opening the window to achieve different compounds by controlling the reaction pathway.

II- 3.1.Solvent-free diacetone-D-galactose functionalization

II- 3.1.1. Functionalization with glycidyl methacrylate

On a first hand, the reaction between DAGA and GMA was performed in bulk. DAGA (1eq) and GMA (1eq) were mixed in the presence of triethylamine (NEt_3, 0.2eq) in a round bottom flask. 0.5 wt% of hydroquinone was added as inhibitor to prevent polymerization. The mixture was left to react for 4 hours at 60 °C under nitrogen atmosphere. The reaction was followed by TLC and a mixture of products could be highlighted (Figure II-2).

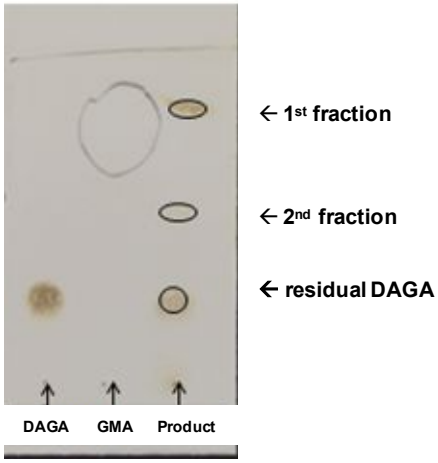
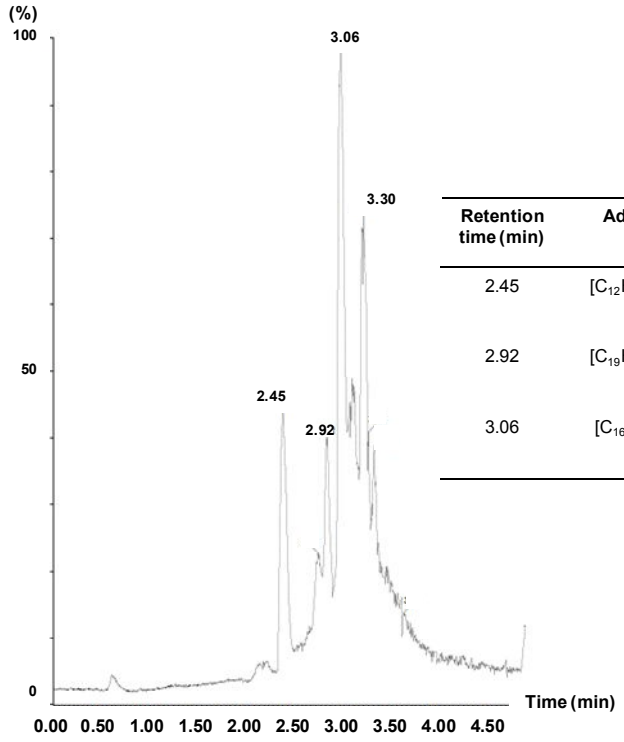


Figure II-2 – TLC plate after elution of the reagent DAGA and GMA as well as the product with an eluent mixture consisting of hexane/ethyl acetate (8:2 v:v)

The resulted crude reaction mixture obtained after four hours was analyzed by UPLC-QTOF, allowing the separation of 3 molecules, identifiable by mass spectrometry (Figure II-3). The main peak eluted at 3.06 minutes and corresponds to the product from transesterification, with the adduct ion $[C_{16}H_{24}O_7+H]^+$ and a mass of 329 g/mol. A molecule resulting from ring-opening mechanism was also detected at 2.92 minutes with a mass of 425 g/mol, and the adduct ion $[C_{19}H_{30}O_9+Na]^+$. The ratio between both pathway products was 90/10 transesterification **1** /ring opening (**2** or **2'**). The reaction allowed the conversion of 80 % of DAGA and its unreacted fraction was also detected by UPLC-QTOF at 2.45 minutes. An additional molecule eluted at 3.30 minutes with a corresponding mass of 606.31 g/mol. This molecular weight suggests a dimer molecule bearing two sugar units; however no consistent potential structure could be identified with the information given.



Retention time (min)	Adduct ion	m/z (g/mol)
2.45	$[C_{12}H_{20}O_6+Na]^+$	283.12
2.92	$[C_{19}H_{30}O_9+Na]^+$	425.18
3.06	$[C_{16}H_{24}O_7+H]^+$	329.16

Figure II-3. UPLC chromatogram of the crude product resulting from DAGA functionalization with GMA and mass spectrometry results

Products were subsequently separated by flash chromatographic column (in a mixture of hexane and ethyl acetate 7:3 (v:v), and the main product of the reaction, a white solid, was characterized by ^{1}H-NMR and ^{13}C-NMR. As can be seen on Figure II-4, the monomer consists of 24 protons, confirming that the main product of DAGA functionalization in bulk with triethylamine results from transesterification pathway, like it was reported in literature for

reactions in solvent. The ^{13}C-NMR spectrum shown in Figure II-5 corroborated that the main product was the result of the transesterification step, namely monomer **1**.

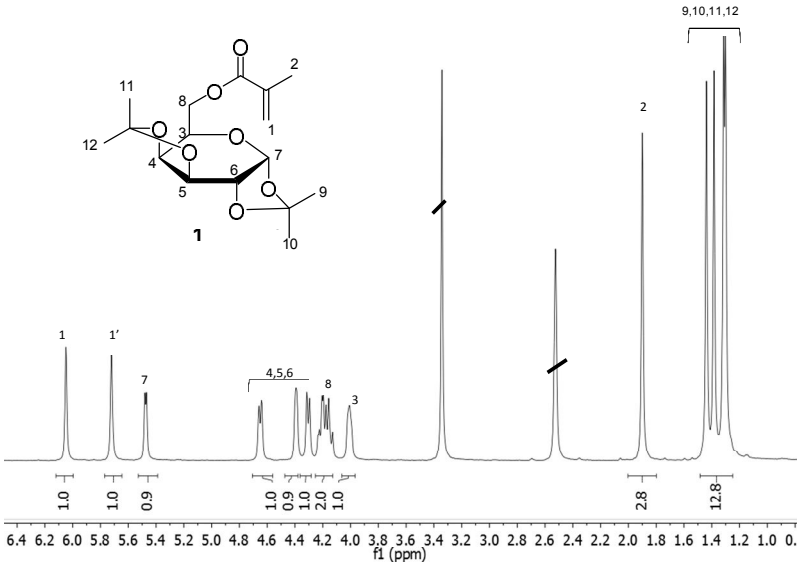


Figure II-4. ^{1}H-NMR of monomer **1**, obtained by transesterification with GMA, recorded in DMSO-d_6

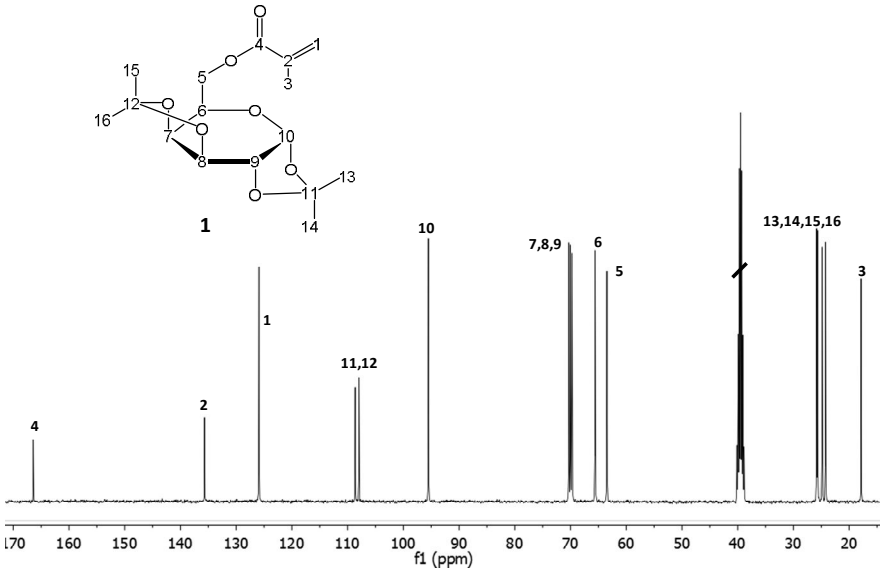
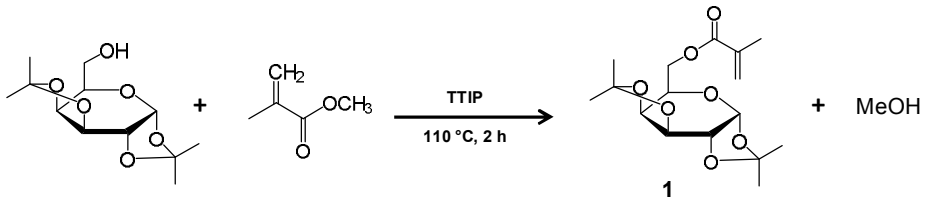


Figure II-5. ^{13}C-NMR of monomer **1**, obtained by transesterification in DMSO-d_6

II- 3.1.2. Functionalization with methyl methacrylate

The results achieved by the first route in which DAGA is functionalized by reacting with GMA in bulk show that transesterification is the most favorable mechanism. Despite of this, because a secondary product is also formed, the yield of the reaction is lower than expected. Therefore, this finding opens the window to use other reactants such as methacryloyl chloride,[24] methacrylic anhydride or methyl methacrylate [24,25] to achieve monomer **1** as single product and therefore with better yields. Since it does not involve the use of solvent and is time efficient, a protocol of functionalizing DAGA with MMA was employed (Scheme II-2).

Scheme II-2. Transesterification functionalization of DAGA with MMA



1

Functionalization of DAGA (30g) with MMA (180 mL) was carried out in bulk (MMA playing the role of solvent) at high temperature (110 °C) in the presence of titanium (IV) isopropoxide (TTIP, 6 mL), using polymerization inhibitor p-methoxyphenol. TTIP is a catalyst that can be used in an environmentally friendly approach, by being grafted onto polymer beads and recycled after reaction.[26–28] A Dean-Stark receiver was used to assure that the methanol produced from the reaction is extracted from the media so that equilibrium is not reached and yield is optimized. During the first 30 minutes, a methanol-methyl methacrylate azeotrope is distilled at 82-85 °C.[29,30] After a couple of hours at 110 °C, under nitrogen atmosphere, the resulting mixture was allowed to cool down by addition of hexane (50 mL) and extracted with distilled water. After drying over Na_2SO_4, the unreacted MMA was evaporated and a yellowish syrup was obtained. The syrup contained a high amount of product and thus purification through simple recrystallization steps in water:ethanol 55:45 (v:v) could be performed. In that case, column chromatography purification was not needed and a white solid was achieved with a melting point of 58-62 °C (yield = 94 wt%).

The ^{1}H-NMR spectrum of the resulting product was the same than Figure II-4, which confirmed that the purified product corresponds to monomer **1**.

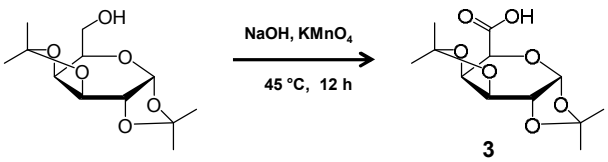
II- 3.2.Ring opening functionalization of diacetone-D-galactose

In this section, in order to obtain galactose monomers carrying spacer between the sugar ring and the functional group, two alternative routes to allow epoxy-ring opening reaction were tested.

II- 3.2.1. DAGA oxidation and functionalization by GMA

In this alternative, DAGA alcoholic function is first oxidized to make it more reactive against the epoxide of GMA (Scheme II-3).

Scheme II-3. Oxidation of diacetone-d-galactose



3

Oxidation was carried out according to the method described by Bentama et al[31] by adding DAGA (3.66 g, 14 mmol) into 61mL of a solution containing NaOH (1.67 g, 42 mmol). When the sugar was solubilized (ca. 30 minutes), 57 mL of an aqueous solution of potassium permanganate (58 g/L, 21mmol, 0.37M) was added dropwise during 15 minutes at ambient temperature. The solution was kept stirring at 45 °C for 12 hours. MnO_2 brown precipitate was filtered off and washed with EtOAc (2 x 50mL). Then a diluted solution of sulfuric acid (H_2SO_4, 0.1 mol/L) was added until pH~2 was reached. The product was extracted in EtOAc and the

organic layer was washed two times with distilled water. The solution was dried over Na_2SO_4 and the solvent was evaporated. The resulting oil was left in a solution of Hexane/EtOAc (9:1 v/v) to give a solid, washed four times to afford compound **3**, with an 80 wt% yield (melting point = 156-158 °C). Figure II-6 shows evidence of oxidation with a signal corresponding to the C=O function at 1750 cm^{-1}.

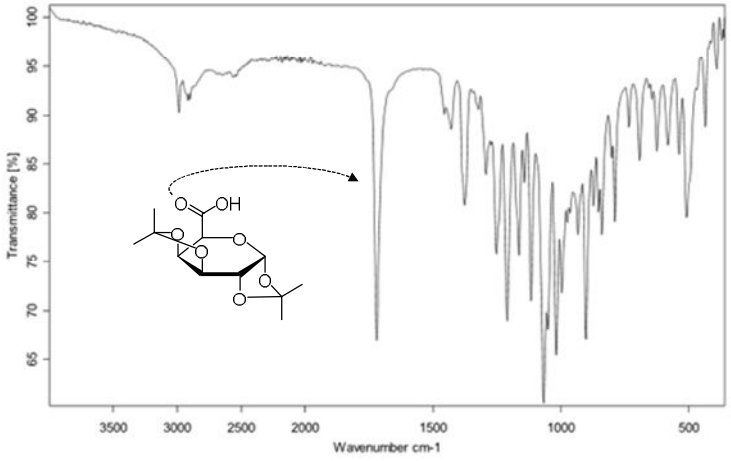


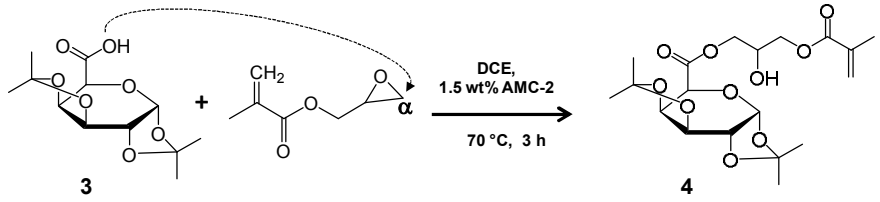
Figure II-6. FTIR spectra of the oxidized diacetone-D-galactose

The oxidized sugar **3** was then allowed to react with GMA in the presence of an industrial catalyst called AMC-2 (Scheme II-4). Specifically designed to promote reactions of epoxides, AMC-2 has shown efficiency in promoting the regiospecific ring-opening reaction of GMA with carboxylic acid groups.[32]

The sugar **3** (3.60 g, 13 mmol) was mixed with glycidyl methacrylate (3 g, 21 mmol) in 150 mL of dichloroethane (DCE) in the presence of 1.5 wt% of AMC-2, with respect to the sugar. A few ppm of benzoquinone were added to the media. The mixture was heated up to 70 °C and

stirred for 3 h. DCE (150 mL) was used to dilute the reaction and the resulting organic phase was washed with distilled water 3x50 mL. Solvent evaporation was performed after drying over Na_2SO_4. The resulting product was isolated by flash column chromatography to obtain a yellowish oil (45 wt% yield).

Scheme II-4. Reaction of oxidized DAGA with GMA



According to the structure of the epoxide group, this reaction may lead to either a primary or a secondary alcohol product. The presence of regio-isomers is difficult to verify by simple flash chromatography or ^{1}H-NMR. Thus UPLC-QTOF analysis was performed and one single peak was observed, indicating the presence of one single regio-isomer of mass 439 g/mol ($[C_{19}H_{28}O_{10}+Na]^+$). According to literature,[33] the most favorable pathway is the nucleophilic attack in the α position of the epoxyde, leading to a secondary alcohol.

The ^{1}H-NMR spectrum of the resulting product is given in Figure II-7 and confirms the structure of molecule **4**, bearing 28 protons. Fully assigned ^{13}C-NMR spectrum in Figure II-8 corroborates these results. DEPT135-NMR spectroscopy was used to highlight the presence of CH_2 carbons coming from the ring opening (negative signals). Surprinsingly 5 CH_2 signals appeared on the spectrum, namely a signal at 125.8 ppm corresponding to the monomer double bond and several peaks with similar shifts in the region of 65 ppm. These peaks were assigned to carbons 5 and 7 resulting from expoide opening. The reason why more than 2

signals can be seen is found in the stereochemistry of the molecule. Indeed, carbon 6 carrying the OH function is a stereocenter. Therefore the monomer is a mixture of two diastereoisomers. An acquisition of ^{13}C-NMR at elevated temperature helped separating the CH_2 signals, confirming these signals are coming from diastereoisomers. Additionally, the signal the stereocenter 6 at 66.3 ppm got split when temperature was increase. This supporting information is available in the Appendix 1.

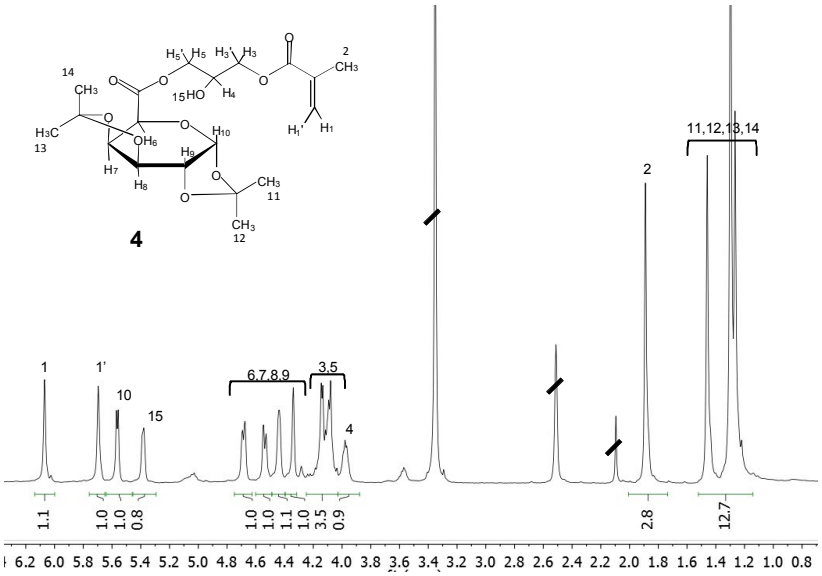


Figure II-7. ^{1}H-NMR of monomer **4**, obtained by ring opening, recorded in DMSO-d_6

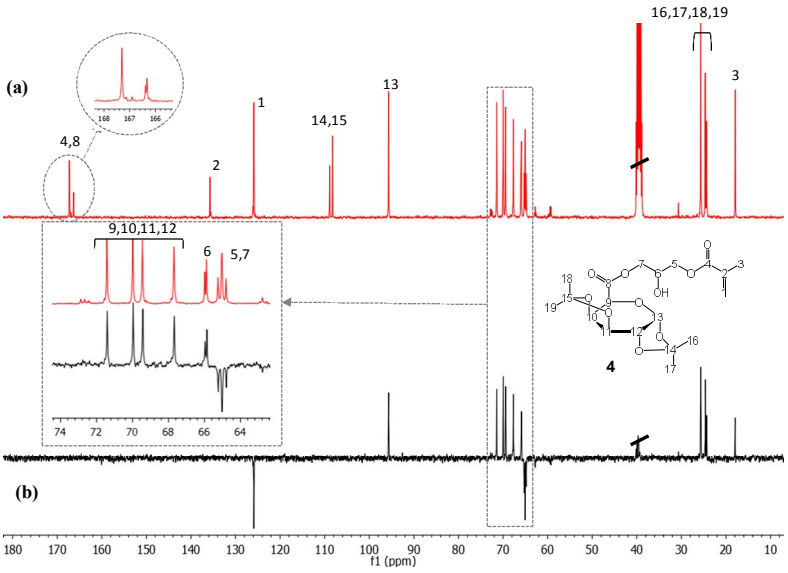


Figure II-8. ^{13}C-NMR (a) and DEPT135-NMR (b) spectra (in DMSO-d_6) of monomer **4**

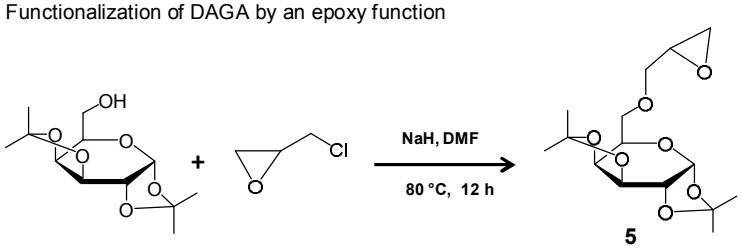
II- 3.2.2. DAGA epoxide fuctionalization and reaction with MAA

A second strategy was investigated to prepare a methacrylate sugar-based monomer carrying a spacer. In that case, monomer **2** obtained as a side product in the bulk reaction of DAGA and GMA (section II-3.1.1) was targeted. The synthesis consisted of grafting an epoxide function onto the sugar scaffold and then make it react with methacrylic acid.

The functionalization of protected galactose by epoxide (Scheme II-5) was performed following the protocol given by Mugunthan et al[34] where the sugar (5.3 g, 20 mmol) was first dissolved in 16 mL of anhydrous dimethyl formamide (DMF) and further activated with an excess of sodium hydride (0.954 g, 40 mmol) at ambient temperature. After 15 minutes, (±)-

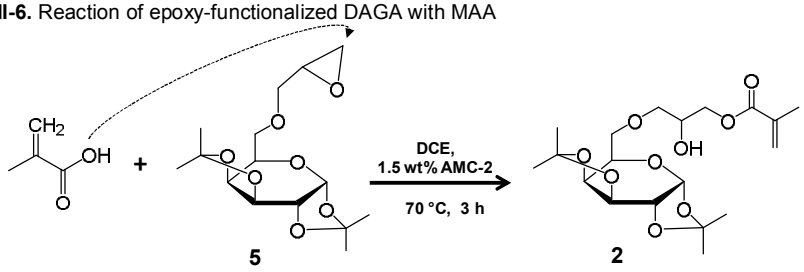
epichlorohydrin (18.9 g, 20.5mmol) was then added and the reaction was kept at 80 °C overnight under nitrogen atmosphere. Then the mixture was diluted with dichloromethane (DCM) and washed 3 times with 75mL of water, dried with Na_2SO_4 and the solvent was removed under vacuum. Product **5** was obtained by purification through a flash chromatographic column, as white solid (85 wt% yield).

Scheme II-5. Functionalization of DAGA by an epoxy function



In a second step, the epoxy carrying sugar **5** reacted with methacrylic acid (Scheme II-6) in the presence of AMC-2 in solvent at 70 °C as follow: to a concentrated solution of **5** (2 g, 6.4 mmol) in dry DCE (50mL) was added methacrylic acid (810 µL, 9.6 mmol) with 1.5 %wt of AMC-2 catalyst. A few ppm of benzoquinone was added. The solution was left to react at 70 °C for 3 hours under nitrogen atmosphere. After dilution in DCE, the organic layer was washed with distilled water (3x50mL) and dried over Na_2SO_4. The solvent was then removed under vacuum and the crude product purified via flash chromatography to obtain a yellowish oil (47 wt% yield).

Scheme II-6. Reaction of epoxy-functionalized DAGA with MAA



5 **2**

Again, this reaction could eventually lead to a secondary or a primary alcohol. Nevertheless, UPLC-QTOF analysis highlighted one single peak, indicating the presence of single isomer of mass 425 g/mol corresponding to the ion adduct $[C_{19}H_{30}O_9+Na]^+$. As mentioned before, AMC-2 catalyst has been reported to allow regio-selective functionalization of epoxides. Thus, the nucleophilic attack in the α position is again favorable and leads to a secondary alcohol product. Monomer **2** was characterized by ^{1}H-NMR spectroscopy (Figure II-9) and ^{13}C-NMR (Figure II-10) and peaks were successfully assigned to the structure.

Monomer **2** is carrying two CH_2 resulting from the epoxide opening, named carbons 5 and 6 in the spectrum. Again in that case, the number of CH_2 carbons highlighted by the DEPT135-NMR spectrum was interestingly higher than expected. The signal coming from the resonance of the vinyl carbon is seen at 126.3 ppm while the peaks corresponding to the epoxide-opening carbons are assigned to the negative signals in the region of 65 to 75 ppm. A ^{13}C-NMR was recorded at elevated temperature and better separation was obtained, indicating the presence of a total of 5 CH_2, so in terms two diastereoisomers (cf Appendix 1).

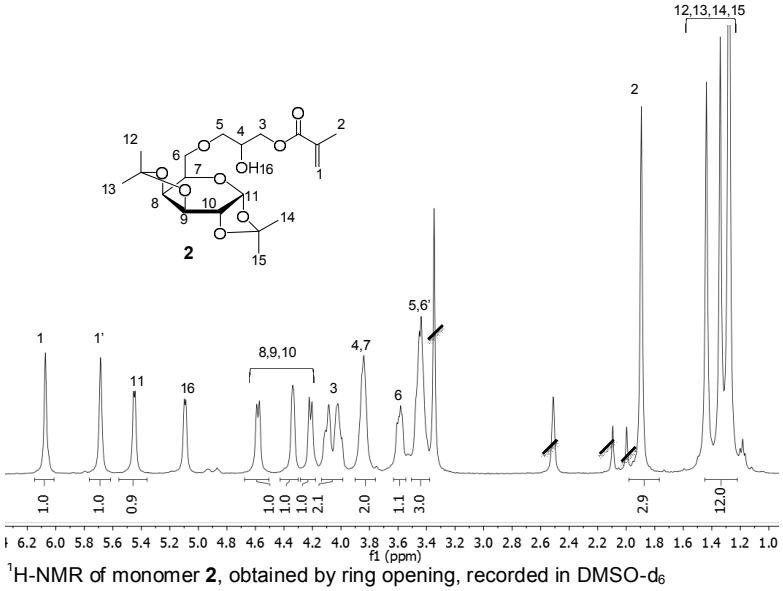


Figure II-9. ^{1}H-NMR of monomer **2**, obtained by ring opening, recorded in DMSO-d_6

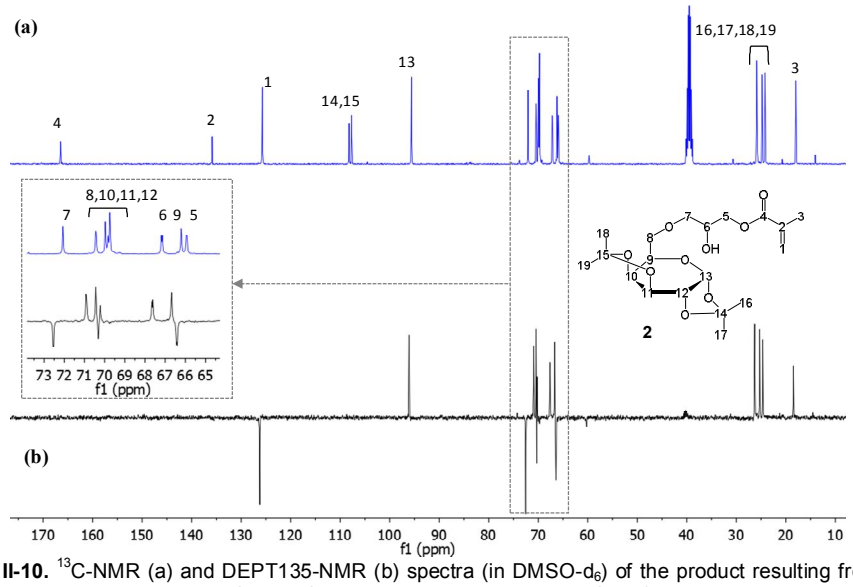
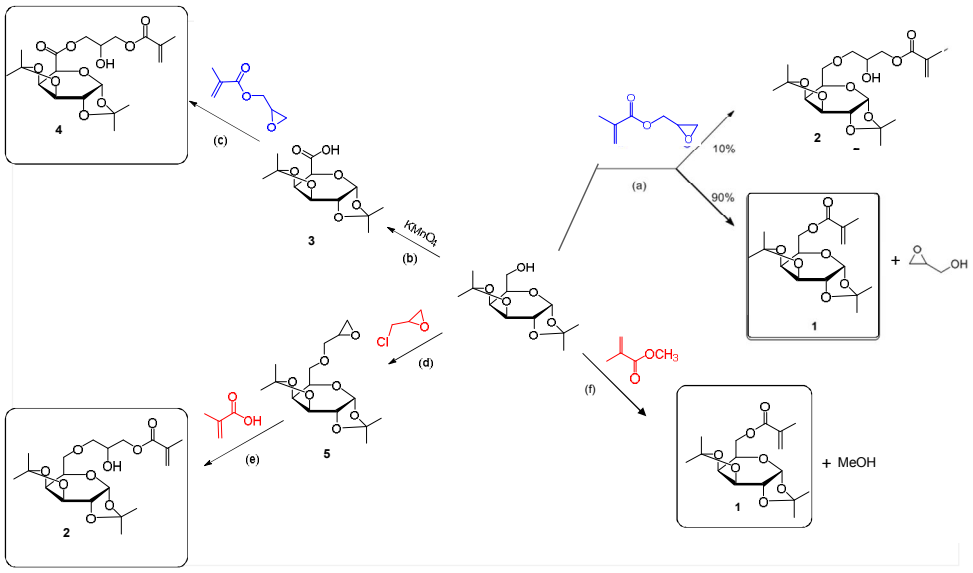


Figure II-10. ^{13}C-NMR (a) and DEPT135-NMR (b) spectra (in DMSO-d_6) of the product resulting from the reaction between the epoxy modified DAGA and MAA, in the presence of AMC-2 catalyst.

In conclusion, three different monomers carrying a methacrylate group were synthesized from protected galactose. Scheme II-7 summarizes the synthetic routes employed and the final monomers achieved. It is worth remarking that monomer **1** is the easiest to produce in opposition to monomers **4** and **2**, which require more synthetic steps and time and involve lower yields.

Scheme II-7. Syntheses strategies summary to produce methacrylate galactose-based monomers from transesterification and epoxy-ring opening mechanisms. Reagents and conditions: (a) GMA, NEt_3, 60 °C, 5 h; (b) NaOH, $KMnO_4$, 45°C, 12h; (c) GMA, AMC-2, DCE, 70 °C, 3h; (d) EPC, DCM, 80 °C, 12h; (e) MA, AMC-2, DCE, 70 °C, 3h; (f) MMA, TTIP, 110 °C, 2h.



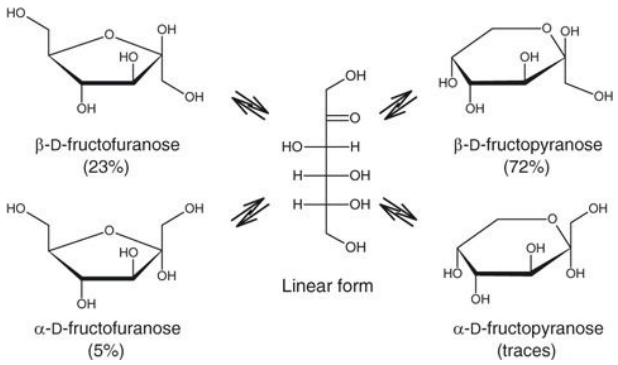
II- 4. SYNTHESIS OF A FRUCTOSE-BASED METHACRYLATE MONOMER

In nature, many different types of monosaccharides are available, namely glucose, ribose, galactose or fructose. In terms of availability, fructose is of high interest since it represents a large portion of fruits containing sugar. Given the fact that the production of methacrylate sugar-based monomers would ideally come mostly from agricultural wastes, it appears interesting to explore the use of fructose as raw material.

Surprisingly, very rare examples of the use of fructose as building block for bio-based polymers can be found in literature although this methacrylate fructose monomer was first described by Koßmehl *et al*[24] in 1989. Methacrylate fructose was cited as potential interesting monomer for the production of waterborne coatings,[35] but as far as we know, actual polymerization results have not been reported yet. More recently, methacrylate fructose was used to synthesize nanocarriers via RAFT polymerization for biomedical application.[36]

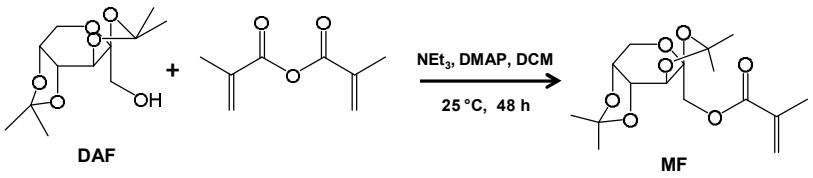
D-fructose is actually present in nature as a mixture of isomers,[37,38] as shown in Scheme II-8. Numbers in parentheses represent the proportion of each form for D-fructose in aqueous solution at room temperature.

Scheme II-8. Equilibrium of D-fructose linear form and α/β pyranose/furanose configurations.[39]



In this work, an isopropylidene protected β-isomeric (diacetone-D-fructose, DAF) form has been used as raw material. A simple transesterification in solvent (DCM) with methacrylic anhydride (1eq) and diacetone-D-fructose (1eq) was carried out at ambient temperature, under nitrogen atmosphere. Triethylamine (2 wbm%) was used as catalyst (Scheme II-9).

Scheme II-9 Functionalization of protected fructose in solvent at ambient temperature



Product isolation was performed through common extraction in DCM, drying over Na_2SO_4, solvent evaporation and followed by column chromatography. In some cases where yield was high enough, purification was permitted by recrystallization in a water:ethanol 55:45 (v:v)

mixture with a 85 wt% yield. The resulting monomer has a melting point in the range of 30 to 35 °C.

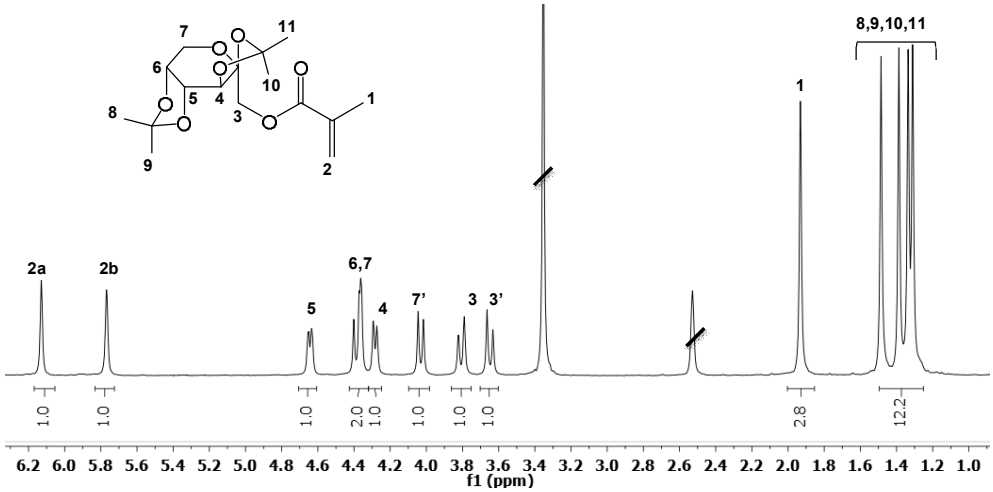


Figure II-11. ^{1}H-NMR spectrum of the methacrylate protected fructose (MF) monomer in DMSO-d_6

Figure II-11 reports the proton NMR spectrum of the pure resulting monomer. The methacrylate functional group is recognizable: vinyl protons are giving signals at 5.8 and 6.2 ppm and methyl protons appear as one singlet at 1.9 ppm. The presence of the sugar ring protons is confirmed by the signals between 3.5 and 4.8 ppm. The ^{13}C-NMR spectrum given in Figure II-12 corroborates these results by highlighting a structure bearing 16 well identified carbons.

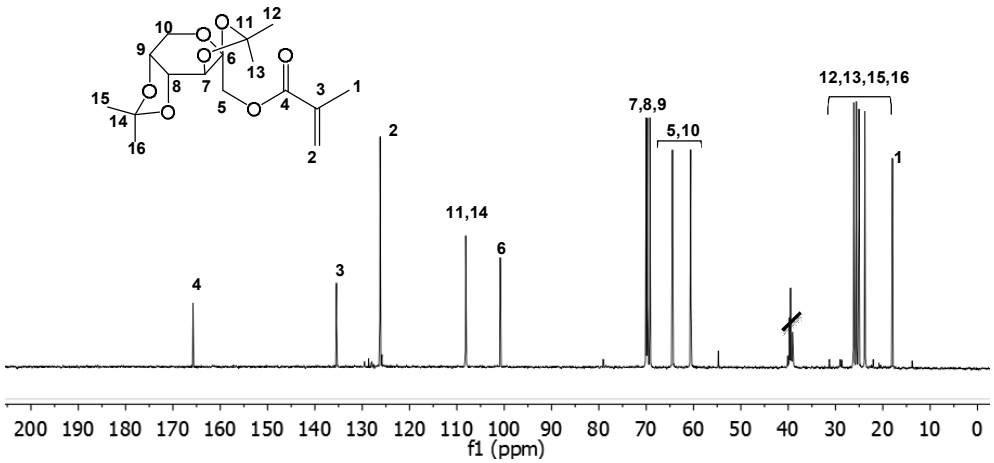


Figure II-12. ^{13}C-NMR spectrum of the methacrylate protected fructose (MF) monomer in DMSO-d_6

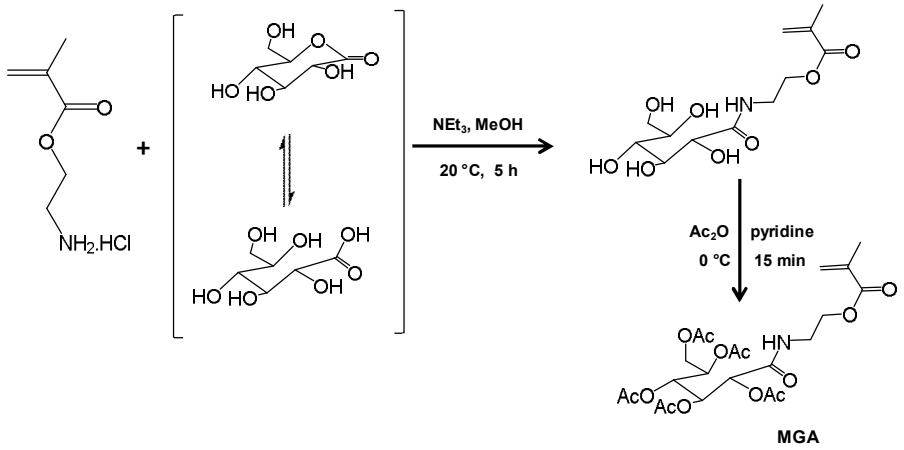
II- 5. SYNTHESIS OF METHACRYLATE HYDROPHOBIC OPEN SUGARS

The cyclic nature of the pre-cited sugar-based monomers makes them very rigid molecules expecting to result in hard polymeric materials. For instance, a glass transition temperature of 167 °C have been reported for homopolymers consisting of furanose (5-membered ring) glucose-based units.[40] Thus, aiming at obtaining sugar-based monomers yielding broad range of Tg polymers, open sugar-based systems were investigated as precursors to low Tg polymers. In this work, gluconic acid and lactobionic acid were chosen as potential raw materials for the preparation of linear methacrylate sugars.

II- 5.1.Synthesis of protected 2-gluconamidoethyl methacrylate monomer (MGA)

In nature sugars are in equilibrium between their open-chain form and cyclic form, and gluconic acid is in permanent equilibrium with gluconolactone in solution.[42] In this work, gluconic acid was chosen as potential raw material for the preparation of linear methacrylate sugar. As mentioned in the introduction, this compound is a high value-added sugar obtained from agro-industrial residues and thus receiving an increasing interest. In fact, its functionalization with a methacrylate function has been fairly investigated but to our knowledge, the preparation of hydrophobic monomer from gluconic acid has not been reported. Herein, the functionalization of gluconic acid and the protection of its hydroxyl function will be presented. Scheme II-10 summarizes the complete synthesis. Final monomer will be called MGA.

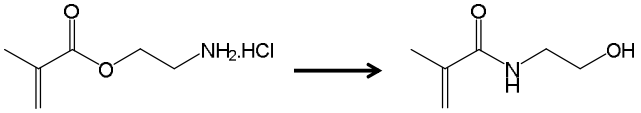
Scheme II-10. Gluconic acid functionalization and protection



It is important to remind that in solution, sugars are naturally in equilibrium between their open-chain form and cyclic form. [41] Thus, in this work the equilibrium between gluconic acid and its cyclic equivalent, that is to say gluconolactone has to be considered.[42–44]

The reaction of gluconic acid in methanol with 2-amino ethyl methacrylate hydrochloride was performed, following a procedure described eslwhere.[45] 2-aminoethyl methacrylate hydrochloride was added in excess to a solution of gluconic acid in methanol at ambient temperature, in presence of triethylamine. The mixture was allowed to react for 5 hours under nitrogen atmosphere at ambient temperature. Product isolation was preceded by selective precipitation in either dichloromethane or 2-propanol.

Scheme II-11. Possible internal rearrangement of 2-aminoethyl methacrylate hydrochloride



It is important to point out that the 2-aminoethyl methacrylate hydrochloride reagent is not very stable and can suffer an internal rearrangement,[46] as shown in Scheme II-11. A particular attention has to be paid to use this reactant in excess. Moreover, the addition of triethylamine to the media is crucial to allow hydrochloride neutralization.

Figure II-13 shows the ^{1}H-NMR spectra of the resulting methacrylate linear sugar. The specific peaks coming from functionalization can be identified and assigned, ensuring a successful incorporation of the methacrylate function onto the sugar scaffold. The signals coming from the hydroxyl functions are visible between 4.25 and 5.70 ppm. Additional 2D-NMR spectroscopy, given in Appendix 1 permitted their identification.

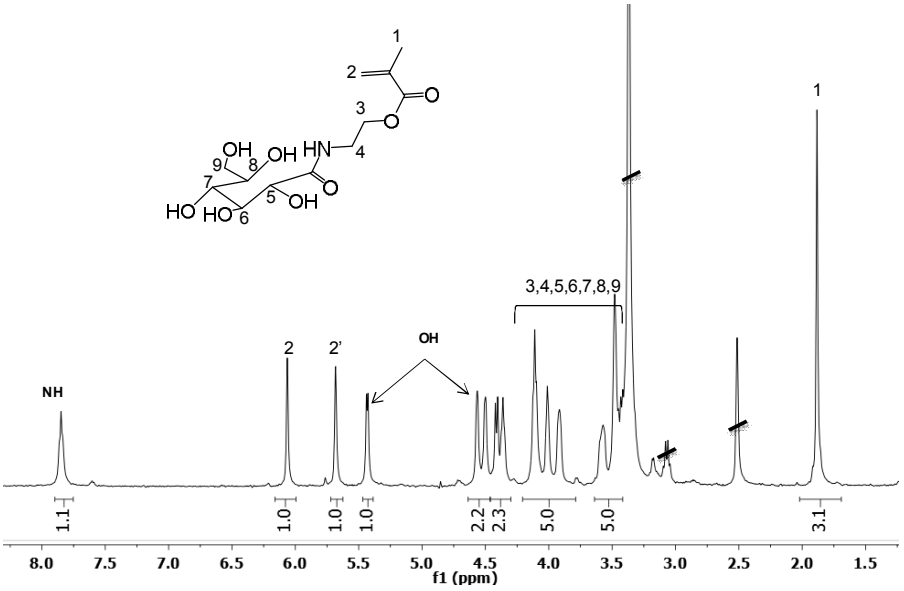


Figure II-13. ^{1}H-NMR spectra of monomer MGA before protection, recorded in DMSO-d_6

As mentioned in the introduction the use of sugars as building blocks for the production of waterborne copolymers requires the preparation of hydrophobic structures. However, the functionalized gluconic acid exhibits full water solubility because of the presence of hydroxyl groups. With this in mind, protection of these hydroxyl groups was carried out. Isopropylidene groups could be used to make the monomer more hydrophobic but it would significantly increase the monomer stiffness and as a result the homopolymer Tg. For this reason, acetyl groups were preferred in that case.

To a flask containing the hydrophilic monomer (1eq) in pyridine, acetic anhydride (5eq) was added dropwise at 0 °C, under nitrogen atmosphere. The reaction mixture was allowed to slowly reach ambient temperature after addition was completed. The solution was then

evaporated to result in brownish syrup, further purified by subsequent DCM extraction, drying and evaporation.

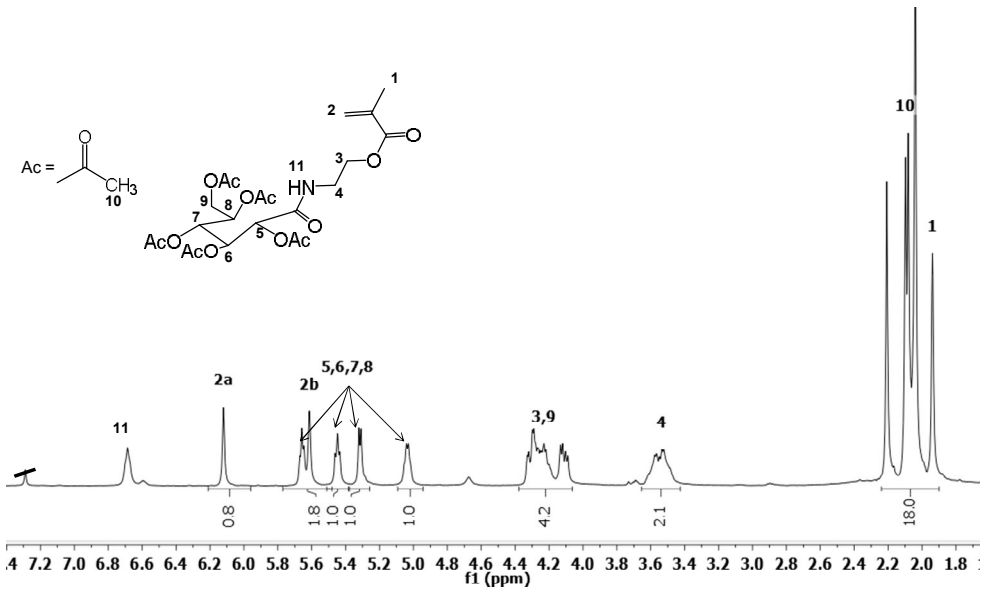


Figure II-14. ^{1}H-NMR spectrum of MGA monomer, recorded in chloroform-d

The resulting hydrophobic monomer was characterized by ^{1}H-NMR spectroscopy. As shown in Figure II-14, full acetyl protection was obtained since peaks between 2.0 and 2.3 ppm, integrate for 15 protons. The presence of the acetyl groups was further corroborated by ^{13}C-NMR (Figure II-15) with the clear presence of extra carbonyl functions around 170 ppm.

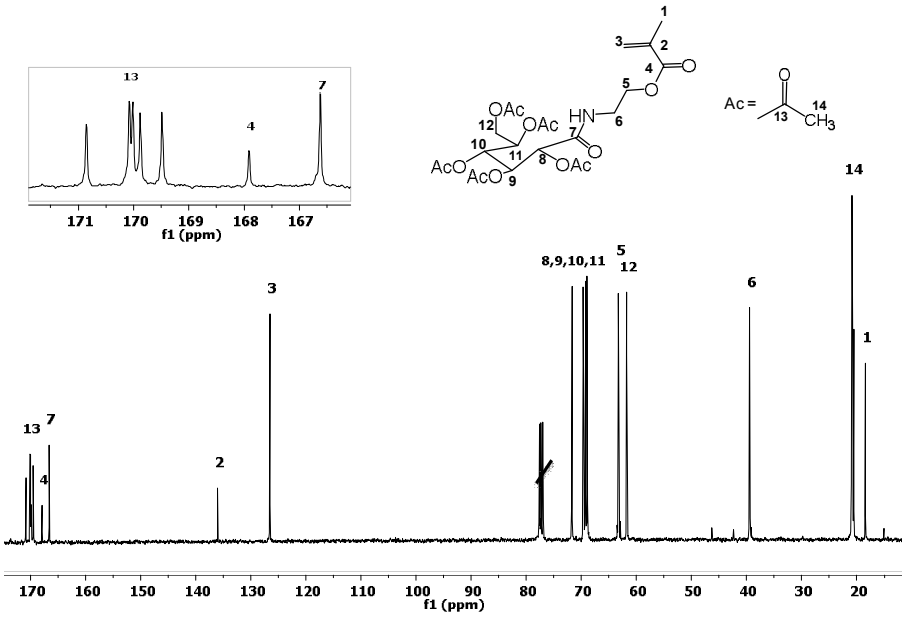
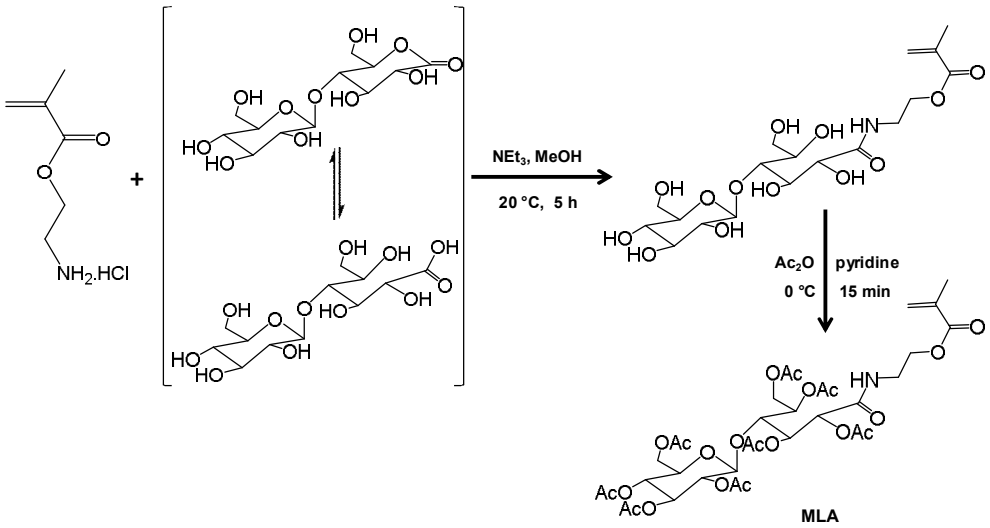


Figure II-15. ^{13}C-NMR spectrum of MGA monomer, recorded in chloroform-d

II- 5.2.Synthesis of protected 2-lactobionamidoethyl methacrylate (MLA)

To further explore the use of cheap sugars coming from agro-industrial residues, lactobionic acid was considered as valuable precursor for the preparation of, in that case, a disaccharide type monomer. Further, although hydrophilic functional lactobionic acid has found interest in many fields, its hydrophobic form has never been published. Following the objective of producing methacrylate monomers able to polymerize in dispersed media, the protection of the resulting water-soluble monomer was carried out to achieve a methacrylate hydrophobic disaccharide monomer, named MLA. Scheme II-12 describes the reaction routes.

Scheme II-12. Lactobionic acid functionalization in solvent at ambient temperature



In a first step, lactobionic acid, in equilibrium with its lactone equivalent, was functionalized in solution with a methacrylate function following the procedure used in paragraph II-5.1 for its gluconic acid homologue. Lactobionic acid was dissolved in methanol and an excess of 2-aminoethyl methacrylate (HCl salt form) was poured to the solution. Triethylamine was added to the media and the mixture was left to react for 5 hours at ambient temperature. Product isolation was performed by selective precipitation in dichloromethane or 2-propanol.

Characterization of the resulting hydrophilic monomer is given Figure II-16. The reactive site of the molecule is preserved and vinyl proton signals are observed at 5.6 and 6.1 ppm, as well as the methyl group protons at 1.8 ppm. Hydroxyl function appear as wider signals in the

region between 4.3 and 5.3 ppm. Additional NMR spectroscopy was performed to fully characterize the molecule (Appendix 1).

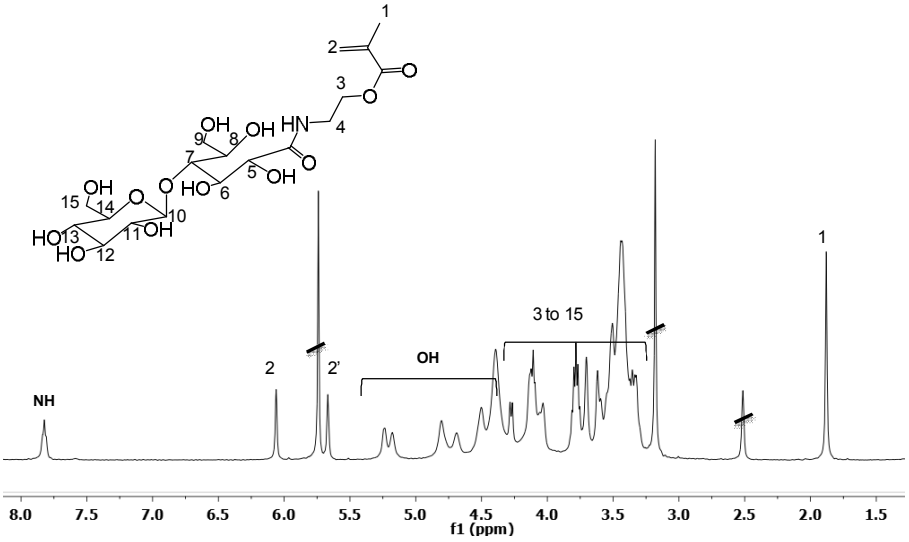


Figure II-16. ^{1}H-NMR spectra of monomer MLA before protection, recorded in DMSO-d_6

As explained above, to be used in emulsion polymerization, monomers need to exhibit hydrophobicity. Therefore, protection of the hydroxyl groups is essential. The procedure employed to acetylate the OH functions of the disaccharide monomer is the same as the one used for the functionalized gluconic acid. The monomer (1eq) was mixed with pyridine and temperature was decreased to 0 °C. Acetic anhydride (8eq) was added dropwise to the medium and the reaction was left to warm up to ambient temperature again. The product was extracted in DCM, dried over Na_2SO_4 and solvent was evaporated. MLa monomer was obtained as a white solid.

Figure II-17 shows the ^{1}H-NMR of MLA hydrophobic methacrylate resulting monomer. It can be concluded that full acetylation is achieved from the integrals of the peaks corresponding to the 8 acetyl groups, carrying 24 protons (1.8 – 2.2 ppm).

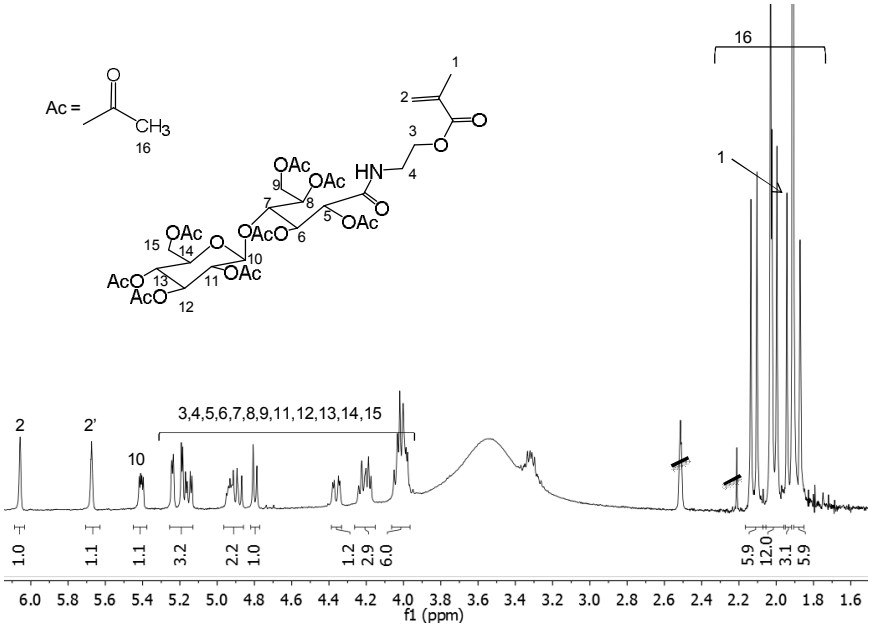


Figure II-17. ^{1}H-NMR spectrum of monomer MLA

II- 6. HOMOPOLYMERIZATION IN SOLUTION

In order to compare the properties of the synthesized monomers, their homopolymerization was performed in solution. Thus, monomers prepared from galactose through both transesterification and ring opening were polymerized and characterized, and the influence of the spacer in the repeated unit structures evaluated. On a second hand, the

monomers produced from open sugars, namely MGA and MLA, as well as their unprotected homologues were homopolymerized and characterized, and the effect of their structural differences, as well as protecting groups on glass transition temperatures discussed.

Monomers were polymerized at 70 °C using conventional radical polymerization in benzene with Azobisisobutyronitrile (AIBN) as thermal initiator. 0.5g of monomer was dissolved in 10 mL of benzene. The reaction mixture was purged with nitrogen for 20 minutes and then heated up to 70 °C. AIBN 1wbm%, dissolved in benzene (ca. 0.1mL) was added as a shot to initiate the polymerization. After 48 hours of reaction, the polymer was precipitated in a large amount of heptane. The product was filtered under vacuum, then dissolved in THF and re-precipitated again in heptane. The resulting fine powder was suction filtrated and dried for one night under vacuum. In all cases yield higher than 80 wt% were achieved.

II- 6.1. Galactose-based monomers. Effect of spacer

Homopolymers coming from galactose-based monomers **1**, **2** and **4** (Scheme II-7) (polymers named as HOMO-1, HOMO-2 and HOMO-4 respectively) were characterized by DSC in order to evaluate their glass transition temperatures. The results presented in Figure II-18 show a significant effect of the spacer between the methacrylate group and the sugar on Tg. Because of its cyclic nature, the protected pendant sugar scaffold provides stiffness to the chains and as it can be seen HOMO-1 exhibits a very high Tg around 110-115 °C. However, the presence of a short chain separating the methacrylate from the sugar helps improving significantly the mobility of the polymer chains and justifies the lower Tg of HOMO-4 and

HOMO-2 around 65 °C and 36 °C respectively. We also believe that the planarity of the extra carbonyl group in HOMO-5 can explain its higher Tg compared to HOMO-2.

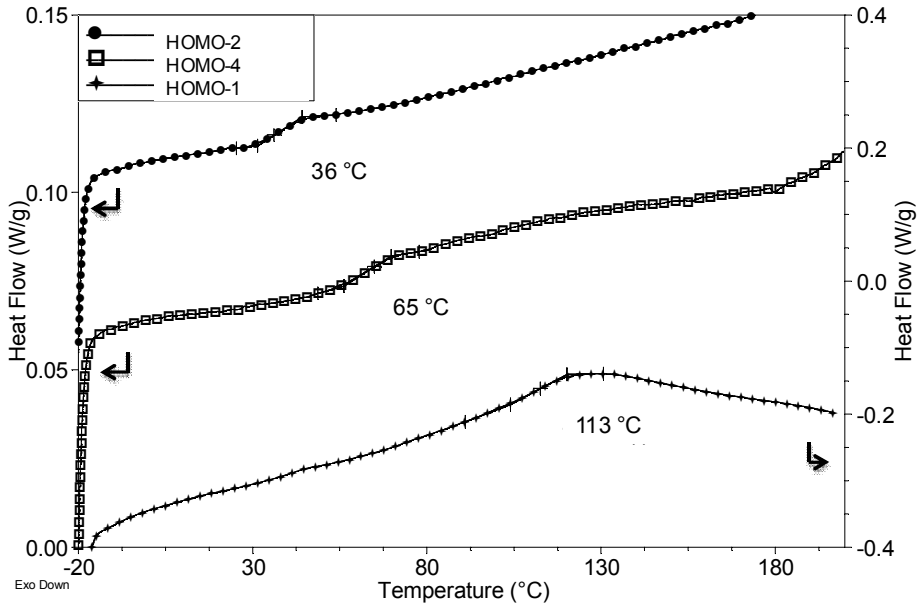


Figure II-18. Overlapped DSC curves of homopolymers from monomers 1, 2 and 4.

II- 6.2.Functionalized open sugars

The properties of homopolymers from monomers MGA, MLA and their unprotected homologues were also evaluated. Figure II-19 shows the glass transition temperatures of the corresponding four homopolymers. Polymers from hydrophobic MGA and MLA (HOMO-MGA and HOMO-MLA) have Tg around 26 and 51 °C respectively. The higher Tg of HOMO-MLA is explained by the presence of a sugar closed ring given that lactobionic acid is a disaccharide.

In addition a clear effect of protecting groups on Tg values can be observed. Indeed, the absence of acetyl groups induces a Tg decrease: HOMO-MGA Tg moves from 26 °C to -10 °C, while HOMO-MLA Tg is shifted from 51 °C to 18 °C when unprotected. Again, the planarity of the extra acetyl groups carrying carbonyl functions is believed to be responsible of chains mobility decrease and Tg rise up.

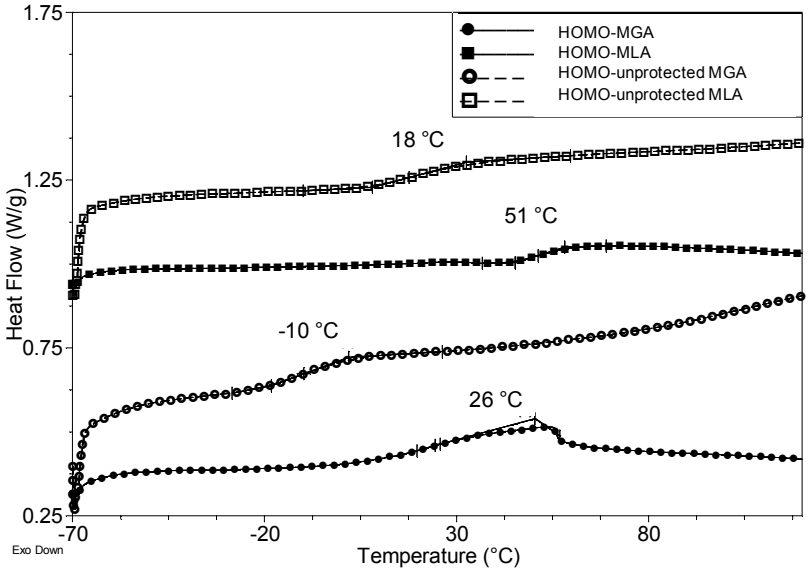


Figure II-19. Overlapped DSC curves of homopolymers from both protected and unprotected MGA and MLA

II- 7. HOMOPOLYMERIZATION IN EMULSION

Given the fact that these sugar-based monomers are meant to be used as comonomers in the preparation of waterborne polymeric binders, their potential ability of homopolymerizing

in a more sensitive process such as emulsion is interesting to be tested. Batch emulsion polymerizations of methacrylate galactose (monomer **1**) and methacrylate fructose (named MG and MF respectively hereafter) were performed in a 250 mL double wall glass reactor, equipped with a turbine stirrer, a nitrogen inlet, a reflux condenser, a temperature probe and a sample device. Reaction temperature was controlled by a Lauda water bath.

The sugar-based monomers were heated up and introduced as a melted substance in the reactor containing an aqueous phase made of deionized water, emulsifier (sodium dodecyl sulfate, 4 %wbm) and buffer ($NaHCO_3$, 4 %wbm) pre-heated at 60 °C. The pre-emulsion was prepared under nitrogen purging and strong agitation stirring (800 rpm). Then, temperature was increased to 70 °C and the initiator (potassium persulfate, 2%wbm), previously dissolved in water, was introduced in the media. Low solids content of 10 wt% was targeted.

Table II-1. Emulsion polymerization characteristics

Run	Final dp (nm)	Tg (°C)	Mw (g/mol)	Đ
HOMO-MG	**63**	**110 – 115**	**$1x10^6$**	**1.9**
HOMO-MF	**67**	**110 – 115**	**$0.9x10^5$**	**2.2**

Full conversion was achieved in both cases, as complete disappearance of the vinyl protons signals was observed after 1 hour of reaction by ^{1}H-NMR. Moreover, small particle sizes were obtained (as observed by TEM image of MG homopolymerization, Figure II-20), and the molecular weights were in the range of emulsion polymers (Table II-1).

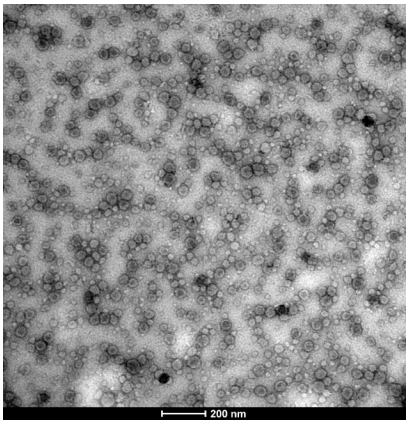


Figure II-20. TEM image of MG homopolymer latex

Thermal properties from both homopolymers were characterized by differential scanning calorimetry. It can be seen from Figure II-21 that MG and MF homopolymers exhibit the same thermal behavior in terms of glass transition temperature. Tg can be measured in the range of 110 – 115 °C. These high values can be explained by the cyclic nature of the sugar combined with the rigidity of the diacetonide protecting groups which limits the mobility amplitude of the chains. As demonstrated above, the absence of spacer between the methacrylate group and the sugar ring also contributes to the high Tg. All things considered, it can be concluded that the position of the methacrylate function in the sugar ring does not significantly affect the behavior of the monomer neither in terms of polymerization nor with respect to thermal properties.

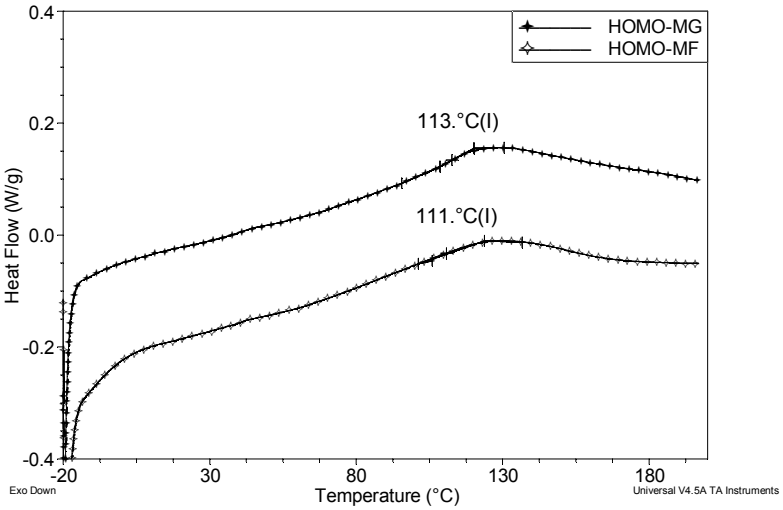


Figure II-21. Overlapped DSC curves of homopolymers from MG and MF polymerized in emulsion

II- 8. CONCLUSIONS

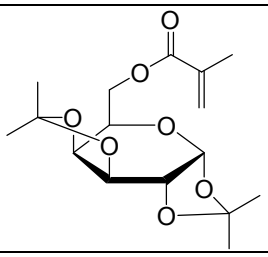
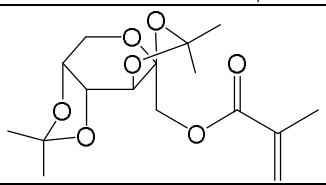
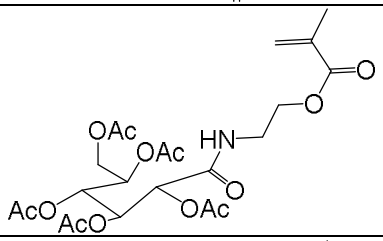
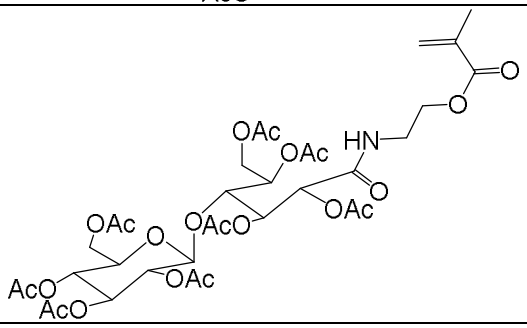
In this chapter, several monomers were produced, namely starting from diacetonide galactose and fructose or from open sugars such as gluconic and lactobionic acid, as summarized in Table II-2. Different synthesis strategies were tested and all succeeded in the obtaining of hydrophobic methacrylate monomers. Nevertheless, not all the pathways employed are worth to be considered. Indeed, the preparation of methacrylate sugars via ring opening is yield inefficient and time consuming whereas preparation of methacrylate galactose and fructose by transesterification is yield efficient and rather simple, making the process easier to scale up.

Still homopolymerization of all prepared monomers was carried out in solution. As expected, the use of open-form sugars as building block leads to lower Tg polymers. Further, it was concluded that the use of protecting groups, in particular acetyl groups here induces an increase in Tg.

As to confirm the great interest for transesterified diacetone galactose (MG) and fructose (MF), emulsion polymerization was carried out at 10 wt% solids content. Stable systems were obtained, supporting a promising behavior for further copolymerization in dispersed media. Additionally, no significant differences were observed between MG and MF in terms of polymer properties.

As a result, the selection of MG and MF as most promising monomers for the preparation of waterborne copolymers emerges relevant. Furthermore, due to their high Tg, these compounds appear as a potential equivalent to the petrochemical methyl methacrylate raw material, which is commonly used in acrylic systems to improve mechanical properties of films.

Table II-2. Summary of the synthesized hydrophobic sugar-based methacrylate monomers

NAME AND ABBREVIATION	STRUCTURE	APPEARENCE	HOMOPOLYMER Tg
Methacrylate galactose, **MG**		White to yellowish solid	110 – 115 °C
Methacrylate fructose, **MF**		White to yellowish oil/solid	110 – 115 °C
Methacrylate gluconic acid, **MGA**		Colourless to slightly brownish oil	26 °C
Methacrylate lactobionic acid, **MLA**		White solid	51 °C

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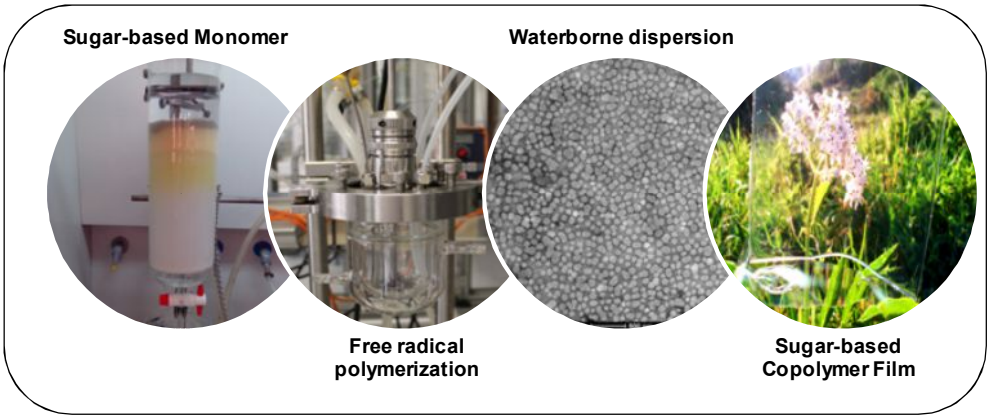
Chapter III- Waterborne Copolymers from Methacrylate Sugars



III- 1. INTRODUCTION

In chapter II the preparation and homopolymerization of sugar-based methacrylate monomers and their corresponding thermal properties was reported. Both methacrylate galactose (MG) and methacrylate fructose (MF) were shown to be able to homopolymerize in emulsion by free radical polymerization and the resulting polymers exhibited rather high glass transition temperatures around 110-115 °C, which appeared as an interesting alternative to the petrochemical methyl methacrylate (MMA) raw material.

Therefore, in this chapter, the substitution of MMA in waterborne acrylic containing formulations –typically used as adhesives or coatings- by either MG or MF will be investigated. As a first approach, low solids content emulsion polymerizations with different sugar-based monomer/butyl acrylate (BA) compositions will be studied. Taking the advantage of the understanding gained in this first part, the study will be extended to high solids content systems, which are very important for effective industrial applications. Indeed, among other factors, the reaction production is maximized, transportation and storage costs are minimized, surface coverage is improved when applied and film-forming and drying time are reduced. In addition, and aiming to go towards a fully bio-based system, a commercial bio-based monomer with low Tg, named Visiomer C13-MA, will be used instead of BA. In all the cases, polymerization kinetics, polymer characteristics, thermal properties and the quality of the resulting films will be reported.

III- 2. MG OR MF/BA SUGAR–BASED LATEXES

Emulsion copolymerization reactions were carried out in batch, by varying the bio-based monomer content (MG or MF), and the initiator amount in some cases. 30 wt% solids content as well as 45 wt% solids latexes were synthesized. For the preparation of the emulsion, the organic phase composed by the monomer mixture (MG or MF and BA) and the aqueous phase containing the emulsifier and the buffer ($NaHCO_3$), used to avoid drops in pH during the reaction, were mixed under magnetic stirring (15 min at 900 rpm). The resultant mixture was poured in a double wall glass reactor equipped with a condenser, a nitrogen inlet, a sample device and a stainless steel turbine stirrer. Reactions were carried out at 70 °C under agitation (250 rpm) at pH around 9. The initiator KPS was injected as a shot when the pre-emulsion temperature reached 70 °C, defining the beginning of the reaction. The system was then allowed to react in batch. Samples were withdrawn at regular intervals and inhibitor (hydroquinone) was used to stop reaction and allow monomer conversion follow-up along time, which was performed by ^{1}H-NMR (See Appendix 1).

III- 2.1. 30 wt% solids content sugar-based copolymer latexes

30 wt% solids content copolymers at different ratio of methacrylate galactose or methacrylate fructose with butyl acrylate were synthesized by free radical emulsion polymerization, by using the formulation presented in Table III.1.

Table III-1. 30 wt% solids content formulation

Ingredient	Amount (g)
Monomers[a]	30.00
Water	73.15
SDS	0.60
KPS	0.30
$NaHCO_3$	0.45

[a] MG or MF and BA

The copolymer composition in both weight and molar fraction together with the nomenclature to be used throughout this chapter is reported in Table III.2. The first number refers to the weight percentage of sugar-based monomer while the second number corresponds to the solids content.

Table III-2. Methacrylate sugar-based copolymers composition and nomenclature

Latex name	Sugar monomer/BA ratio (wt%)	Sugar monomer (molar composition)	BA (molar composition)
MG20-BA/30	20/80	0.089	0.911
MG30-BA/30	30/70	0.143	0.857
MG40-BA/30	40/60	0.206	0.794
MF20-BA/30	20/80	0.089	0.911
MF30-BA/30	30/70	0.143	0.857
MF40-BA/30	40/60	0.206	0.794

III- 2.1.1. Polymerization kinetics

Figure III.1 presents the kinetics and the particle size evolution of the different methacrylate galactose-based copolymers. All reactions occurred very fast and no differences among the different compositions are observed. Final particles were in the range of 80 nm, and independent from the initial monomer composition. A TEM picture of MG40-BA/30 latex is given in Figure III-2.

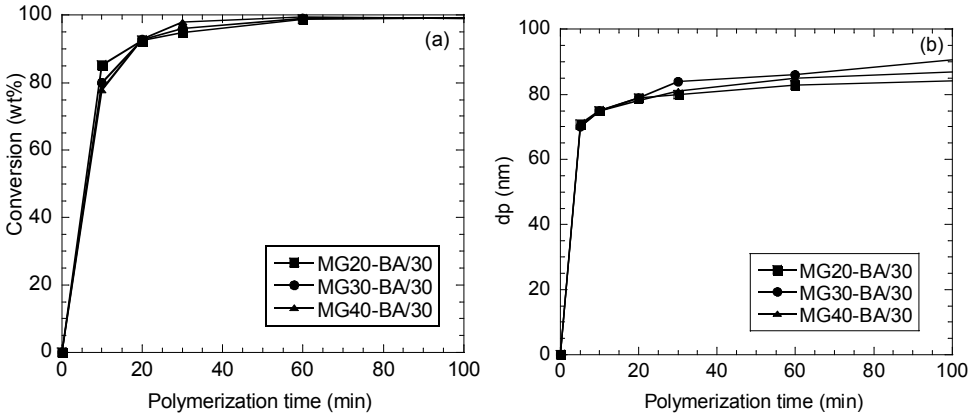


Figure III-1. Methacrylate galactose-based copolymers. (a) Kinetics and (b) particle size evolution

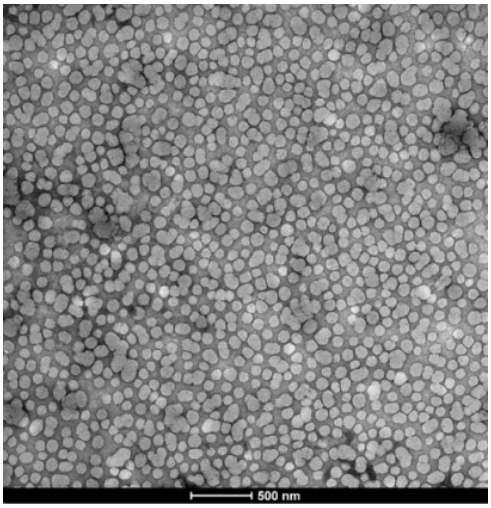


Figure III-2. TEM image of galactose methacrylate and butyl acrylate copolymer (40/60).

The copolymerizations carried out with the methacrylate fructose and BA (Figure III-3) presented similar behavior than the previous ones, except for the faster kinetics of the MF40-BA/30.

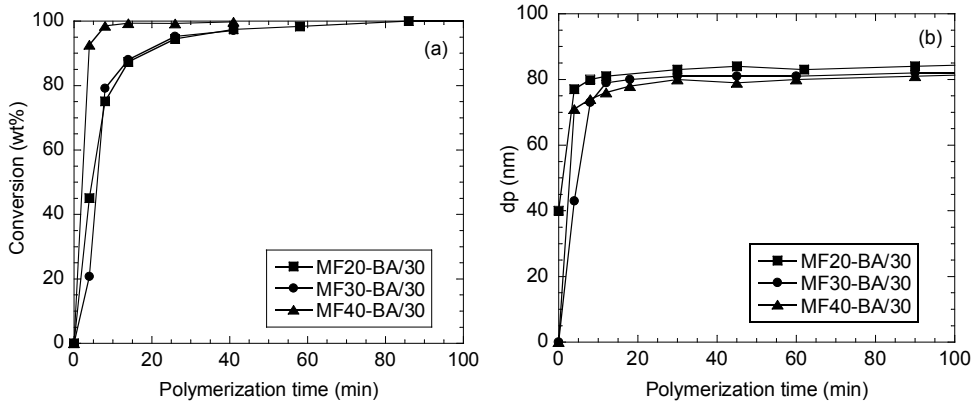


Figure III-3. Methacrylate fructose-based copolymers. (a) Kinetics and (b) particle size evolution for copolymerizations carried out with 1.0 wbm% of KPS.

In order to clarify the behavior of MF40-BA/30, another set of reactions using lower amount of initiator (0.5 wbm%) were performed (Figure III-4). As expected, slower kinetics were observed, but again the reaction containing 40 wt% of MF was the fastest. Due to the much higher reactivity of the methacrylates with respect to BA, the effect of the composition on the overall conversion would be more pronounced when dealing with higher methacrylate composition.[1]

The reason why this difference was not observed in the case of the MG system can be attributed to the fact that kinetics are so fast that the experimental error measuring the conversion (^{1}H-NMR) may be in the range of the overall conversion differences. Indeed, differences are observed in kinetics reported in the following section. Therefore, it can be concluded that likely kinetics follow the trend of methacrylate/acrylate systems in batch, and the position of the methacrylate function in the sugar ring did not affect the polymerization, in accordance with what was observed for homopolymerations in the previous chapter.

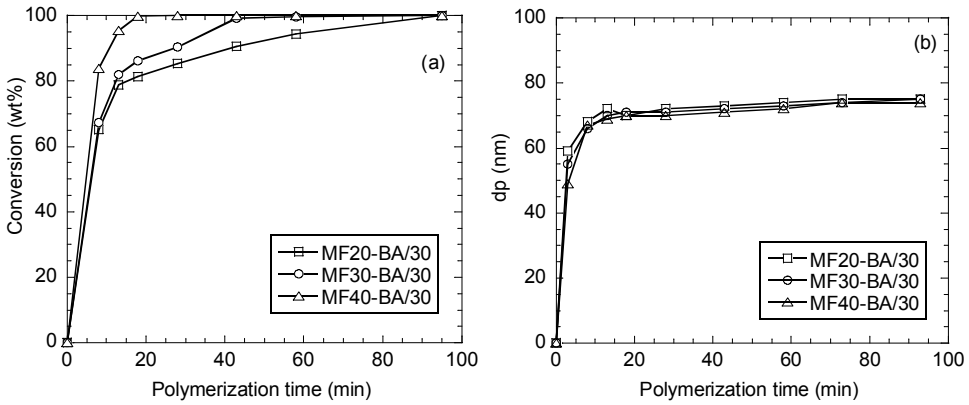


Figure III-4. Methacrylate fructose-based copolymers. (a) Kinetics and (b) particle size evolution for copolymerizations carried out with 0.5 wbm% of KPS

III- 2.1.2. Polymer characterization

Gel content (insoluble polymer in THF) was measured by soxhlet extraction. Rather high amounts of gel were obtained in all cases as shown in Figure III-5. By one hand, high amount of gel is expected when dealing with large BA content latexes, because of intermolecular chain transfer to polymer followed by termination by combination, as reported in literature.[2–4] However, it is also mentioned that the incorporation of methyl methacrylate (MMA) units in butyl acrylate chains promotes the decrease of gel content.[4] Indeed, MMA terminated chains are less reactive towards hydrogen abstraction and the absence of abstractable hydrogens in the MMA units together with the fact that MMA radicals terminate predominantly by disproportionation explain the significant reduction of gel content. Thus, since the bio-based monomer is carrying a methacrylate function a decrease in gel could be expected as more methacrylate sugar is incorporated. This effect was observed for the two sets of reactions involving MF/BA copolymerization, i.e. using 0.5 %KPS on one side and 1%KPS on the other. Additionally, gel content was found lower when a smaller amount of KPS was employed. Latexes containing 40 % of methacrylate fructose exhibited gel content of 28% when prepared with 0.5 %KPS and 72% when prepared with 1%KPS (Figure III-5 (a)). This unexpected result goes against theory according to which higher initiator concentration induces a superior number of radical per particle, promoting termination reactions, and hence lower gel contents.

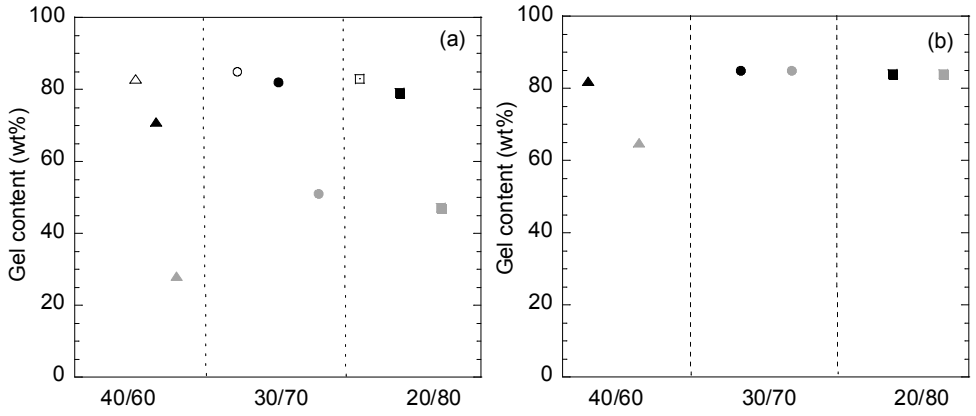


Figure III-5. Gel content of sugar-based latexes prepared with (Δ○□) MG/BA at 1wbm%KPS (▲●■) MF/BA at 1wbm%KPS and (▲●■) MF/BA at 0.5wbm%KPS. Measurement repeated after 15 days (right plot).

It could be speculated that the system is evolving during storage. Indeed, recent studies in our lab have shown that the gel content of MMA/BA (50/50) latex increases during storage, when the latex does not contain hydroquinone (HQ), likely because of the presence of tertiary radicals which are very stable and can survive long time. In our case, gel was not measured systematically after reaction but eventually a few days after. Further, the latexes were stored without HQ. Therefore, this hypothesis could further contribute to explain the results.

Another aspect to take into consideration is the probability of sugar side chains to suffer partial deprotection, yielding –OH groups that could aggregate due to hydrogen bonding. It is well known that the isopropylidene protecting groups can be cleaved under acidic conditions.[6] Figure III-6 shows the deprotection mechanism of the diacetone.D-galactose. However it was reported that protecting groups within non-water soluble polymer chains are rather hard to cleave. Black et al[7] reported the particular resistance to deprotection of methacrylate galactose

homopolymers, showing that heating at 100 °C in HCl for several hours failed deprotecting the carbohydrate units. However, emulsion polymerization involves the transfer of monomer through the aqueous phase. It can be speculated that most likely low molecular weight accessible sugar units in water are the most likely to be deprotected. In our work, reactions were performed at basic pH by using $NaHCO_3$ as buffer, so hydrolysis of the protecting groups should be reduced.[8] In any case, it is worthy to note that if the polymer chains have significant molecular weight, just few –OH in the chain would be enough to lead to association by hydrogen bonding and to form polymer chains that are not soluble in THF.

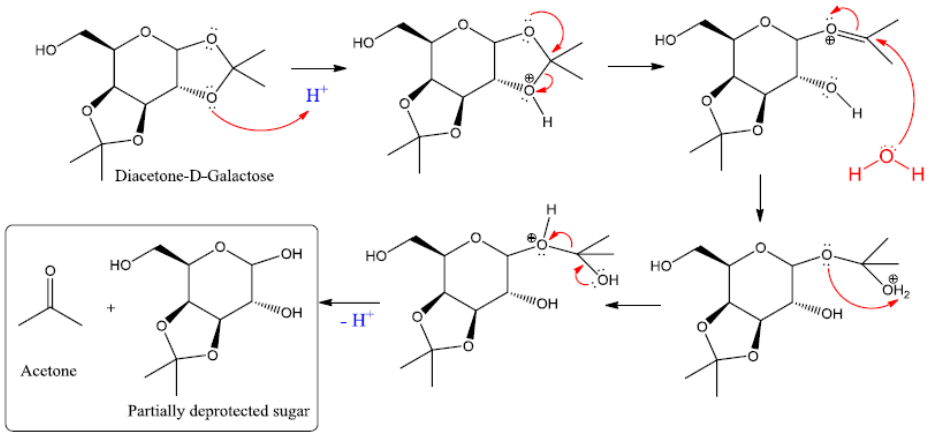


Figure III-6. Deprotection mechanism of diacetone-D-galactose

Some attempts to prove hydrolysis of the diacetonide groups were performed. For instance, MG40/BA latexes were analyzed by ATR-FTIR and ^{13}C-NMR after approximately six months of storage, but no presence of –OH was detectable. We observed a decrease on the pH from almost 9 to around 5. The difficult quantification of a small portion of deprotection is

essentially due to the detection limits of the analytical techniques, especially when sugar units consist of less than half of the sample. Besides, the insolubility of polymers in deuterated solvents, restraining chains mobility reduced considerably spectra resolution.

To verify if the latexes evolved during storage, gel measurement of the latexes containing MF/BA were repeated. As a result, MF-40BA/30 sets of experiments prepared with 0.5%KPS were characterized after only 3 days of storage while the serie of reactions performed with 1 %KPS was analyzed after 26 days. Soxhlet extractions were repeated for both series, copolymer dispersions being respectively 18 days and 41 days old. Figure III-5 (b) summarizes the results. As a first sight, 0.5%KPS series “fresh” latexes were found to exhibit low gel, but a clear increase was observed after only 15 days of storage. On the other hand, the comparison of the 1% KPS series also shows that the latex is evolving during storage. From the observed evolution, it can be concluded that the gel fraction of the three series are in fact comparable and that most likely the insoluble fraction in THF increases upon time because of a very small deprotection of the sugar chains and hydrogen bonding formation.

III- 2.1.3. Thermal characterization and film properties

The glass transition temperatures of the resulting MG/BA and MF/BA copolymers were measured by DSC, and are reported in Figure III-7 (a) and (b) respectively. It was observed that all copolymers exhibited one single Tg and the values were in good agreement with the predicted by the Fox-equation, considering the Tg of the sugar-based homopolymer 115ºC and that of the butyl acrylate -50 °C, as revealed in Table III.3.

Table III-3. Measured and predicted Tg of the different methacrylate sugar-based copolymers

Monomer composition	20/80	30/70	40/60
MG/BA	-30	-10	2
MF/BA	-30	-25	-2
Fox-equation prediction	-29	-17	-4

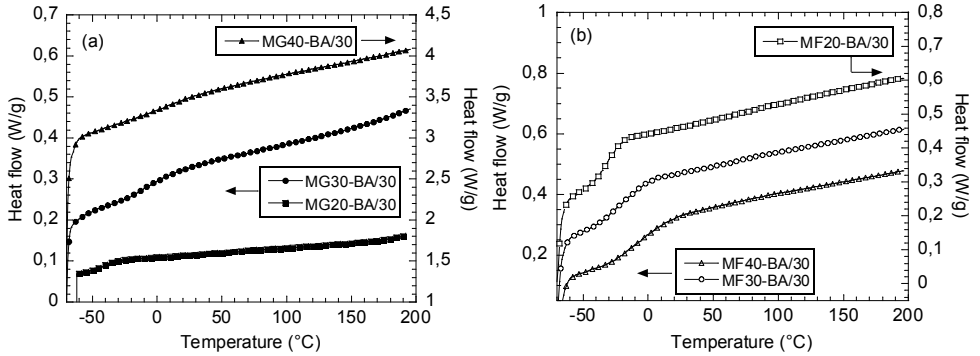


Figure III-7. Differential scanning calorimetry analysis of copolymers of different comonomer composition from BA with (a) MG and (b) MF

The thermal stability of the different sugar-based copolymers was evaluated by thermogravimetric analysis. It can be seen in Figure III-8 that thermal stability resulted independent from the carbohydrate-monomer content. Besides, the copolymers exhibited very good thermal stability, since no significant degradation was observed below 350ºC. Moreover, copolymer degradation was achieved in one single step, suggesting homogeneity in chains composition.

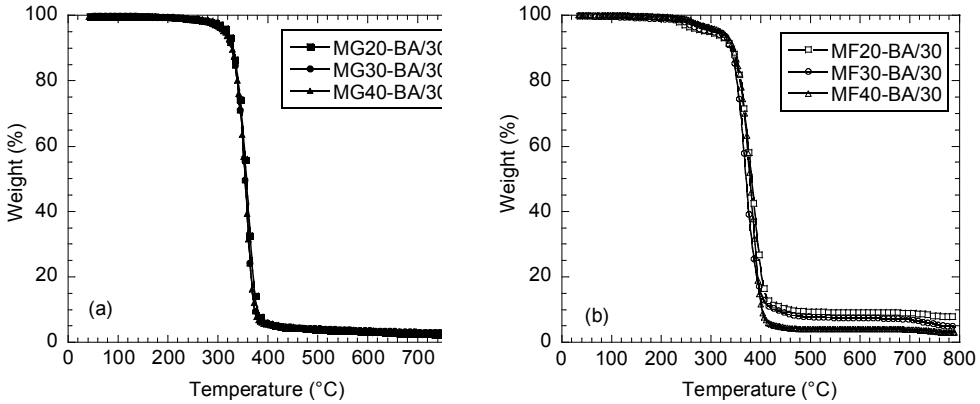


Figure III-8. Thermogravimetric analysis of copolymers of different comonomer composition from BA with (a) MG and (b) MF

The visual appearance of films casted on silicon moulds at ambient temperature and 55% humidity from the different MG/BA and MF/BA copolymers is given in Figure III-9 and Figure III-10 respectively. In all cases, continuous transparent films were obtained. This result was in accordance with the thermal properties of the copolymer shown above, indicating a good incorporation of the sugar units in butyl acrylate chains.

Figure III-9. Pictures of polymer films containing (from left to right) 20, 30 or 40 wbm% methacrylate galactose content.

Figure III-10. Pictures of polymer films containing (from left to right) 20, 30 or 40 wbm% methacrylate fructose.

III- 2.2. 45 wt% solids content sugar-based copolymer latexes

30 wt% solids content latexes were prepared from sugar-based methacrylate monomers and present excellent colloidal and thermal stability, as well as a homogeneous incorporation of the sugar units in butyl acrylate chains. However it was not possible to properly cast thin films because of wettability issues. Latexes tend to shrink when applied on various substrates such as glass, metal or plastic, making their mechanical or adhesive properties difficult to evaluate. Therefore the formulation was modified to produce 45 wt% solids content latexes. To enhance electrostatic stabilization of the particles a disulfonate carrying emulsifier (Dowfax 2A1), whose structure is given Figure III-11, was used.

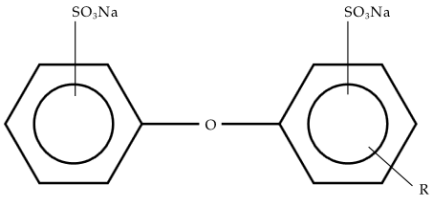


Figure III-11. Dowfax2A1 molecular structure

Moreover acrylamide is a water soluble monomer which can generate oligomers or polymers in the aqueous phase causing more interactions and resulting in an increase in viscosity. Rising up viscosity helps having a better wetting when casting films; moreover amide functions are well-known to promote adhesion.[9] Thus, 2 wbm% of acrylamide was added to the formulation, as shown in Table III-2. 0.75 wbm% of KPS was used.

Table III-2. 45% solids content formulation

Ingredient	Amount (g)
Monomers[a]	45.000
Water	58.200
Dowfax	0.918
Acrylamide	0.900
KPS	0.344
$NaHCO_3$	0.459

[a]MG or MFw and BA

The latexes achieved were very stable, with final average particle sizes about 110 nm and narrow distribution (Đ < 0.04; Đ = (width/mean)² in DLS Malvern equipment). The evolution of the reaction was also very fast, as exemplified in Figure III-12, although some inhibition was observed for MG system, which could be related with some impurities arising from the monomer synthesis. Indeed, during all the work it was observed that monomer purity was crucial to achieve good reproducibility. Moreover, faster kinetics for latexes containing 40% of sugar-based monomer were achieved for both fructose and galactose-based monomers.

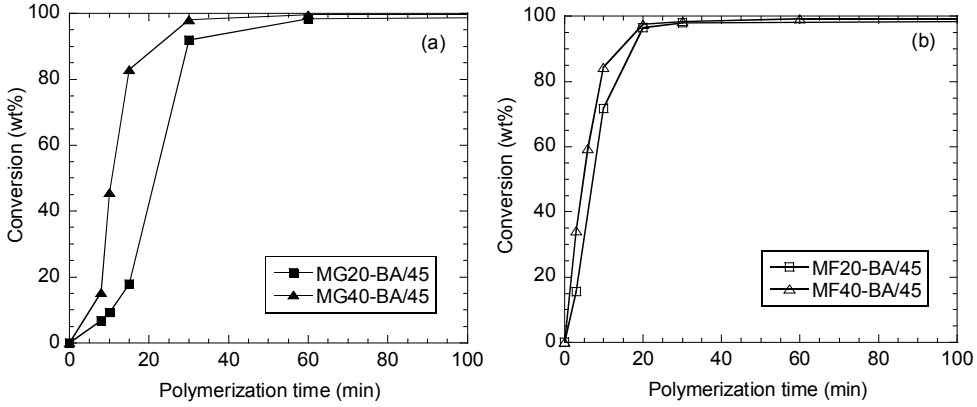


Figure III-12. Evolution of overall conversion along polymerization time for the copolymerization of (a) MG and (b) MF with BA at different ratios.

As expected, the formulation allowed obtaining high viscosity latexes: 250 MPa.s^{-1} for 40/60 ratio copolymers and 400 MPa.s^{-1} for 20/80 compositions. Excellent wettability features were observed on various substrates, such as plastic, glass and metal, and the resultant films were continuous and transparent. A summary of the final copolymers characteristics is given in Table III-3. Here also rather high amounts of gel -fraction of polymer insoluble in THF- were obtained. Values around 80% were measured independently of monomer type and composition. It is important to note that soxhlet extractions were carried out after several days of storage.

Table III-3. Final copolymer characteristics

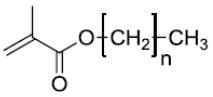
Run	dp (nm)	Tg (°C)	Gel (wt%)
MG20-BA/45	119	-23	83
MG40-BA/45	110	2	80
MF20-BA/45	107	-24	76
MF40-BA/45	106	3	75

III- 3. TOWARDS FULLY BIO-BASED COPOLYMER LATEXES

We have shown so far that it was possible to substitute petroleum-based methyl methacrylate by renewable sugar-based monomers in common butyl acrylate formulations. Now the objective is to replace butyl acrylate by a more environmentally friendly monomer to go towards 100 % renewable based polymers.

For that purpose a bio-based monomer called Visiomer Terra C13-MA (named V in this chapter) from Evonik Industries was selected (Table III-4). This monomer is of high interest not only because of its renewable origin (from natural oils) but also because it helps lowering down the VOC latex content significantly since the molecule is not volatile. Thus using non-volatile sugars in combinaison with natural oil based monomers greatly improves the added-value of the latexes.

Table III-4. Structure and properties of the renewable Visiomer Terra C13MA monomer

Name	Structure	Mw	Boiling T	Tg (homopolymer)
Visiomer C13-MA*	 n≈12	268 g/mol	> 300 °C	≈ -44 °C

** properties given by the supplier*

Because of the high hydrophobicity of the monomer, miniemulsion polymerization was selected as polymerization procedure. MG/Visiomer and MF/Visiomer copolymerizations at different comonomer compositions were tried, as presented in Table III-5.

Table III-5. Bio-based copolymer latexes composition

Latex name	Sugar monomer/V ratio (wt%)	Sugar monomer (molar composition)	V (molar composition)
MG20-V	20/80	0.169	0.831
MG30-V	30/70	0.259	0.741
MF20-V	20/80	0.169	0.831
MF40-V	40/60	0.352	0.648

Monomers were mixed together with the costabilizer hexadecane (HD) and added dropwise to the aqueous phase containing both emulsifier and buffer. The mixture was magnetically stirred at high shear rate for about 20 minutes and then sonicated at 80 % amplitude (0.5 pulse ON/OFF) during 15 minutes with a Branson sonifier. Samples were withdrawn to follow droplets size evolution with time and thus, achieve the optimum conditions yielding minimum droplet size. Then the miniemulsion was poured in a 250 mL double wall

glass reactor, equipped with a turbine stirrer, a nitrogen inlet, a reflux condenser, a temperature probe and a sample device. Reaction temperature was controlled by a Lauda water bath. Stirring was ensured by a mechanical stirrer working at 250 rpm. When reaching 70 °C, a shot of persulfate (KPS) was done to start the polymerization. The targeted solids content was 30 wt% in all the cases. The general formulation is presented in Table III-6.

Table III-6. Miniemulsion copolymerization formulation.

Ingredient	Amount (g)
Monomers[a]	18.00
Water	43.17
SDS or Dowfax	0.36
HD	0.72
KPS	0.18
$NaHCO_3$	0.27

[a] MG or MF and Visiomer

III- 3.1. Waterborne copolymers from MG and V

Figure III-13 shows the polymerization kinetics of the reaction. A certain inhibition was observed at the beginning of the process. It can be seen that reaction went from 20 % conversion to high values very fast, namely within 20 minutes after inhibition. Again, this phenomenon of inhibition was attributed to the presence of impurities in the medium coming from monomer synthesis, which somehow affect the polymerization process. Besides, different batch of monomer were being used throughout this chapter, making the results interpretation more delicate and leading sometimes to reproducibility issues.

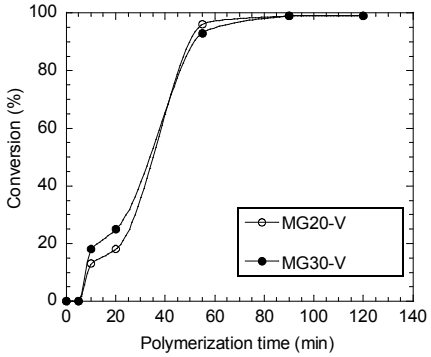


Figure III-13. Polymerization kinetics

The characteristics of the final latexes are given in Table III-7. As shown by the previous plot, rather high conversions were achieved. Final droplet sizes of the MG30-V are lower than those of MG20-V. Indeed, in miniemulsion process, the initial size of monomer droplets is mainly controlled by two factors, namely colloidal stability and droplet break-up during sonication or homogenization. At rather low solids content, colloidal stability which is closely related to the concentration of surfactant, is the predominant variable affecting droplet size.[7,8] Besides, because interfacial tension of an organic phase dispersed in water increases with respect to its intrinsic hydrophobicity, the amount of surfactant needed to stabilize a droplet of high hydrophobicity monomer with respect to low hydrophobicity monomer is higher. As a result droplet size of miniemulsified monomer mixture is expected to be lower in the case of more hydrophilic monomer phase, containing a higher fraction of sugar-based monomer, as effectively observed in Table III-7. Further, independently from monomer composition final polymer particle sizes were substantially lower than initial droplet diameters. Hence, the estimated ratio between the number of particles in the reactor and the number of initial droplets showed secondary nucleation in all cases.

Table III-7. Summary of copolymerization kinetic data and copolymer microstructure

Run	Conversion (wt%)	dd (nm)	dp (nm)	Np/Nd final
MG20-V	> 99	201	137	2.7
MG30-V	> 99	185	129	2.5

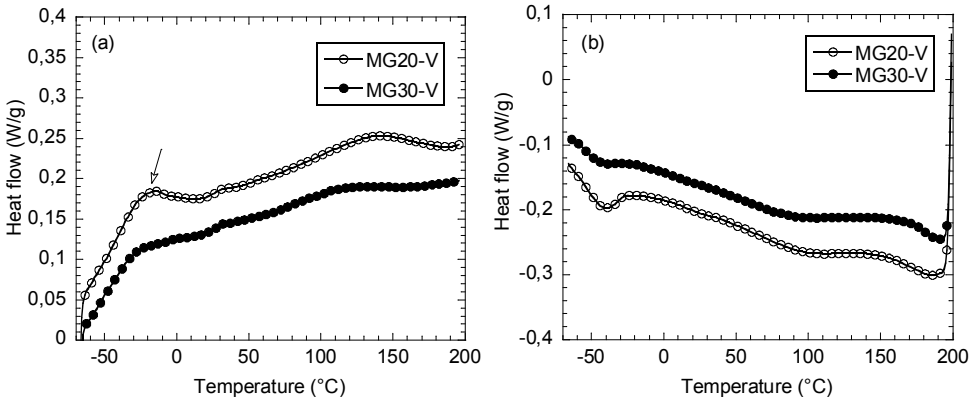


Figure III-14. Differential scanning calorimetry of copolymers from V and MG, (a) heating cycle, (b) cooling cycle.

The differential scanning calorimetry analysis of the copolymer containing 20 wt% of MG and 80 wt% of Visiomer are given in Figure III-14. The evolution of the heat flow in function of temperature is difficult to interpret, and shows various apparent transitions. A first transition is seen around -26 °C (pointed out with the arrow). The peak-shape of this transition suggests a melting phenomenon. The transition observed at low temperature during the cooling cycle (Figure III-14, b) seemed in accordance since it highlights a crystallization peak. It must be reminded that 80 wt% of the copolymer consist of a long alkyl chain methacrylate. Because of its bio-based nature, coming from natural oils, Visiomer C13-MA consists of a mixture of

monomers, carrying either 12 or 14 carbons in their alkyl chains, with an approximate ratio of 70:30 respectively. The presence of long-side chains $(CH_2)_{n>10}$ can induce crystalline domains in the polymer.[12] Rehberg et al.[13] reported the melting temperature of polymers having long-side chain as a function of carbons number (n) in the n-alkyl chain. In the case of poly(methacrylates), side chains of 12 carbons are giving a melting point at -30 °C. This data is in quite good agreement with the result obtained by DSC. Further, when increasing the amount of methacrylate galactose in the copolymer, the same general trend is observed by DSC but interestingly the melting point coming from long-side chains around -26 °C is slightly broaden. This effect is due to the higher incorporation of "short-chain" methacrylate units, herein MG, in the copolymer sequence that separates the crystalline domains. This effect was demonstrated by O'Leary.[14]

This heterogeneity in polymer chain composition can be due to monomers reactivity differencies or more likely to the process itself. Indeed, copolymerizing a hydrophobic monomer (V) with a more hydrophilic one -here sugar-based- can induce differences in droplet composition eventually coming from hydrophilic monomer transfer through the aqueous phase.

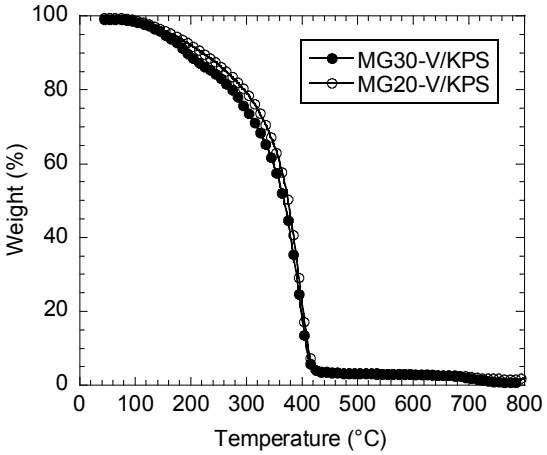


Figure III-15. Thermogravimetric analysis of copolymers from MG and V at different ratios.

This hypothesis of heterogeneous copolymer chains is supported by the thermogravimetric analysis given in Figure III-15. It can be seen that the degradation profile of the copolymers does not follow a sharp decrease in weight.

Further the opacity of the resulting films dried at ambient temperature under 55% relative humidity, whose pictures are shown Figure III-16, corroborate these results.

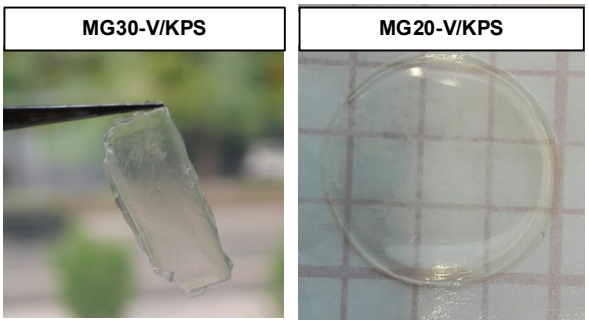


Figure III-16. Pictures of copolymers from MG and V

III- 3.2. Waterborne copolymers from MF and V

For the copolymerization of MF and V, Dowfax 2A1 was used as anionic emulsifier. Potassium persulfate was employed at 70 °C as thermal initiator. Solids content was maintained at 30 wt% but the addition of a small amount of functional monomer was attempted. 2 wbm% of acrylamide was incorporated into the chains to promote more adhesion and achieve better wettability when casting films. Aiming at preparing binders for adhesives and coatings, two different monomer ratios were used, 20/80 and 40/60, as shown in Table III-8. Rather high conversions were obtained in both cases. Final polymer particle sizes were about 150 nm and the ratio Np/Nd was close to one, which suggested that the majority of the droplets were nucleated and neither significant secondary nucleation nor coagulation occurred.

Table III-8. Latexes characterization

Run	Conversion (wt%)	dd (nm)	dp (nm)	Np/Nd final
MF20-V	> 99	170	153	1.2
MF40-V	> 99	162	141	1.3

Figure III-17 shows the differential scanning calorimetry analysis of the resulting copolymers. In this case, no melting peak is detected. This can be due to the fact that both methacrylate fructose and acrylamide are short-side chain monomers which avoid the formation of crystalline domain from successive V units. Interestingly, dynamic scanning calorimetry analysis seemed to highlight two main transitions for both copolymers, as shown in

the Figure. This behavior revealed a difference in composition of the chains composing the copolymer latexes.

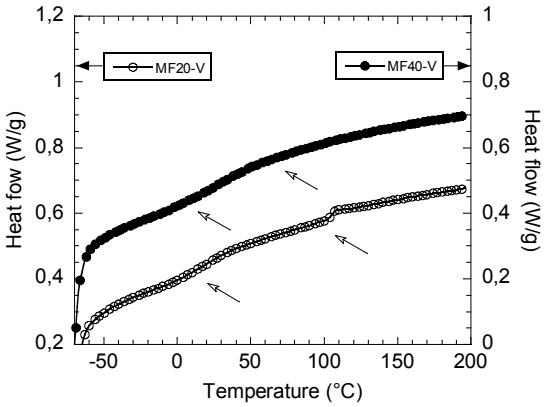


Figure III-17. DSC analysis of copolymers from MF and V

In fact, an analysis of the ^{1}H-NMR recorded from samples withdrawn during the reaction helped determining a potential reason for such result. First of all, it can be seen from Figure III-18 that no inhibition occurred in these cases; signals from both V and MF vinyl group (5.3, 5.4 and 5.8, 5.9 ppm) disappeared within 10 minutes. Secondly, a clear reactivity difference between both bio-based monomer and acrylamide was highlighted in batch. In fact, signals pointed out by a square on the graph, at 5.7 and 6.2 ppm belong to the vinyl protons of the functional monomer. Their full vanishing required more than one hour, indicating a drift in polymer chain composition certainly occurred. This could explain the particular behavior in DSC.

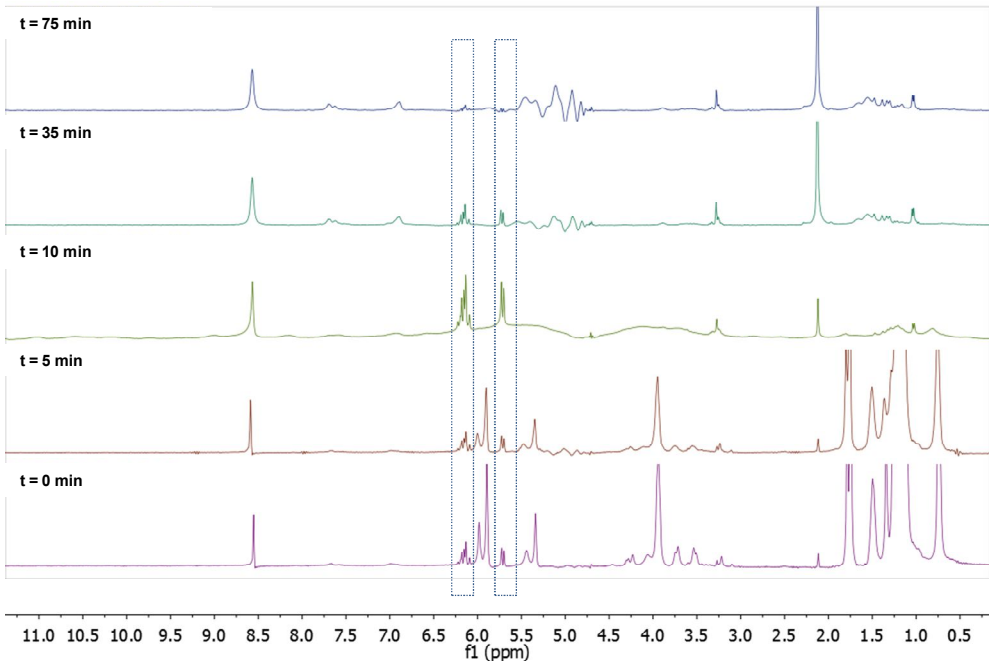


Figure III-18. Evolution of the ^{1}H-NMR spectra of samples withdrawn during the copolymerization reaction of MF40-V

In fact, this postulate was greatly supported by the appearance of the resulting films casted at ambient temperature under controlled relative humidity (55 %). MF40-V copolymer latex was not forming film at ambient temperature while MF20-V resulting in a continuous hard and brittle film. This phenomenon can be attributed to the presence of acrylamide units either at the surface of the polymer particles or as homo-oligomers in the aqueous phase. In fact, according to theoretical glass transition temperatures, based on Fox equation (-24 °C for 20/80 copolymer and 1 °C for 40/60), both latexes were expected to exhibit MFFT below ambient temperature. Instead, particle coalescence and chain interdiffusion did not occur properly at 23

°C. This observation supports the idea according to which acrylamide units may be located at the surface of the polymer particles and/or at the near environment of the latter. The consequence of such phenomenon can be compared with surfmer-stabilized latexes, where functional monomers are used as stabilizers being grafted onto the polymer particles. In that particular case, the interactions between particles, induced by these groups, disturb particle coalescence and chain interdiffusion during film formation. In addition, the potential presence of acrylamide homopolymers or copolymers rich of acrylamide units at the surface of the particles enhance this effect since poly(acrylamide) is a high Tg polymer (165 °C) whose hardness can significantly and negatively affect chain interdiffusion during film formation.

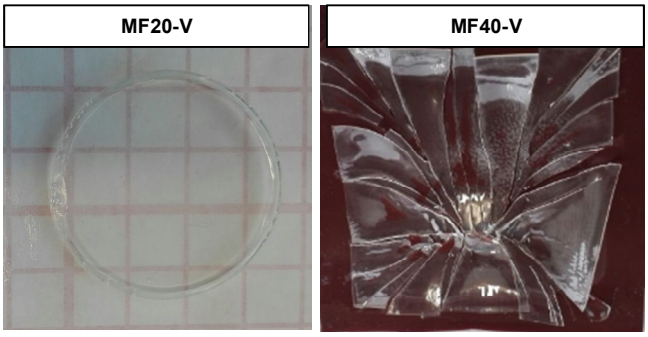


Figure III-19. Pictures of films from copolymers of V and MF

The thermogravimetric analysis of the copolymers showed very good thermal stability (Figure III-20), indicating a rather good incorporation of the sugar units in the oil-based monomer chains.

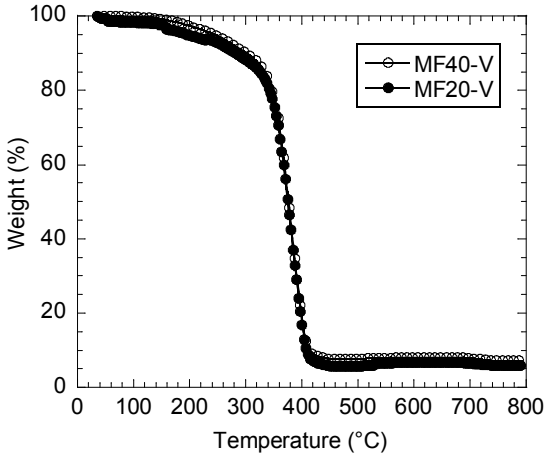


Figure III-20. Thermogravimetric analysis of copolymers from V and MF

III- 4. CONCLUSIONS

In this chapter, the synthesis of different waterborne systems derived from renewable-based monomers was studied.

First, the copolymerization of both galactose and fructose-based monomers with butyl acrylate in batch emulsion polymerization was investigated. Polymer particle size was shown independent from bio-based monomer content and in all cases high conversions were achieved. Furthermore, latexes led to continuous transparent films with a unique glass transition temperature, in the range of the theoretical value calculated from the Fox equation together with an excellent thermal stability.

In a second step the possibility of preparing high solids content latexes from these systems was demonstrated by synthesizing copolymer sugar-based latexes at 45 % solids content with good colloidal stability and high monomer conversion.

In parallel, the use of renewable feedstock was extended to the copolymerization of sugar-based monomers with a commercial product based on natural oils. Miniemulsion polymerization was carried out at 30 % solids content, leading to two series of experiments. On one hand, stable methacrylate galactose and Visiomer C13 latexes of different composition were synthesized. On the other hand, methacrylate fructose and visiomer copolymerized in the presence of 2 wbm% of acrylamide were produced. The incorporation of the functional monomer in batch was shown to affect significantly film formation, opening the window to improved mechanical properties copolymer made from soft materials.

Finally, it can be concluded that both MG and MF are able to copolymerize with low Tg monomers in both emulsion and miniemulsion copolymerization to achieve stable copolymers in good conversions.

It is worthy to note however that difficulties were found in the reproducibility of some results, likely because of the use of different monomer batches eventually carrying impurities, which were not detectable by ^{1}H-NMR analysis.

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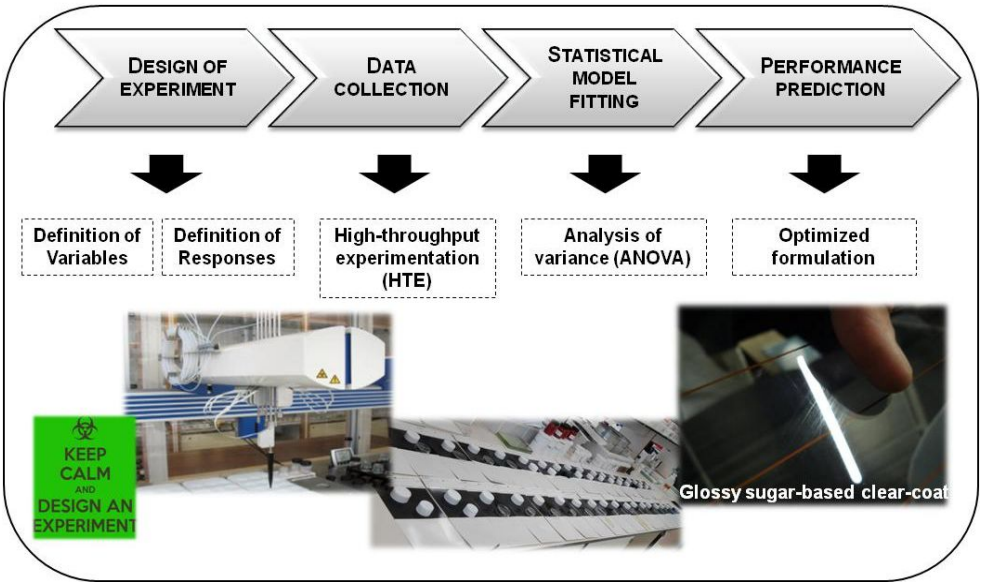
Chapter IV- Incorporation of Waterborne Sugar-based Polymers in Paint Formulation



IV- 1. INTRODUCTION

Waterborne coatings are becoming increasingly demanding due to environmental concerns. As a result organic solvents are being replaced by water-based paints across a broad range of application, as for instance in the residential market, for which they account as 80 %.[1] Legislation is in place to support this trend.[2] Indeed, waterborne paints have several advantages with respect to solvent-borne paints, starting from reducing health risks to all involved by limiting VOCs emission and thus improving air quality. Besides for multiple hues and striping, waterborne paints have an advantage when it comes to spraying due to a thinner application. It takes less clear-coat to even out the surface for the different layers.

The performance of a coating is affected by a wide range of factors. The structure and composition of the polymeric binder itself is obviously an important parameter but, although used in rather small amounts, additives play a crucial role in paints performance. These formulation agents can significantly improve paint rheology properties, adhesion, appearance, hardness, corrosion protection and storage stability among others.[3,4] As a result, the use of additives in paint technology is essential. However, the choice of the appropriate ingredient is not straightforward and requires preliminary screening. Indeed, the difficulty here lays in the fact that one additive can be beneficial for a given property but at the same time disadvantageous with regards to another. Consequently, a compromise has to be found to balance additives effects and achieve optimized formulations. Given this, combinatorial methods appear to be a powerful tool for the optimization of these systems.[5] In fact, the design of experiments (DoE) is a worthwhile strategy allowing the analysis of variable correlation.[6] In addition, high-throughput experimentation (HTE), whose key principle is parallelization, is

receiving an increased interest. As for instance, Bohorquez et al showed the interest of the use of HTE-solutions in combination with a statistical analysis in the behavior and the interaction of a complex polymer colloid in a pigmented paint.[7] The successful optimization of bio-based high solids content hybrid films containing casein was reported by Minari et al.[8] In complementation to formulation optimization, these methods can be employed as useful tool to conduct coating assessment. Chisholm and coworkers[9–15] published a series of combinatorial method driven studies, wherein the strategy is applied to a wide type of systems including polysiloxane, hybrid organic/inorganic coatings, marine coatings, etc. Indeed, rather than carrying out single experiments one after another, several tests can be performed simultaneously, making the procedure cost and time effective.

The aim of this work is to study the possible use of waterborne sugar-based copolymers as binder in paints. Sugar-based copolymers were claimed to be promising materials for their use in paints,[16,17] but as far as we know proper evaluation of formulated paints and comparison with commercial equivalent grades has not been reported yet. Therefore, in this chapter, the effect of the incorporation of sugar-based polymers in waterborne formulated paints on their performance will be investigated. Two latexes based on methacrylate fructose were prepared and formulated to be evaluated as clear-coat for decorative application. As to give an overview of the coating performance, several tests were performed, such as measurement of hardness, gloss, haze, chemical resistance and blocking, following specific protocols used by manufacturers. To achieve paint formulation optimization, a design of experiment was conducted. This work was carried out at the Research and Development laboratories of Allnex in Bergen-op-Zoom, The Netherlands.

IV- 2. EXPERIMENTAL

IV- 2.1. Materials

Regular chemicals and monomers such as methyl methacrylate (MMA), butyl acrylate (BA), methacrylic acid (MAA) and acrylic acid (AA) were purchased as technical grade by Allnex and used as supplied.Methacrylate fructose (MF) was prepared by Carbosynth (UK), company who scaled up the synthesis procedure described in chapter II-4.Cosolvent (butyl diglycol) was supplied by Helm Chemicals BV. Commercial defoamers from both Evonik and Dow were used; as well as additives from BYK company. Distilled and deionized water were used in all formulations.

IV- 2.2. Synthesis of high solids content sugar-based resins

Two resins were selected as binder to be formulated for paint evaluation. A MF/BA (40/60) latex synthesized in our laboratory following the study reported in the previous chapter, and a binder based on a commercial latex formulation (Nu-Stq), where MMA was substituted by MF.

IV- 2.2.1. Batch copolymerization MF/BA (40/60)

A copolymer latex was prepared from MF and BA following the protocol given in Chapter III (synthesis of MF40-BA/45). Batch polymerization conditions were employed, at 70 °C using a thermal initiator (KPS) and an anionic emulsifier (Dowfax 2A1). A solids content of 45 wt% was targeted. In order to be able to carry out several sets of formulated paints, reaction was scaled up from 100 mL to 400 mL. Conversions higher than 99 wt% were achieved. Final particle size was 100 nm. Accumulation of polymer was observed around the stirrer, and final experimental solids content was 42 wt%. A residual amount of butyl acrylate of 1900 ppm was detected by gas chromatography. The resulting latex will be referred as MF40BA/45 throughout this chapter.

IV- 2.2.2. MMA substitution by MF in Nu-Stq recipe

To properly evaluate the effect related to the incorporation of the sugar-based monomer on paint performance, MMA was substituted by MF in a commercial recipe from Allnex containing 48 wt% of MMA. Seeded semi-batch polymerization was carried out at 80 °C. A thermal persulfate initiator and sodium dodecyl sulfate emulsifier were used (Table IV-1). The reaction was carried out at 40 wt% solids content in a 0.5 L double wall glass reactor, equipped with a stirrer, nitrogen and pumps inlets and a thermocouple. Full conversion of volatile monomers was verified by thermogravimetric analysis and a final particle size of 93 nm ($Đ<0.02$, measured by DLS) was obtained. pH of the final resin was adjusted to 7 by use of ammonia. Neither coagulation nor fouling was observed.

Table IV-1. MF-Stq polymerization formulation

Chemical	Amount (g)	Ratio (wbm%)
Methacrylate fructose	55.85	**48.1**
Butyl acrylate	49.53	42.7
Acrylic acid	6.56	5.6
Monomer 1	4.15	3.6
Persulfate	0.55	0.47
Sodium dodecyl sulfate	1.89	1.6
Distilled water	172.90	-

The resulting latex will be named MF-Stq throughout this chapter. Comparison of its performance with the corresponding reference product from Allnex (Nu-Stq) was done, MF content being the unique difference between them.

IV- 2.3. Design of experiments in combination with High-throughput experimentation

As briefly explained in the introduction, the combination of design of experiments (DoE) and high-throughput experimentation (HTE) is a method associating mathematical models and testing in an effective manner.

On one hand, high-throughput experimentation is a valuable solution to conduct more cost effective R&D projects. Indeed, parallelization of experiments allows for increased experimental load without subsequently increasing personnel costs or development time, thus

shortening the time to market for new products. Besides, small scale testing allows for a reduction in materials and feed, ultimately reducing the total cost.

On another hand, the design of experiments is the plan used to collect the data. Investigator defines one or more factors of interest (herein formulation variables), and each factor contains two or more levels. Levels can be numerical (can be expressed as a number on a continuous scale) or categorical (words, names, numbers on a non-continuous scale). Typically a component concentration will be defined as a numerical level while a type of additive will be defined as categorical. Different levels produce different groups. Experimental units are assigned randomly to groups (Figure IV-1).

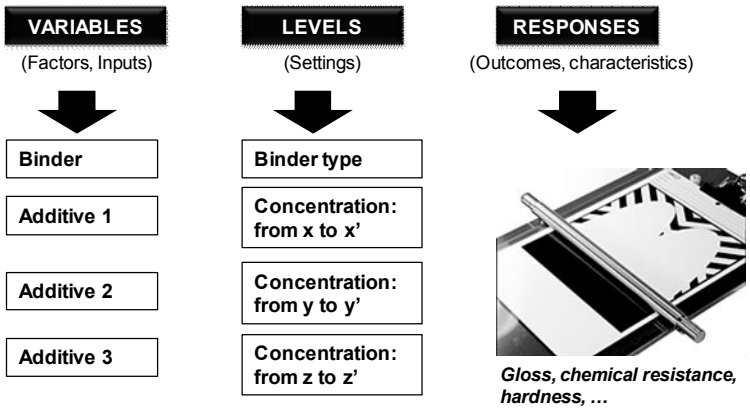


Figure IV-1. Design of experiment settings

Paint formulation and testing is done following the plan of experiments given by the DoE. Results obtained from each paint testing can be treated as responses from the DoE and fitted into a mathematical model describing the interaction between the formulation variables and the performance measured.

To do so, analysis of variance (ANOVA) was used. It determines if the responses obtained from each experiment can be used to describe a tendency. Responses are exploited to determine the variation caused by a factor, which can be related to various sum of squares. A more detailed description of ANOVA principles is given in Appendix 2.

IV- 2.4. Paint preparation

Binders were formulated to produce clear-coat for decorative application. Clear-coats are non-pigmented coatings; therefore the use of mill-base is not required. Paints were prepared in an automatic robot (SynchronXperimate: Robotic XYZ automated liquid handling systems for bench, integration and custom applications). A high speed mixer was used at 3000 rpm for 3 minutes once all additives were introduced.

IV- 2.5. Paint characterization

IV- 2.5.1. Gloss and haze

In terms of visual appearance, gloss is one of the key features of paints. Gloss might vary from polymer film to paint film due to the incorporation of additives that may affect particle stability and increase heterogeneity. Haze represents a complementary characteristic that helps fully characterize paint gloss. Figure IV-2 shows two examples of glossy surfaces. High values of haze (bottom example) induce a non well-defined reflected image. Indeed,

microscopic defects on the surface of the coating are causing an increased amount of scattered light. As opposite, when haze is low (top example) the reflected image appears clear and distinct. The distribution of the light intensity shows a sharp gloss peak, corresponding to a true high-gloss surface.

In this work, gloss at 20° and haze of the coatings were determined with a goniophotometer Rhopoint IQ. Formulated dispersions were applied on Leneta cards using a barcoater (120 µm wet film thickness) and dried at 22 °C and 50 % of humidity for 24 hours.

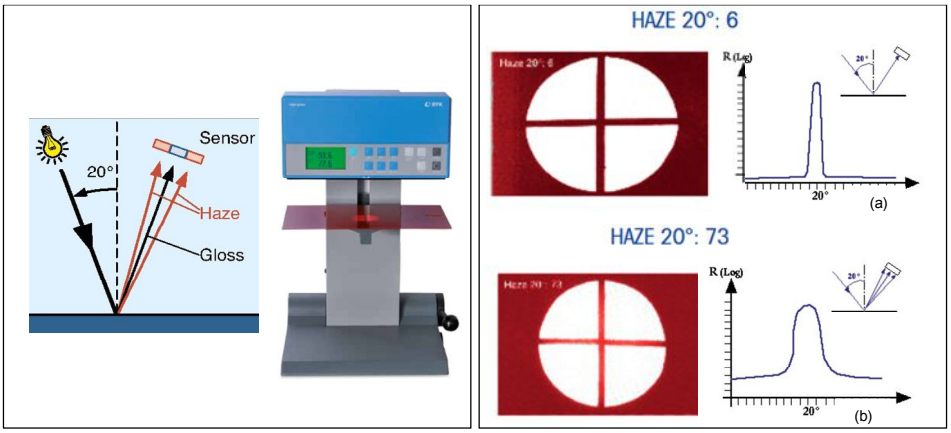
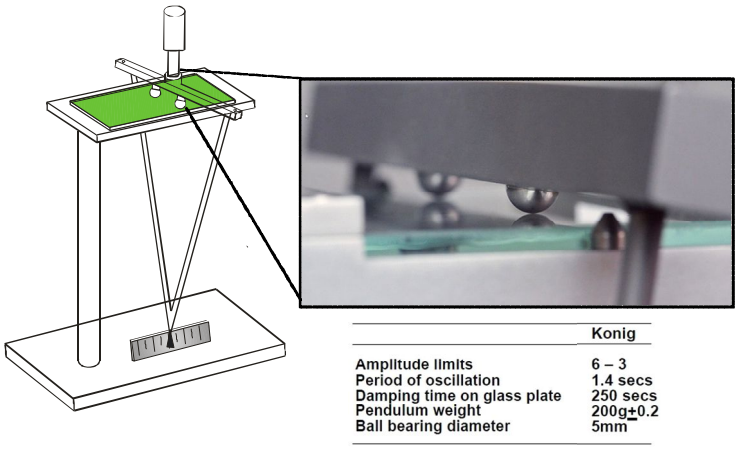


Figure IV-2. Representation of gloss and haze film measurement

IV- 2.5.2. Hardness

Hardness is defined as the resistance of a coating to a mechanical force such as pressure, rubbing or scratching.[18] Formulated dispersions were casted on glass substrates using a doctor blade film applicator (120 µm wet film thickness) and dried at 22 °C and 50 % of relative humidity and hardness was measured with a König pendulum apparatus (Erichsen

model 299/300). The instrument consists of a pendulum which is free to swing on two balls resting on a coated glass plate, as can be seen on Figure IV-3. A physical relationship is set between oscillation time, amplitude and pendulum geometric dimensions. When the pendulum is set into motion, the balls roll on the surface and apply pressure on the coating. Depending on the elasticity, the oscillation amplitude damping will be stronger or weaker. The softer the material is the faster damping occurs. As a result, coating hardness is defined by the number of oscillations made by the pendulum within the specified limits of amplitude and is reported as a damping time in seconds. Hardness evolution can be followed upon film drying time.



	Konig
Amplitude limits	6 – 3
Period of oscillation	1.4 secs
Damping time on glass plate	250 secs
Pendulum weight	200g±0.2
Ball bearing diameter	5mm

Figure IV-3. Hardness measurement using a Konig pendulum device

IV- 2.5.3. Chemical resistance

The chemical resistance of a paint is important especially for wood application, where furniture coatings must be particularly resistant to stain. Therefore the chemical resistance to water, ethanol and coffee was evaluated.

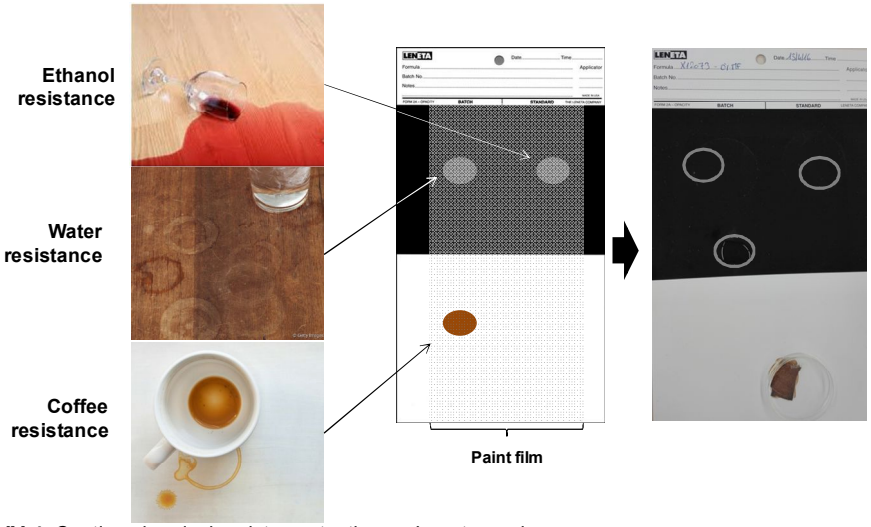


Figure IV-4. Coating chemical resistance testing on Leneta cards

Formulated dispersions were applied on glass substrates using a barcoater (120 µm wet film thickness) and dried at 22 °C and 50 % of humidity for 24 hours. The "spot test" was carried out by depositing drops of liquids on top of the film as shown in Figure IV-4. In the case of water and ethanol resistance, water drops were deposited on the film on one side and a 50 wt% aqueous solution of ethanol on the other side and left for 24 hours. Drops of a coffee solution (2 g of espresso instant coffee in 50 mL of distilled water) were placed on top of the

film (white part of the Leneta card) and left for 4 hours. After these given times, liquids were carefully wiped off and films damage was evaluated visually, grading from 0 for extremely damaged films to 5 for visually intact films.

IV- 2.5.4. Anti-blocking properties

Blocking is a common problem encountered by coating manufacturer. It refers to the adhesion of two adjacent layers of film and it is mostly associated with furniture pieces being stacked on top of each other during manufacturing process or transportation.

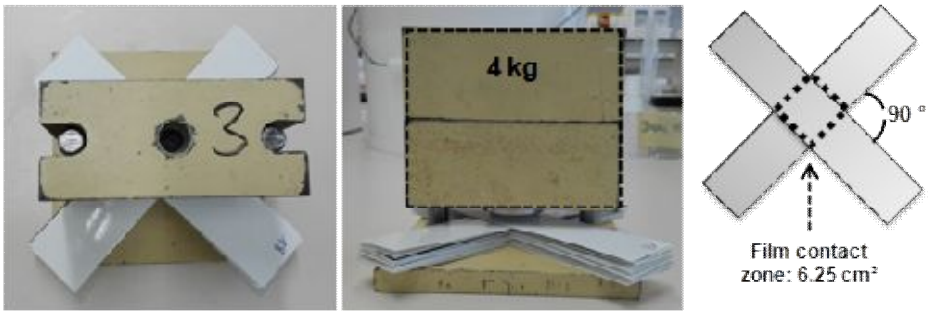


Figure IV-5. Films blocking performance test

Paints blocking was evaluated by casting films on Leneta cards (120 µm wet thickness) and dry them at 22 °C and 50 % relative humidity for 24 hours. Leneta cards were then sliced into 2.5 cm wide strips, brought together by pair to achieve a squared film surface contact of 6.25 cm², as shown in Figure IV-5. A weight of 4 kg was positioned on top of 12 strips pair and placed in an oven at 50 °C for 4 hours. When removed from the oven, films were allowed to cool down to room temperature. Assessment of blocking performance was done by evaluating

qualitatively the force needed to separate each pair of strip, as well as the damage induced. Grades were attributed to each paint ranging from 0 for a bad film separation (adhesion, film damages) to 5 for an easy and damage-free separation of the strips.

IV- 3. RESULTS AND DISCUSSION

IV- 3.1. Evaluation of a MF/BA waterborne copolymer as binder

IV- 3.1.1. Preliminary study. Evaluation of non-formulated latexes

Latex MF40-BA/45 was evaluated, without prior additive incorporation, as potential binder for paint application.

The first important parameter to verify is the ability of film forming at ambient temperature. Film formation from waterborne dispersed polymers is often described as a process involving three steps in series as shown in Figure IV-6.[19] When a waterborne dispersion of polymer particles is deposited onto a certain substrate, water evaporation will occur, and consequently, the particles will come into close contact forming a close-packed array of particles. This contact between particles will force particle deformation in order to fill the voids between them. In this point where no space between particles is present, optical clarity in the film appears because light is not scattered anymore by the heterogeneities in refractive index. This onset of optical clarity is defined as the Minimum Film Formation Temperature (MFFT). Once the particles are deformed, and at temperatures above the glass transition temperature of the polymer (Tg),

interdiffusion of the polymer chains across the boundaries of the particles occurs leading to a continuous film.

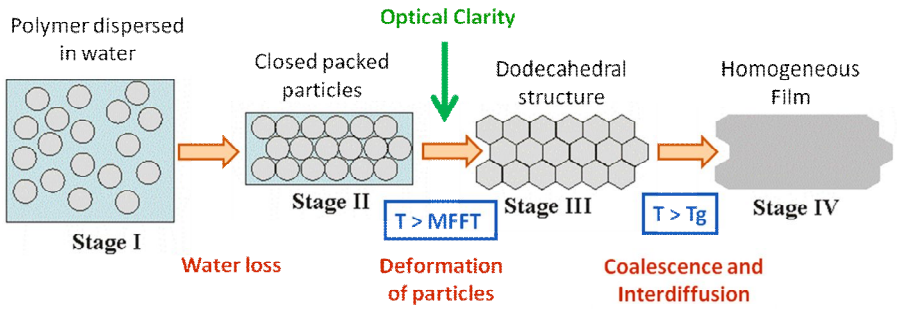


Figure IV-6. Stages of film formation

In practice, film formation can be assessed by casting a thin layer of latex onto a flat substrate (commonly glass) or using a MFFT stainless steel bench device. In both cases, visual inspection of the dried film by the operator is required. If optical clarity is observed, then the latex is considered as a film forming resin. However, film formation is a very critical process whose quality directly impacts on paint performances. A film can be optically clear but presenting micro-cracks, small enough not to cause significant refractive index disruption, but sufficient to induce lower chemical resistance, corrosion protection or gloss. To better evaluate film formation, a trick is to cast a film onto a cardboard and test its permeability after a few hours of drying by dropping water on the surface. Permeable films indicate a non optimum coalescence and interdiffusion process upon drying. Figure IV-7 gives an example of a bad film forming resin (left image) and a good film forming latex, which corresponds to MF-40BA/45 resin (middle image).

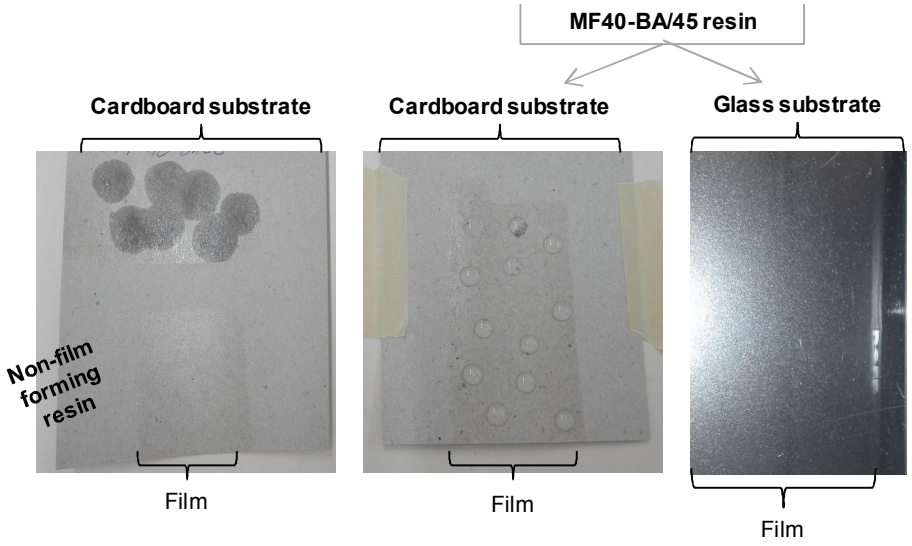


Figure IV-7. Film formation evaluation

Additional information can be extracted from these preliminary tests. In fact, a homogeneous film can be obtained via good film formation as described above, but resin needs also to properly spread out over the substrate and not shrink during drying process to fully exhibit good film forming properties. This capacity is referred to as wetting or wettability. Figure IV-7 (right image) shows the picture of a film casted from MF40-BA/45 latex on a glass substrate. As can be seen perfect wetting occurred during film formation since the applied film edges are straight and correspond to the deposited wet latex. No shrinkage appeared during film formation. In conclusion, it has been seen that the non-formulated MF40-BA/45 resin exhibits good film forming properties, and excellent wetting on glass substrate. Consequently, properties such as hardness, gloss and haze were tested on the dried non-formulated film (Table IV-2). As can be seen, gloss is rather low and the film can be defined as quite hazy. But

the most concerning result is the hardness, measured after 7days of drying with a value of 6 seconds. Hardness of a commercial paint should always be above 40 at least. The reason for hardness to be that low is explained by the glass transition temperature of the copolymer, measured around 4 °C. Consequently, in this work the formulation of latex MF40-BA/45 definitely needed the incorporation of additives meant to help improving hardness.

Table IV-2. Characteristics of non-formulated MF40-BA/45

Hardness (s)	Gloss (20°)	Haze (20°)
6	45.3 ± 3.6	± 30.6

IV- 3.1.2. Preliminary study. Incorporation of additives.

In parallel, some common additives, chosen for their compatibility with waterborne acrylics and when possible environmentally friendly, were tried to be added to the resin. Table IV-3 indicates the type, name and amount of ingredient used. In many cases, additives were causing latex destabilization, especially cosolvents, as represented on the table (xxx significant coagulation, xx important coagulation, x minor coagulation).

As an alternative to common commercial additives, alkali soluble resins (ASR) are often used as paint component. ASR are random or block copolymers from hydrophobic monomers, such as MMA, butyl methacrylate (BMA), styrene (S) and carboxylic acid containing units (MAA or AA). The presence of COOH groups combined with low molecular weight copolymers allows chains solubility to be pH dependant. The pH of a coating is typically above 7, therefore chains are expected to be fairly soluble in the aqueous media. The presence of charged oligomers in the aqueous phase affects viscosity positively (by augmenting interactions). Wetting,

stabilization as well as flow and leveling properties are thus improved. Furthermore because of their high glass transition temperature, ASR can help improving film hardness.

Table IV-3. Additives names, characteristics and amounts used in the preliminary study

Name	Composition	Amount(wt%)	Result
COSOLVENT			
BG	Butyl glycol	0.5	xxx
BdG	Butyl diglycol	0.5	xxx
Dowanol DPnB	Dipropylene glycol n-butyl ether	0.5	xxx
Dowanol DPM	Dipropylene Glycol Methyl Ether	0.5	xxx
DEFOAMER			
Tego Twin410020	Siloxane-based gemini surfactant	0.5	xxx
Tego foamex83021	Organic polymer, silicon free	1.0	xx
BYK-171022	Emissions-free polymer-based defoamer	0.5	xx
WAX/SURFACE PROTECTION			
Aquacer-51323	HDPE-based VOC free wax emulsion	6.0	x
SLIP/ANTI-BLOCKING			
Tego Glyde 48224	Polydimethylsiloxane	1.0	x
SCRATCHING/ABRASION			
Nanobyk-362025	Surface treated silica nanoparticle dispersion in water. VOC free	10.0	-

A commercial ASR solution from Allnex, as well as its bio-based equivalent, containing itaconate derivatives (upon investigation in Allnex laboratories) were selected. In addition NanoBYK-3620, consisting of surface treated silica nanoparticles dispersed in water was chosen. The latter is used to enhance scratch resistance, usually closely related to poor hardness. As added value, NanoBYK-3620 is VOC free.

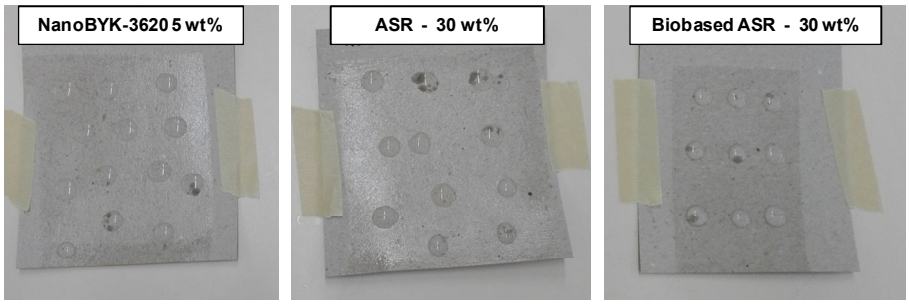


Figure IV-8. Cardboard film forming test for MF-40BA/45 incorporating NanoBYK-3620, ASR and bio-based ASR

The maximum load capability of additive in the latex was tested via “cardboard test”. In order to maximize the improvement of film hardness, a rather high amount of ASR was used. A maximum of 30 wt% of both synthetic and bio-based ASR could be added without disrupting film forming as can be seen on Figure IV-8. In contrast, NanoBYK-3620 could only be incorporated up to 10 wt%.In conclusion, preliminary tests showed very good film forming properties of MF40-BA/45 resin, but low hardness and poor stability against additive incorporation. As a result, alternative ingredients such as alkali soluble resins were selected to formulate the paint, together with a silica-based specific additive for scratch resistance improvement. These three components were set as variables in a DoE study.

IV- 3.1.3. Design of experiments and paints formulation

In this study, the effect of a commercial ASR, its bio-based equivalent ASR and NanoBYK-3620 incorporation on the performance of a clear-coat prepared from sugar-based latex was investigated by means of a combination of DoE and HTE. The main goal is to describe the interactions between the carbohydrate-based binders and the different

components in formulation. The DoE was carried out with the aid of the commercial software "Design Expert®" (version 9). The type of design used was Surface Response since it offers the possibility of including both numerical and categorical variables.[26] The design was defined as shown in Table IV-4. ASRs and NanoBYK-3620 additives were set as numerical variables, with levels from 0 to 30 and 0 to 10 respectively in a concentration continuous scale expressed in wt% of total resin mass. The type of resin, e.g. MF40-BA/45 and Nu-Stq (reference commercial binder from Allnex) was defined as categorical variable. Responses consisted of paint testing, including hardness, gloss, haze, chemical resistance, and blocking.

Table IV-4. DOE settings for the testing of MF40-BA/45 and Nu-Stq

Variables	Levels	Responses
Binder	Type1: MF40-BA/45 Type2 : Nu-Stq	Hardness
ASR additive	From 0 to 30 wt%	Water resistance Ethanol resistance
Bio-based ASR additive	From 0 to 30 wt%	Coffee resistance Blocking
NanoBYK-3620	From 0 to 10 wt%	Gloss Haze

Twenty-four paint formulations were prepared, including five replicate points, as detailed in Table IV-5.

Table IV-5. Paints formulation proposed by DoE (quantities given in grams)

Paint	Nu-Stq	MF40-BA/45	ASR	BIO-based ASR	NANOBYK 3620	Total
1	0.000	11.765	3.529	3.529	1.176	**20.000**
2	14.286	0.000	0.000	4.286	1.429	**20.000**
3	14.815	0.000	2.222	2.222	0.741	**20.000**
4	0.000	14.815	4.444	0.000	0.741	**20.000**
5	0.000	16.293	2.077	0.000	1.629	**20.000**
6	0.000	16.427	3.080	0.493	0.000	**20.000**
7	0.000	13.418	1.509	3.744	1.329	**20.000**
8	18.779	0.000	0.000	0.000	1.221	**20.000**
9	0.000	16.333	0.000	2.033	1.633	**20.000**
10	14.815	0.000	2.222	2.222	0.741	**20.000**
11	14.286	0.000	4.286	0.000	1.429	**20.000**
12	0.000	14.826	0.000	4.448	0.726	**20.000**
13	15.385	0.000	0.000	4.615	0.000	**20.000**
14	15.385	0.000	4.615	0.000	0.000	**20.000**
15	0.000	12.500	3.750	3.750	0.000	**20.000**
16	0.000	16.333	0.000	2.033	1.633	**20.000**
17	0.000	20.000	0.000	0.000	0.000	**20.000**
18	12.214	0.000	3.664	3.664	0.458	**20.000**
19	0.0000	14.826	0.000	4.448	0.726	**20.000**
20	14.815	0.000	2.222	2.222	0.741	**20.000**
21	0.000	13.774	3.347	2.355	0.523	**20.000**
22	12.808	0.000	3.573	2.402	1.217	**20.000**
23	0.000	14.886	1.563	3.550	0.000	**20.000**
24	0.000	13.774	3.347	2.355	0.523	**20.000**

IV- 3.1.4. Statistical evaluation of the responses and formulation optimization

The responses obtained from the twenty-four formulations were studied and fitted into a statistical model based on ANOVA. The software used to design experiments and treat the data is able to calculate various parameters aiming at helping the operator to determine whether the statistical model generated is reliable or not. That is to say if the model can be used to predict and optimize formulations.

Model validation is possibly the most important step in the model building sequence. Ideally responses are exploited to determine the variation caused by a factor. But the risk here is to assimilate variations caused by random error to a factor effect. As a result, two types of variance are distinguished: the variation due to factor and the variation due to random error. These latter can be related to a sum of squares and later to a mean sum of squares (see details in Appendix 2). The mean squares obtained allow the calculation of F-value, basically giving a signal to noise ratio value and thus information on the consistency of the model. Typically a high F-value indicates that the variation due to a factor is not in the range of the variation due to random error. In addition to this, the probability of having a high F-value due to noise is calculated and referred as p-value.

Table IV-6 gives a summary of the most common parameters checked to verify the model fitting performance, including F and p-values. For instance, here in the case of gloss, F-value is 54.25 and there is less than 0.01 % chance (p-value) that F-value this large could occur due to noise. In contrast, for coffee resistance, F-value is much lower i.e. 5.58 and there is 9 % of chance (p-value) that a F-value this large could occur due to noise. In brief, coffee resistance response cannot be related to factors effects. Consequently a model equation to represent the

coffee resistance behavior with respect to factors influence cannot be generated. All three chemical resistance responses gave unacceptable F and p-values here.

The lack of fit is also extracted from the software. A regression model exhibits lack-of-fit when it fails to adequately describe the functional relationship between the experimental factors and the response variable. Thus a good model fitting implies a non significant lack-of-fit. Here although F and p-value were flawed for chemical resistance responses, lack-of-fit is given non significant in all cases. This result highlights the particular importance of analyzing more than one single model fitting parameter to properly conclude on model performance.

Still, the fitting of predicted vs experimental responses needs to be ensured by verification of additional parameters. Residuals are estimates of experimental error obtained by subtracting the observed responses from the predicted responses. Examining residuals is a key part of all statistical modeling, including DoE's. They represent elements of variation unexplained by fitted model. Herein R^2 was unacceptable for chemical resistance responses.

Table IV-6. Responses' model fitting performance

Response	F-value	p-value	Lack of fit	R^2
Hardness	131.60	<0.0001	Not significant	0.98
Blocking	13.62	<0.0001	Not significant	0.91
Gloss	54.25	<0.0001	Not significant	0.98
Haze	34.15	<0.0001	Not significant	0.95
Water resistance	5.79	0.032	Not significant	0.55
Coffee resistance	5.58	0.09	Not significant	< 0.5
Ethanol resistance	5.19	0.0327	Not significant	< 0.5

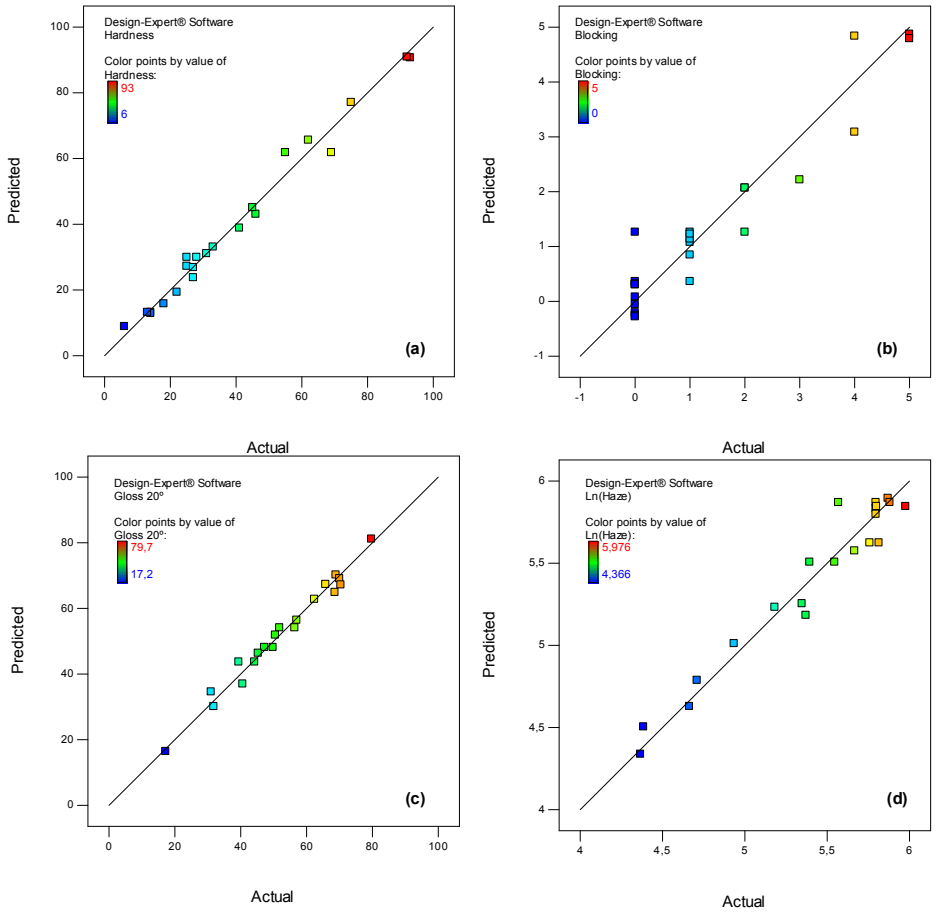


Figure IV-9. Performance of the statistical model ANOVA for predicting (a) hardness; (b) blocking; (c) gloss; (d) haze

In conclusion, responses including hardness, blocking, gloss and haze could be fitted quite nicely into a statistical model, as shown namely by the sum of squares (R^2) close to one, the significance of the model (indirectly given by F and p values) and the insignificance of lack of fit. Furthermore Figure IV-9 shows a good correlation between the values predicted by the

ANOVA model and the experimental responses in the pre-cited cases of hardness, blocking, gloss and haze.

However, chemical resistance performances could not be fitted properly. It is important to point out that chemical resistance responses are more difficult to fit into a mathematical model since their measurement is based on visual evaluation. As a result, operator error can be important, especially when technician is not experienced. Moreover, the evaluation scale is quite restricted, namely from 0 to 5, decreasing data accuracy.

As a result the influence of factors, herein additive types and concentration, on paint performances, except chemical resistance could be extracted from the model. Figure IV-10 shows the parametric 2D plots of the effect of additives on given properties for both MF40-BA/45 on one side and Nu-Stq on the other. In such graphics, higher values are represented by warm colors while cold regions point out the lower ones. Plot (a) compares the hardness of both MF40-BA/45 and Nu-Stq with regards to additives concentration. In general, MF40-BA/45 exhibits lower hardness than Nu-Stq independently of additives amounts. We can note however that the addition of ASR helps improving significantly the hardness of both paints. In contrast, the effect of NanoBYK-3620 on hardness is negligible. With maximum concentrations of ASR, MF40-BA/45 hardness can be increased up to about 33 seconds while Nu-Stq gave a value above 85 seconds. This result is explained by the significant difference in Tg and indirectly in MFFT between both binders, MF40-BA/45 having an MFFT below 0 while Nu-Stq ones is around room temperature.

Additive concentrations influence blocking performance in a similar extent for both MF40-BA/45 and Nu-Stq. Surprisingly while blocking performances resulted independent from bio-based ASR concentration, the synthetic ASR was shown to exhibit a positive effect. Two main

factors are responsible for blocking. On one side the glass transition temperature of the binder and on the other side the potential physical interactions between the functional groups of the resins. Most likely the nature of the bio-based monomer that had been substituted in the ASR synthesis is responsible for the increased interactions between two adjacent films.

Variation of gloss and haze with respect to additives concentration is given in Figure IV-11. A similar trend is observed for both binders. NanoBYK-3620 has a negative effect on gloss performances. Indeed, the presence of hybrid nanoparticles creates a micro-roughness at the surface of the film, inducing more light scattering and thus lower gloss. Increasing nanoparticle concentration results in a matting effect. Evolution of haze corroborates this result. Incorporation of NanoBYK-3620 tends to increase film haziness.

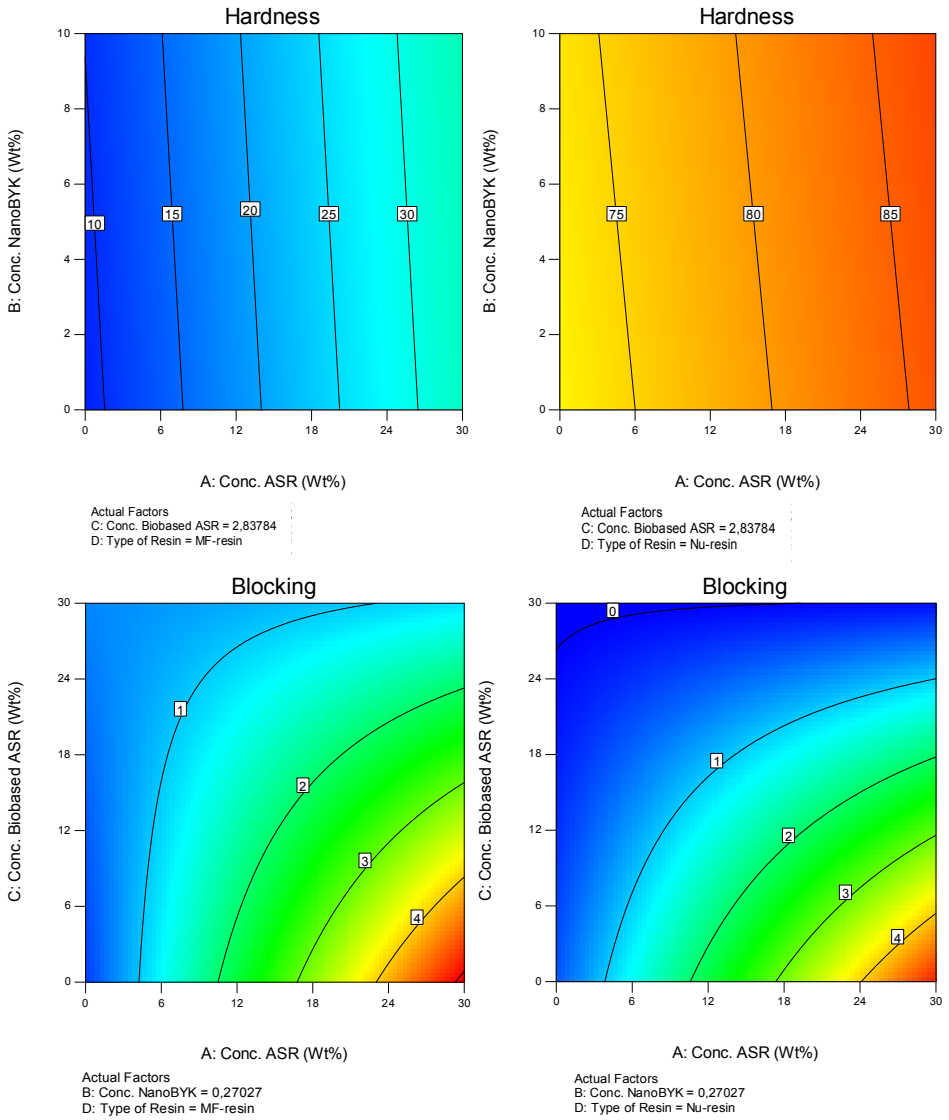


Figure IV-10. Effect of additives concentrations on hardness and blocking performance of formulated MF40-BA/45 (left) and Nu-Stq (right)

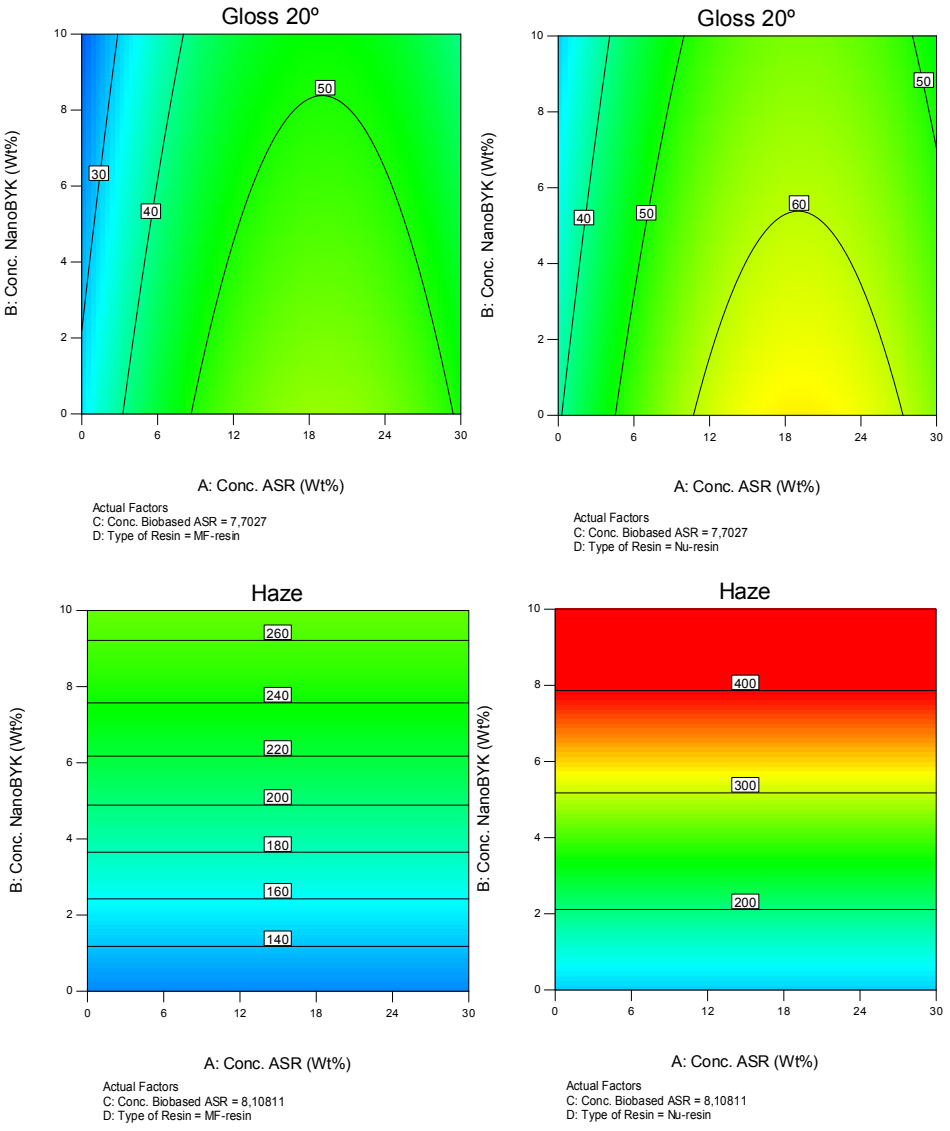


Figure IV-11. Effect of additives concentrations on gloss and haze performance of formulated MF40-BA/45 (left) and Nu-Stq (right)

ANOVA statistical tool offers the possibility of optimizing performances by predicting results. Performance can be maximized or minimized individually or in parallel. Herein we tested the reliability of the model generated from the above design for the optimization of MF40-BA/45 and Nu-Stq formulations with the ultimate goal of maximizing both hardness and gloss. Table IV-7 shows the formulation obtained for each resin. MF40-BA/45 was formulated with 30 wt% synthetic ASR exclusively while both ASR and NanoBYK-36250 were incorporated in Nu-Stq.

Table IV-7. Formulations for the maximization of hardness and gloss

Binder	NanoByk3620 (wt%)	ASR (wt%)	Bio-based ASR (wt%)
MF40-BA/45	0	30	0
Nu-Stq	8.5	30	0

The values predicted by the model and the actual measurements performed are gathered in Table IV-8. Theoretical values of responses are in reasonable agreement with measured performances, highlighting the efficiency of the method and model used. Additives incorporation increased successfully the fructose based paint hardness up to a maximum value of 32 s while Nu-Stq has a high hardness of 86 s. Still, both paints exhibited a high gloss in the range of 70 as well as equivalent blocking properties. The reference binder from Allnex resulted very hazy (216.3) whereas haziness of the sugar paint was very reasonable (60.2). Haziness of formulated Nu-Stq results from the use of Nanobyk additive which, as explained earlier, promotes film surface roughness and therefore causing an increased amount of scattered light.

Table IV-8. Comparison of model prediction with experimental data for the optimization of hardness and gloss

		MF40-BA/45		Nu-Stq	
Optimization	Response	Predicted value	Measured value	Predicted value	Measured value
Maximization	Hardness	33	32	91	86
Maximization	Gloss	70	67.8	80	71.4
	Haze	55	60.2	235	216.3
	Blocking	5	4	5	4

IV- 3.2. Substitution of MMA by MF in a commercial binder formulation

The unformulated sugar-based binder used in the previous study presented quite low Tg, so the coatings presented very low hardness. In this section, and aiming at evaluating the effect of the sugar-based monomer on paint formulations by comparison with an analogous commercial one, MF-Stq binder was produced following the polymerization corresponding to Nu-Stq commercial binder from Allnex but substituting MMA by MF in the formulation (Table IV-1). Nu-Stq contained 48 wbm% of MMA, and consequently MF-Stq equivalent consisted of 48 wbm% of MF. Raw latexes minimum film formation temperatures were determined: Nu-Stq resin exhibited a MFFT around 14 °C, while MF-Stq shows a higher MFFT around 24 °C. This difference can be explained by the glass transition temperatures of poly(MMA) and poly(MF), respectively of 105 and 115 °C. Thus the incorporation of MF to replace MMA is expected to result in a slight increase in the copolymer glass transition temperature.

IV- 3.2.1. Design of experiment and paint formulation

In contrast with MF40-BA/45, MF-Stq latex was shown to be fairly compatible with several types of additives. No coagulation was observed when adding wetting agent, cosolvent or defoamers.

It was mentioned before that the choice of additives depends strongly on the properties of the initial polymeric binder to be considered. In this particular case, since MFFT were in the range of ambient temperature, the use of cosolvent to allow good film formation was necessary. Here butyl diglycol was employed.

In addition, defoamers, chemical additives used to hinder the formation of foam and possibly the type of additive mostly used, were tested to formulate the paints. Typically, a defoamer must exhibit very specific properties, including low surface tension and insolubility in the formulation to be defoamed. Recently novel silicone polyether based antifoams that offer effective foam control balance against ease of incorporation have emerged.[27,28] A variety of copolymer structural possibilities exist, such as block, branched, or comb-shaped copolymers.[29] The siloxane block provides surface activity while the polyether chain enhances the degree of compatibility. Both defoamers used in this work are of polyether siloxane type.

Table IV-9 describes the additives used to formulate the paints derived from MF-Stq and Nu-Stq binders. All of them were sold as compatible additive for acrylic waterborne coatings.

Table IV-9. Additives name and composition used to formulation MF-Stq and Nu-Stq

Name	Composition	Surface tension
COSOLVENT		
BdG	Butyl diglycol	24.7 mN/m (30 °C)
DEFOAMER		
Tego foamex810[31]	Polyether siloxane copolymer, containing fumed silica	N/A
Dow corning71[32]	Active polymeric silicone	26.4 mN/m (20 °C)

Design expert software was used to define a plan of experiments to be able to study the effect of additives on paint performance. Binder type was set up as categorical variable, being either MF-Stq or Nu-Stq resin. Defoamers and cosolvent concentrations were included as numerical variables whose level limits were in accordance with suppliers recommendations. Paints performance such as hardness, gloss, haze and chemical resistance were defined as responses (Table IV-10).

Table IV-10. DOE settings for the testing of MF-Stq and Nu-Stq

Variables	Levels	Responses
Binder	Type1: MF-Stq Type2 : Nu-Stq	Hardness
Cosolvent	From 0 to 5 wt%	Water resistance Ethanol resistance
TEGO Foamex 810	From 0 to 1 wt%	Coffee resistance Blocking
Dow corning 71	From 0 to 0.5 wt%	Gloss Haze

Table IV-11 shows the plan of experiments proposed by the DoE, consisting of 24 experiments, including 5 replicate points.

In this study, particular care was given to the incorporation of the additives in binder dispersions. Defoamers require to be insoluble in the formulation so they must be present in the form of finely divided droplets to enhance their incorporation. The choice of defoamer is therefore always a compromise between compatibility and targeted incompatibility.[33] Defoamers were prepared as 50 wt% solutions in water to facilitate their addition. Firstly cosolvent was added to the resin and mixed with a high speed mixer at 3500 rpm for 3 minutes. Secondly defoamer 1 (Tego foamex 810) together with defoamer 2 (Dow corning 71) were incorporated in the paint and mixed at 3500 rpm (maximum speed) for 3 minutes.

A first set of paints were applied on glass substrates and significant surface defects were observed for both binders. Drift in composition, that is to say bad homogenization of the paint components may result in the appearance of surface defects upon drying process. Consequently, paint mixing was repeated using the speed mixer at 3500 rpm for 5 minutes. Film application on glass substrates showed shrinking and surface defects. An additional 10 minutes of mixing failed in improving film formation. The surface defects observed were corresponding to a cratering effect, as shown Figure IV-12 (a).

Table IV-11. Paints formulation proposed by DoE (quantities given in grams)

Paint	MF-Stq	Nu-Stq	Butyl diglycol	Foamex 810 (50 wt%)	Dow corning71 (50 wt%)	Total
1	15,000	0,000	0,656	0,186	0,073	15,915
2	0,000	15,000	0,375	0,150	0,075	15,600
3	0,000	15,000	0,375	0,150	0,075	15,600
4	0,000	15,000	0,653	0,191	0,000	15,843
5	15,000	0,000	0,319	0,000	0,000	15,319
6	0,000	15,000	0,750	0,300	0,093	16,143
7	0,000	15,000	0,431	0,000	0,049	15,480
8	15,000	0,000	0,750	0,000	0,077	15,827
9	15,000	0,000	0,750	0,300	0,150	16,200
10	15,000	0,000	0,000	0,300	0,076	15,376
11	0,000	15,000	0,273	0,039	0,150	15,462
12	0,000	15,000	0,000	0,000	0,053	15,053
13	15,000	0,000	0,540	0,085	0,150	15,775
14	0,000	15,000	0,375	0,150	0,075	15,600
15	15,000	0,000	0,319	0,000	0,000	15,319
16	15,000	0,000	0,000	0,125	0,000	15,125
17	0,000	15,000	0,750	0,000	0,150	15,900
18	0,000	15,000	0,000	0,300	0,150	15,450
19	15,000	0,000	0,000	0,000	0,150	15,150
20	15,000	0,000	0,750	0,300	0,000	16,050
21	15,000	0,000	0,000	0,300	0,076	15,376
22	0,000	15,000	0,750	0,000	0,000	15,750
23	0,000	15,000	0,000	0,300	0,000	15,300
24	15,000	0,000	0,656	0,186	0,073	15,915

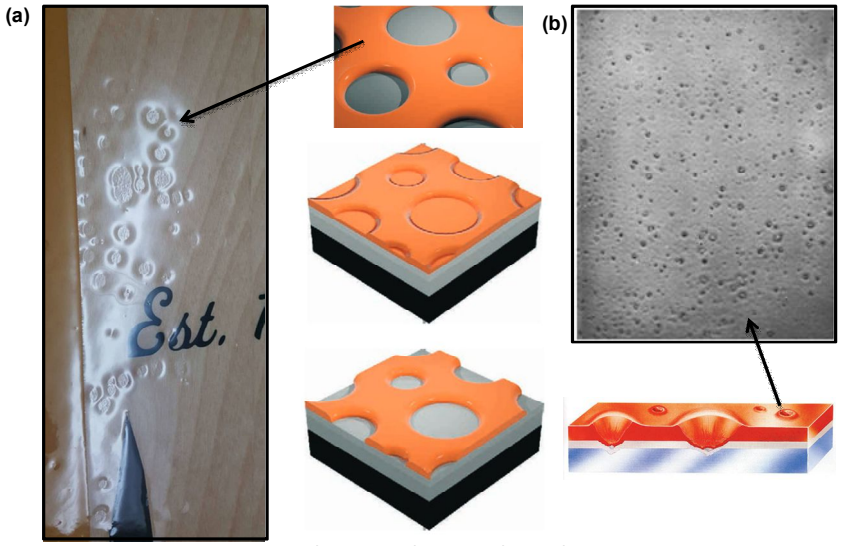


Figure IV-12. Picture and representation of cratering/fish eyes film defects

Cratering is the appearance of small round depressions in a coating surface generally with a slightly raised crest.[34] They often look somewhat like volcanic craters, hence the name. They may also be compared with “fish eyes”. Both phenomena refer to a pesky finish issue involving surface tension effects in fluids. Most commonly cratering may be due to surface tension gradients of the wet paint. Fundamentally it results from a small particle or droplet contaminant of low surface tension which can be on the surface, in the coating or under the applied film.[34] The paint flows away from the low surface tension area, leaving a circular defect as shown in Figure IV-13. This phenomenon derives from the so-called Marangoni effect which is defined as the mass transfer along an interface between two fluids due to surface tension gradient.[35]

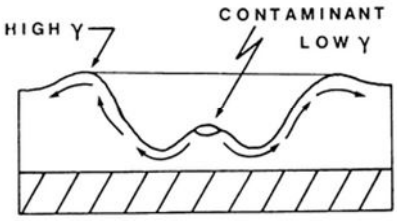


Figure IV-13. Representation of a crater showing the flow away from the area of low surface tension (contaminant) to an area of higher surface tension and the buildup of paint to form the rim of the crater.[36]

In practice, contaminants well-known to cause cratering include hydrocarbon and fluorocarbon oils, lubricants, silicones, plasticizers, resin gel particles, oven condensate, dirt, fibers, filter material, overspray, deodorants and other personal care products, poorly dissolved. But in fact dispersed additives, especially silicones can act as such pollutant. Indeed, as explained before defoamers are typically mineral oils or silicones of low surface tension. Their incorporation in paints can thus be critical. Several studies demonstrated the risk of adding low surface tension additives to waterborne paints on film defects and various crater formation mechanisms by antifoams were reported.[37] Furthermore, agitation conditions during defoamer incorporation was shown to be extremely important since it contributes to the definition of defoamer particle sizes, closely related to the defoaming process efficiency. For instance, Tego Chemie service performed a study highlighting the influence of agitation conditions on cratering defects for several defoamer systems.[38] Figure IV-14 (a) shows the crater occurrence ranging from 1 for defect-free films to 4 for high crater concentration. It can be seen that higher shear forces resulted in lower crater formation. Indeed, higher shear conditions were shown to induce lower defoamer particles sizes as shown Figure IV-14 (b). It

can be concluded that bigger defoamer droplets promote dewetting and obviously method of incorporation has a great influence on paint film quality.

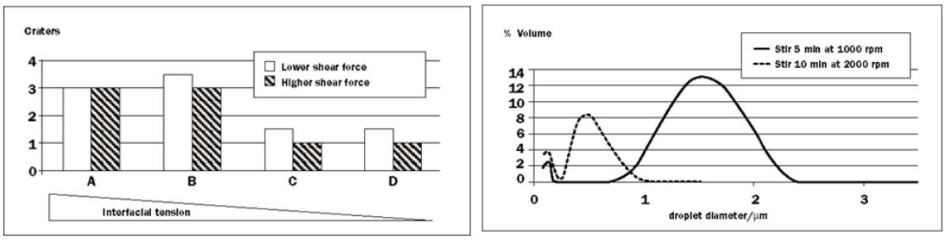


Figure IV-14. (a) Crater occurrence caused by different incorporation methods of active matter. (b) Droplet size distribution in an alkyd system using different incorporation methods (defoamer: poly(ethersiloxane).[38]

Given this, we believe that the choice of defoamer together with the addition conditions used herein were not suitable to achieve good paint formulation. In fact defoamer selection and their use in waterborne coatings is complex and formulating binders requires many critical steps to achieve optimal performance.[39,40]

IV- 3.2.2. Statistical evaluation of the responses and formulation optimization

The initial aim of such design of experiments was the study of the relationship between input variables, here additives and latexes and output responses, namely coating performance. However, because of paint defects issues above mentioned, several experiments could not be tested properly. In addition to this, experiments where MF-Stq resin was used without incorporation of cosolvent (namely paint N° 10, 16, 19 and 21 in Table IV-11) were not exploitable because of the presence of cracks in the film, due to the higher MFFT of MF-Stq. In fact the level of the numerical variable “cosolvent concentration” should have been set different from 0 in the design to ensure good film formation in all cases (instead of being set from 0 to 5

wt%). Therefore the cited experiments were ignored during the design treatment. Having a diverse set of experiments is essential for the successful outcome of the procedure. In fact, a smaller matrix of experiments turns out to be insufficient to find all the parameters needed to adequately describe the functional relationship between the experimental factors and the response variable.

Anyhow, responses outcome from the rest of experiments were included in the design and a model was generated by the software using ANOVA concepts. Model fit evaluation was carried out keeping in mind the previous comments on experiment matrix reduction. As explained in paragraph IV-3.1.4 of this chapter, F-value, p-value as well as the lack of fit and R^2 are the most common statistical parameters to analyze for model fitting performance assessment. Table IV-12 gives the parameters obtained for various responses. Note here that ethanol resistance was excluded since its evaluation was difficult to proceed because of films poor quality.

Again here, the importance of a combined evaluation of parameters is highlighted. Although lack-of-fit is given not significant in all cases, model fitting was not suitable for all responses. In particular chemical resistance response fitting were giving very low R^2 and so was the case for both hardness and blocking. In contrast, gloss and haze responses gave quite good model fitting parameters with high F-value of 64.07 and 15.79 respectively together with a probability of having F-values that high coming from noise less 0.01 % in both cases.

Table IV-12. Responses' model fitting performance

Response	F-value	p-value	Lack of fit	R^2
Hardness	6.77	0.0047	Not significant	0.59
Blocking	13.86	0.0002	Not significant	0.75
Gloss	64.07	<0.0001	Not significant	**0.99**
Haze	15.79	<0.0001	Not significant	**0.93**
Water resistance	10.42	0.0066	Not significant	0.44
Coffee resistance	9.01	0.0024	Not significant	0.53

Figure IV-15 corroborates these conclusions by showing a good agreement between actual and predicted values of gloss and haze while blocking and water resistance predicted values are poorly corresponding to actual ones. In fact among all the properties tested, gloss and haze are the ones which are being measured with the most accuracy. Indeed since an electronic device is used and a minimum of 5 repetitions per sample is carried out, experimental error is minimized. In the context of a reduced experiment matrix, it makes sense that properties of gloss and haze are the most likely to be adequately fitted in a model despite the poverty of experimental data. The effect of additives on these two properties was thus plotted on bidirectional graphs, given Figure IV-16 and Figure IV-17.

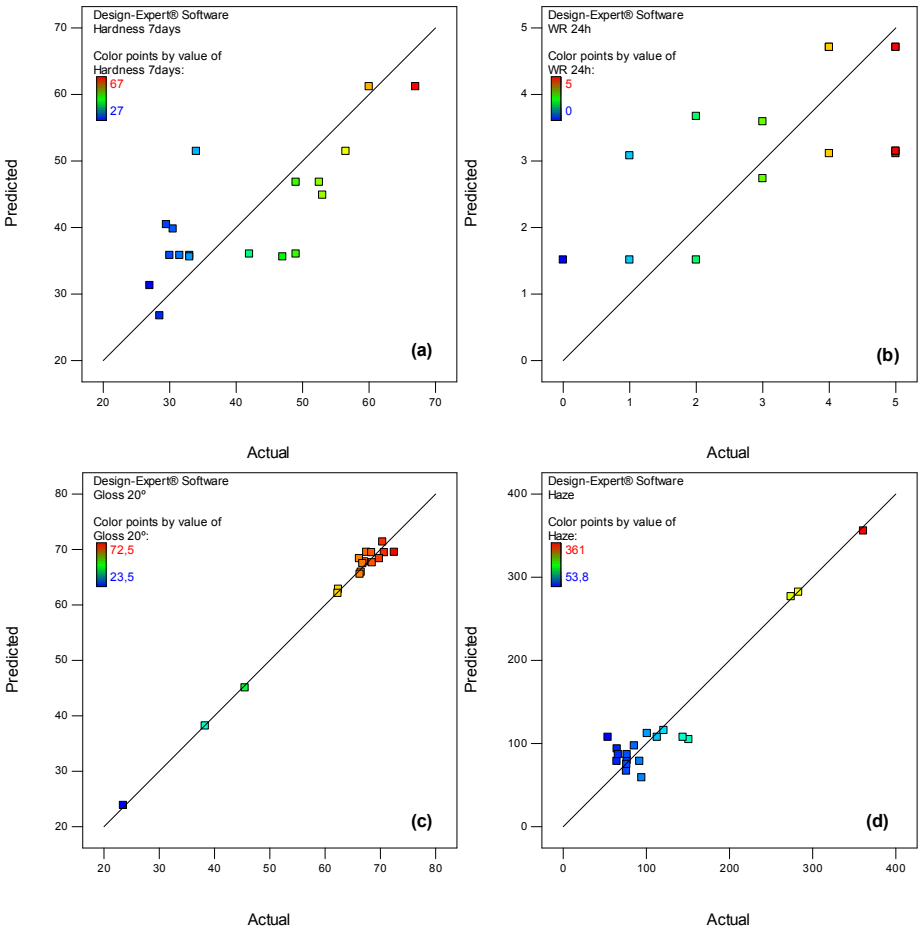


Figure IV-15. Performance of the statistical model ANOVA for predicting (a) hardness, (b) water resistance, (c) gloss and (d) haze.

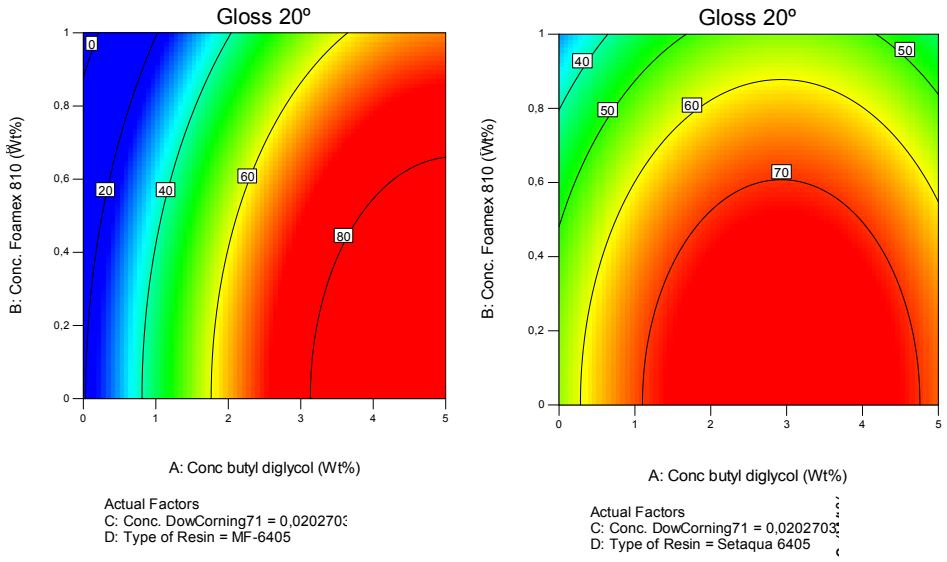


Figure IV-16. Effect of the cosolvent and Tego foamex 810 concentrations on gloss for formulated MF-Stq (left) and Nu-Stq (right) films

MF-Stq gloss performance is completely dependent on cosolvent concentration. This result is in agreement with the theory of film formation given in paragraph IV-3.1.1. When the Tg and by consequence the MFFT of a latex is too high, e.g. higher than ambient temperature, particle coalescence and chains interdiffusion does not occur properly, creating refractive index heterogeneity in the film and negatively affecting gloss. The plot transposes this effect, giving low values of gloss at low cosolvent concentrations and high gloss values when better particles and chains interpenetration is allowed by the cosolvent plasticizing effect. Interestingly, for the same range of additives concentration, a greater effect on gloss is noticed for MF-Stq, with gloss values varying from 0 to 80, while variation for Nu-Stq is going from 40 to 70.

The presence of defoamer influences negatively gloss for both MF and Nu Stq paints. In fact, defoamers are in the form of tiny droplets or solid particles, eventually at the film surface. A side effect of their presence is based on local modifications of refractive index resulting in increased light scattering and therefore low gloss. This is one the main reasons why great efforts have been done lately to enhance defoamers efficiency at low concentrations. Again, additive incorporation is a compromise between paint performance and additive efficiency.

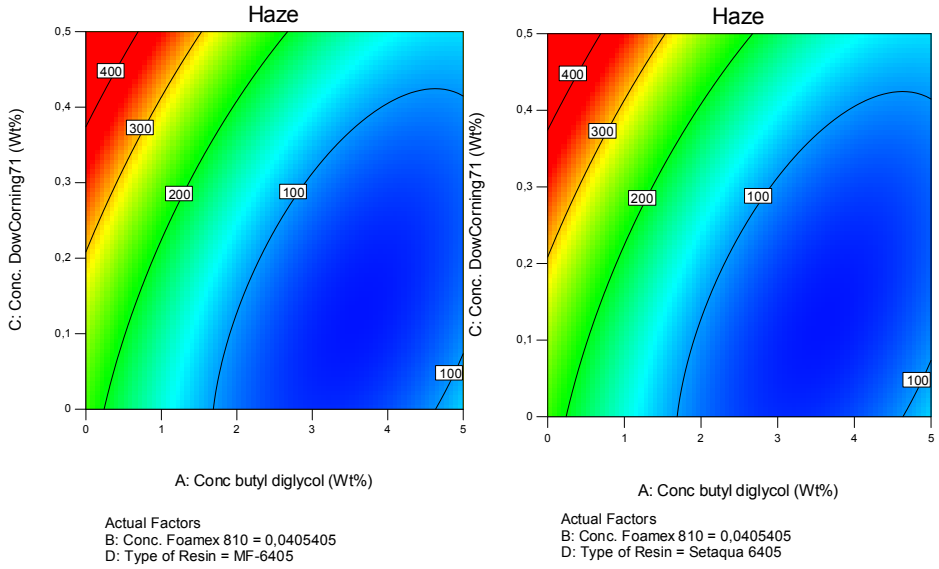


Figure IV-17. Effect of the cosolvent and Dow corning71 concentrations on haze for formulated MF-Stq (left) and Nu-Stq (right) films

The influence of additive concentration on haze is represented in Figure IV-17. The tendency obtained corroborates the results obtained above for gloss, i.e. cosolvent addition results in better film formation and thus lower haze. As opposite, defoamer promotes increment of haziness.

Mathematical models build up from the responses were not used herein to predict behavior and optimize formulation since many responses could not be fitted. However, two formulations proposed by the DoE were giving very good results and were further studied. On one hand a MF-Stq resin containing 2.125 wt% of cosolvent and no defoamer was considered (Paint N°5 in Table IV-11). On a second hand Nu-Stq formulated with 5 wt% of cosolvent and no defoamer was selected as commercial reference (paint N°22).

Table IV-13 lists the performance of both paints with respect to hardness, chemical resistance and blocking. While commercial binder Nu-Stq has a hardness of 42 s, the sugar-based latex MF-Stq presents a great hardness of 64 s. These results can be explained by the glass transition difference of copolymers bearing MMA units or MF units. MF-Stq also exhibits lower haziness than its commercial equivalent and excellent gloss. The sugar-based paint was found to have a better water resistance whereas resistance to coffee staining was rather low. Blocking appeared to be not efficient in both cases, although the fructose-based paint shows a slightly better anti-blocking behavior.

Table IV-13. Performances of formulated MF and Nu Stq

Resin	Hardness (s)	Gloss	Haze	Coffee resistance	Water resistance	Blocking
MF-Stq	64	69.5 ± 0.8	68.2 ± 10.5	2	5	2
Nu-Stq	42	68.5 ± 1.7	101.0 ± 18.0	5	4	0

IV- 4. CONCLUSION

"Tiny amount, huge effect!", *Dr. Sonja Schulte – European Coating chief editor.*

In the past years, coating manufacturers have been developing effective alternatives to reduce paints VOC content by giving greater importance to water-based systems. More environmentally friendly waterborne paints are though multifaceted systems involving complex phenomena. The formulation of dispersed copolymers is a compromise between additives incorporation for performance enhancing and paint integrity preservation. Nowadays the additive market offers a multitude of possible ingredients to be used in paints, making the process of formulation optimization a difficult task. In that sense, design of experiments is a powerful tool to resolve this puzzle in a time-effective manner.

In this chapter, the use of DoE was explored to optimize the formulation of sugar-based binders. Two copolymers were selected, namely MF40-BA/45 prepared in our laboratories and MF-Stq produced at Allnex following a commercial binder recipe but substituting MMA by MF. Sugar-based binders had a bio-based content of 40 and 48 wt% based on total monomer amount respectively.

On a first hand, the potential performance of the sugar based MF40-BA/45 latex to be used as binder for coatings was evaluated. It was shown to exhibit very good film forming properties and wettability, but rather low hardness, because of its low glass transition temperature. The incorporation of ASR and NanoBYK-3620 (additive commonly used to enhance scratching resistance) was investigated by a combination of DoE and HTE, obtaining an optimized formulation that allowed the preparation of a low haze glossy clear coat with acceptable hardness.

On a second hand, the latex MF-Stq prepared from Allnex Nu-Stq commercial reference, in which all MMA was substituted by MF, was investigated as binder, using a design of experiment including cosolvent and defoamer. These experiments highlighted the critical aspect of defoamer addition within paints. Indeed, because of inappropriate incorporation methods and/or media incompatibility, resulting films exhibited lots of surface defects of cratering type. Nevertheless, both MF-Stq and Nu-Stq were formulated with cosolvents and their performance was compared. MF-Stq exhibited very good paint properties, namely better hardness, equivalent gloss, lower haze and better water resistance and blocking. As such, these results definitely demonstrate the potential of sugar-based latexes to be used as binder in paints.

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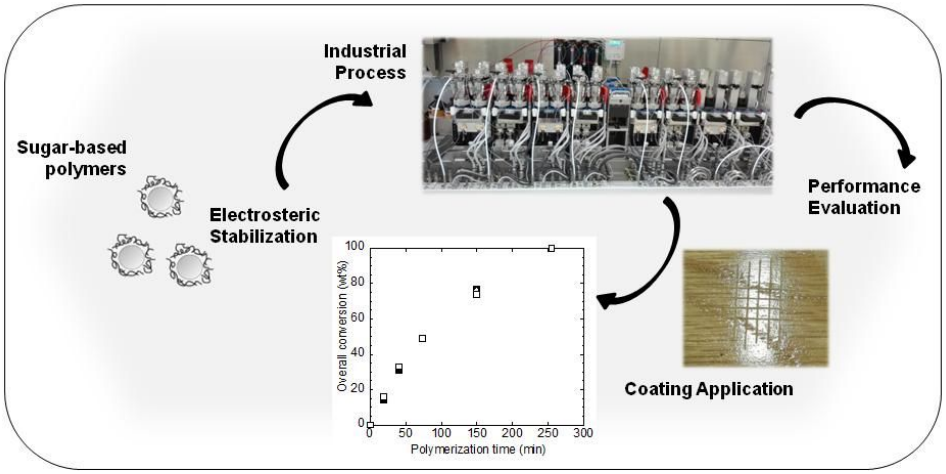
Chapter V-Synthesis of Electrosterically Stabilized Latexes using Sugar-based ASRs. Their Performance in Decorative Paints.



V- 1. INTRODUCTION

Emulsion polymers are widely used as film-forming materials for coatings and adhesives. Because hydrophobic particles dispersed in aqueous media are thermodynamically unstable, surfactants are used to provide kinetic stability. However, conventional anionic or nonionic surfactants create many drawbacks in terms of application properties. This poor performance is mainly due to the weak interaction between the surfactant and the polymer phase.[1–10] Surfactant migration creates a heterogeneous distribution of hydrophilic species within the film and thus negatively affects the properties. The use of polymeric surfactants is an effective method for overcoming most of these problems because they strongly adsorb on the polymer particles.

Alkali soluble resins (ASRs) are random or block copolymers composed by hydrophobic monomers, such as methyl methacrylate (MMA), butyl methacrylate (BMA) and styrene (S), and monomers containing carboxylic acid units, namely (meth)acrylic acid (MAA or AA), with usually low molecular weight (below 20000 g/mol). The presence of COOH groups combined with the low molecular weight of the copolymers make chains solubility to be pH dependant. Typically, when the pH is lower than the carboxylic acid pKa, the functional COOH groups are protonated and the ASR is not soluble in water. At high pH however, carboxylic acid groups get deprotonated, increasing chains water solubility. Under these conditions, ASRs are able, above certain concentration, to form aggregates in aqueous phase, as it was demonstrated via surface tension measurements.[11–13] As a result, ASRs are meant to be good candidates to act as electrosteric stabilizer in emulsion polymerization. In fact, they have been employed as such by the industry since the early 70's,[14,15] because among others, ASRs may induce better film

properties, namely excellent mechanical and freeze-thaw stability, good pigment dispersion and wetting properties.[16–20] Moreover, because of its strong adsorption onto polymer particles, leaching is strongly retarded, resulting in a better aging of the coatings.

However, main monomers of the ASRs proceed from non-renewable feedstoks. As it has been explained in the previous chapters, the incorporation of biomass derivative compounds in polymeric materials has gained plenty of interest due to the potential added-value of such resources in terms of renewability, novelty and eventually degradability. Therefore, the aim of this Chapter is to explore the use of sugar-based building blocks in the production of ASRs for the preparation of robust polymeric binder systems.

This work was carried out in the laboratories of Allnex in Bergen-op-Zoom, The Netherlands. Thus, two commercial products from Allnex, prepared using ASRs as stabilizers were taken as references (Product A and Product B in this chapter). Both are acrylic latexes used as binders in either pigmented or clear coat paints, containing around 50% of ASRs (mainly MMA/BMA/MAA) as a hard phase and a softer phase which includes some amount of MMA. In this work, two sugar-based monomers, consisting of methacrylate fructose (MF) and methacrylate gluconic acid (MGA) were incorporated at different ratio within the ASR chains to substitute methyl methacrylate and butyl methacrylate respectively, as well as within the softer phase. The selection of such sugar-based monomers was done taking into consideration the similar Tg of MF and MMA on one side and MGA and BMA on the other. All products prepared thereof were formulated for clear coat paint application and properties were measured and compared with the commercial products.

The chapter is organized in two parts. Firstly the incorporation of the MF as replacement of MMA is investigated, and the second part deals with the study of the substitution of BMA by MGA.

V- 2. EXPERIMENTAL

V- 2.1. Polymerization conditions

Polymerizations were carried out in small scale stainless steel reactors (100 mL) in a Chemspeed Accelerator A100 miniplant from Chemspeed Technologies. The reactors head included an integrated reflux condenser, and was equipped with a nitrogen inlet, and pumps inlets allowing small flow rates of feeding (for either pre-emulsions or monomer feeding). A stainless steel anchor impeller was used at 200 rpm. Pre-emulsions were prepared in a separated reactor equipped with a stainless steel turbine, stirred from 400 to 1200 rpm and constantly maintained at 20 °C. .

V- 2.2. Preparation of ASR stabilized latexes

As mentioned in the introduction, two different products were investigated by substituting conventional monomers by sugar-based units. Both strategies involved the use of polymeric stabilizers. Typically ASRs are high Tg polymers, therefore they will be often referred as hard phase in this work. Besides to allow obtaining latexes with good MFFT, e.g. able to

form film at ambient temperature, ASRs were used for the polymerization of a softer phase, eventually referred as polymer particle core. Product A was prepared in a two step process while B required one single synthesis stage.

Figure V-1 describes the procedure employed for preparing product of type A. Firstly, 30% solids content ASR was synthesized by in-situ seeded semibatch emulsion polymerization. The initial charge containing water and a very small amount of conventional anionic emulsifier was heated at 80 ºC under nitrogen atmosphere and agitation. Then, low amount of pre-emulsion (including chain transfer agent, CTA) and the initiator was added as a shot. 20 minutes later, the rest of the pre-emulsion was fed during 60 minutes. The resultant latex contained MMA/BMA/crosslinker/MAA (65/15/12/8 wt%). After full conversion, neutralization was carried out using ammonia to reach a pH of 8.5. In a second step, half of the monomers that constitute the soft phase were charged as a shot, under mechanical agitation and were left to stabilize for 30 minutes. Initiator was then added to the reactor in such a way that polymerization occurred fast and in theory directly within the swollen aggregates formed. The rest of monomer mixture was introduced after 40 minutes. In this process, the ASR represented about 50% of the final formulation.[21]

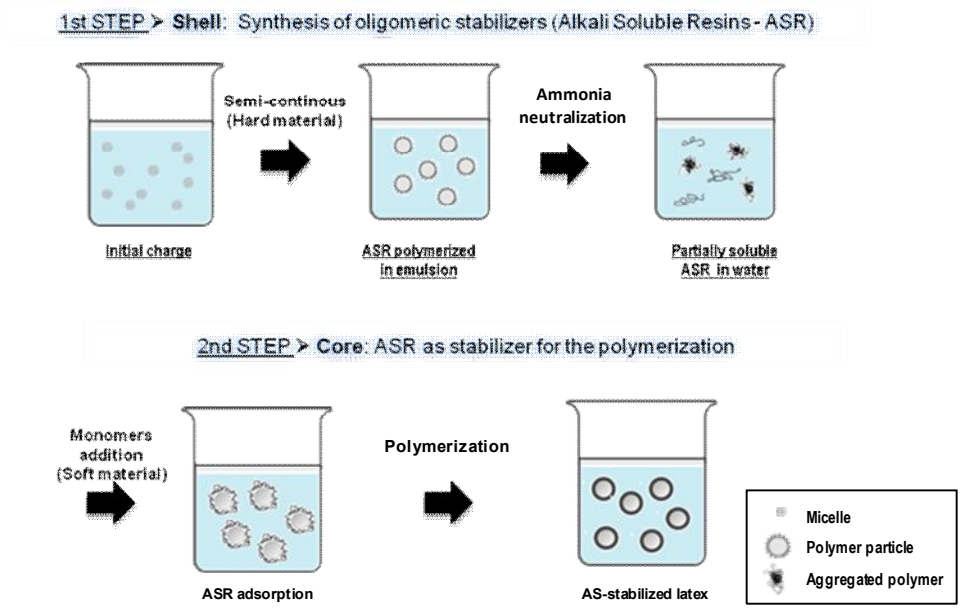


Figure V-1. Procedure for the synthesis of product type A

In the case of product B, the composition of the latex as well as the strategy employed were different. Here ASRs were synthesized in-situ and copolymerization of the soft phase was carried out in the presence of partially neutralized ASR resins. The ASR (MMA/BMA/crosslinker/MAA: 69/15/10/6 wt%) was copolymerized in a semibatch process using an anionic emulsifier and CTA. After 75 minutes, the monomer mixture corresponding to the soft phase was fed to the reactor together with ammonia solution and more initiator, varying the neutralization degree of the ASR resin along the polymerization. During this stage of the reaction, the pH of the polymer dispersion was kept low, below 6, and only after the end of the polymerization the pH of the dispersion was raised up to 7 in order to phase-invert the particle.

During this phase inversion, (part of) the hard and more hydrophilic ASR migrated towards the particle surface, providing stabilization of the latex.[22]After cooling down at room temperature, products were filtered off over a 260 µm grid.

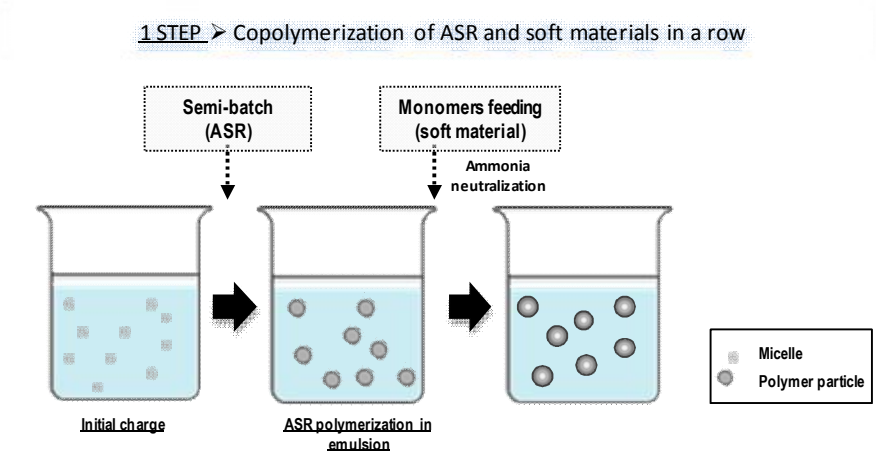


Figure V-2. Procedure for the synthesis of product type B

V- 2.3. Characterization methods

Ultra performance liquid chromatography (UPLC) analysis was performed to monitor unreacted monomer amount during reaction, following the protocol published by S.P.Kossen.[23] 100 mg of waterborne dispersion was dissolved in acetonitrile and precipitated in a saturated NaCl solvent. Solutions were analyzed using a Acquity UPLC BEH C18 column (1.7 µm, 2.1 x 50 mm) at a 1.0 mL/min flow. Results were treated thanks to external calibration curves.

Average particles diameter (dp) was determined by dynamic light scattering, using a Zeta-Sizer instrument from Malvern. Latexes were diluted with deionized water before measurements.

Gel Permeation Chromatography measurements were conducted in an Agilent chromatograph equipped with a PLgel 5µm Mixed-C 600x7.5 mm column. Samples were dissolved in THF with 2 % acetic acid at a concentration of 1.5 mg/mL. Injection was done at a 0.8 mL/min flow rate at ambient temperature

Coating hardness was measured with a Konig pendulum apparatus (Erichsen model 299/300). [24] Gloss at 20° and haze of the coatings were determined with a goniophotometer Rhopoint IQ.

V- 3. ASR STABILIZED LATEXES CONTAINING METHACRYLATE FRUCTOSE (MF).

Since the methacrylate fructose monomer (MF, Figure V-3) and methyl methacrylate lead both to homopolymers of high Tg, respectively 110-115 °C and 105 °C, the exploration of the use of MF instead of MMA in commercial ASR containing latexes was performed. The aim was to evaluate the possibility of incorporating renewable resources in such products and to determine the resulting effects on paints characteristics.

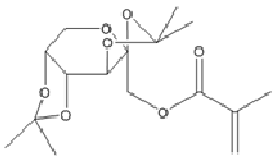


Figure V-3. Methacrylate fructose (MF) monomer structure

In this chapter, the nomenclature describing the different runs is given in Figure V-4. The code is as follows: the letter (A- or B-) refers to the product type procedure and the following letters-numbers to the biobased monomer (MF for methacrylate fructose and MGA for methacrylate gluconic acid) with the percentage of conventional monomer replaced (i.e. A-MF20 for product A with 20 wt% of MMA replaced by MF). When describing the ASR stabilized latex, the code is preceded by the letter L.

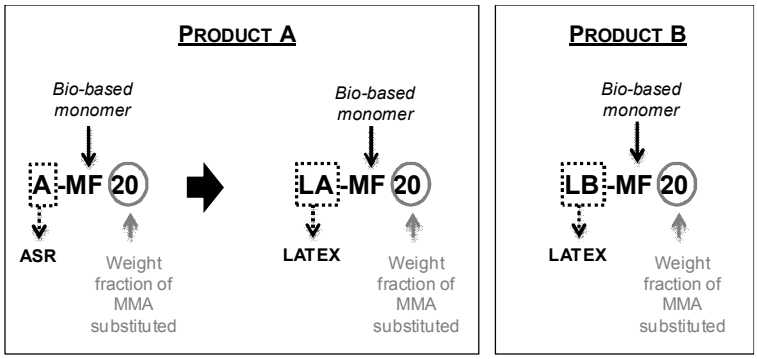


Figure V-4. Nomenclature of the synthesis runs

In both product A and B, MMA content was substituted incrementally. From 20 to 100 wt% of MMA amount in the ASR was substituted by MF while 100 wt% of its fraction was replaced within the soft phase (references excluded LA-MF0 and LB-MF0) (Figure V-5).

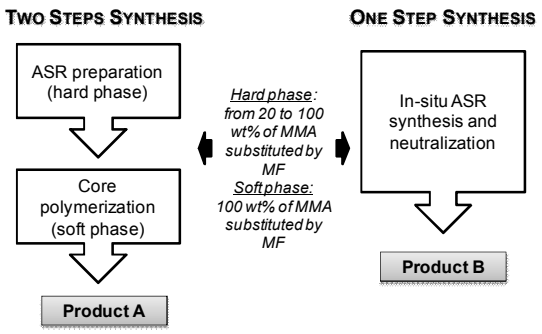


Figure V-5. Distinction of processes for the production of ASR-stabilized sugar-based latexes

V- 3.1. Product A: Sugar-based latex synthesis

Latexes mimicking product A were prepared replacing from 20 to 100 wt% of MMA by MF in the ASR formulation and 100 wt% of the MMA in the soft phase. In consequence, the sugar-based ASRs contained from 13 to 65 wt% of bio-based monomer, when substituting from 20 to 100 wt% of MMA respectively.

V- 3.1.1. ASR synthesis

Figure V-6 shows the overall fractional conversion evolution of both MMA and MF along polymerization for all runs where 0 to 100 wt% of MMA was substituted in the ASR. As can be seen, no obvious difference was observed in terms of conversion evolution. However, it is worthy to note that fractional conversions were calculated from the amount of unreacted monomer at time t (which is meant to be very small since we are working in a semibatch process) divided by the total amount of monomer to be fed in the reactor (large amount). Therefore the reactivity differences between the two monomers, if there is any, will not be

easily highlighted with this graph. By plotting the instantaneous conversion (that is to say the amount of unreacted monomer at time t divided by the amount of monomer fed at time t) along polymerization, we can observe a slight reactivity difference. MF reacts slower than MMA, likely because of the steric hindrance induced by the bulky protected sugar scaffold.

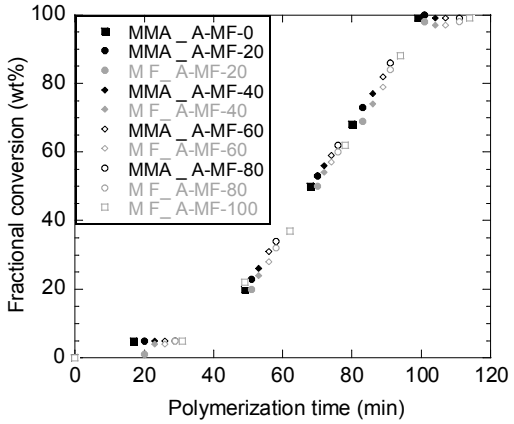


Figure V-6. Synthesis of ASR, product A: Time evolution of fractional conversions of MMA (black points) and MF (grey points) for the different runs.

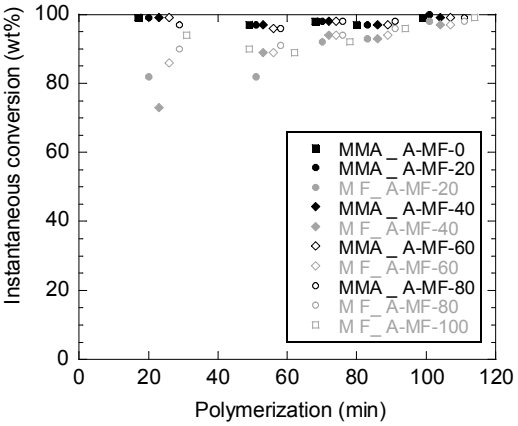


Figure V-7. Synthesis of ASR, product A: Time evolution of instantaneous conversions of MMA (black points) and MF (grey points) for the different runs.

Polymer particle sizes were followed along reactions by dynamic light scattering. Figure V-8 shows a similar evolution as well as very close final polymer particle sizes for all the runs with the exception of A-MF40 where particle size is slightly higher. From the evolution of the number of particles presented in Figure V-8 it could be concluded that neither coalescence nor new nucleations are occuring during the semibatch process. The first sample defined the size of the seed and the number of particles, which then remained relatively constant. Table V-1 compiles the amount of residue obtained after filtering the final latex with a mesh of 260 µm. It was observed that, in general, amounts of residues are more important as the fraction of MF is increased. Moreover, material accumulation around the stirrer was also observed, decreasing the final solids content (the target was 30 wt%).

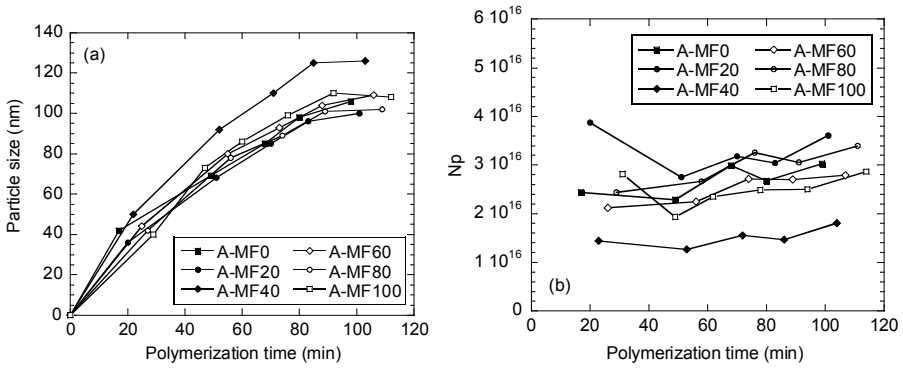


Figure V-8. Synthesis of ASR, product A (a) particle size and (b) number of particle evolution

ASR molecular weights (Mw) are given in Table V-1. The larger the amount of MF, the lower the molecular weight measured. By definition, ASRs are meant to be polymers of rather low chain length. To achieve this characteristic using free radical polymerization, chain transfer agents must be used. Chain transfer is a polymerization reaction by which the activity of a

growing polymer chain is transferred to another molecule. Herein it is important to note that CTA/monomer ratio was calculated in weight. As a result, since the molar masses of MMA and MF are significantly different (100 and 329 g/mol respectively), the CTA/monomer molar ratio was considerably affected whether MMA or MF were used in the formulation. CTA molar fraction was increased when MF amount was raised up and consequently ASR molecular weight was lowered. Further, it could be point out that values given are relative molecular weights obtained from a poly(styrene) standards calibration. Consequently copolymers with different monomer composition cannot be compared rigorously. Indeed replacement of MMA by MF induces an important change in polymer polarity which definitely affects chains affinity with solvent, and thus chains hydrodynamic volume. Since gel permeation chromatography is based on an exclusion principle, variations of polymer hydrodynamic volume automatically implies variations of Mw average values.

Table V-1. Product A: ASR solution characteristics

Run	A-MF0	A-MF20	A-MF40	A-MF60	A-MF80	A-MF100
Mw (g/mol)	15500	13400	12600	10700	11200	9800
Đ	2.0	2.1	1.9	1.9	1.9	1.8
SC (wt%)	29	28	27	29	26	28
Filtered residue (wt%)	0.01	0.08	0.10	0.33	0.31	0.09

Figure V-9 shows a picture of the different ASR solution after neutralization with ammonia. Opaque latexes turned more transparent after neutralization, indicating a partial solubilization of the polymer chains.

Figure V-9. Picture of the different ASR solutions after neutralization (from A-MF0 on the left to A-MF100 on the right)

The interest of changing ASR solubility by adding ammonia to the media is to obtain charged oligomers with a different degree of solubility and therefore a different state of entanglement in aqueous phase. Indeed, partially soluble oligomers will tend to form aggregates, that is to say chains having a hydrodynamic volume lower than water soluble polymers of the same Mw. This particular behaviour opens the possibility to use these aggregates as seed for the polymerization of another phase.

V- 3.1.2. Preparation of ASR-stabilized latex

In a second step, polymerization in dispersed media using ASRs as stabilizers was carried out. The so-called soft monomer mixture of the reference product from Nuplex contains 28 wt% of MMA. In the following study this complete amount was substituted by MF in all cases, except for the reference run LA-MF0. As a result, final latexes contained from 21 to 46 wt% of MF with respect to the total amount of monomer.

A first shot of monomer (representing half of the total monomer amount from the soft phase) was added to the ASR solutions under agitation and left to stabilize for 30 minutes.

Figure V-10 shows the evolution of average particle diameter along polymerization time. At time zero, that is to say after monomer addition and before initiation, we can measure the size of the swollen aggregates. In all cases, aggregates containing MF are bigger than the reference (A-MF0). This can be explained in terms of monomers hydrophobicity. Indeed, the parking area of a surfactant (a_s), that is, the area that covers one molecule of surfactant onto a specific surface at saturation, decreases with polymer hydrophobicity.[25] For instance Caballero et al[26] calculated that the a_s of the ASR (MMA/BA/MAA: 37/49/14 % wt) for PMMA was 3 times higher than for poly(styrene). Moreover they found that the size of aggregates containing butyl acrylate and styrene were bigger than those of MMA, highlighting the influence of monomer hydrophilicity for the formation of aggregates. Thus, being the MF more hydrophobic than MMA, the parking area of the ASR would be lower so more ASR would be needed to stabilize the particles. As a consequence, lower amount of aggregates would be formed being bigger in size.

After initiation, a minor augment of particle sizes was noticed and number of particles is constant. At 40 minutes, a second shot of monomer was carried out followed by an increase of particle diameter. Similar evolution was observed for the six runs. The evolution of the number of particle after this second monomer shot is relatively constant, indicating polymerization occurred within ASR initial aggregates.

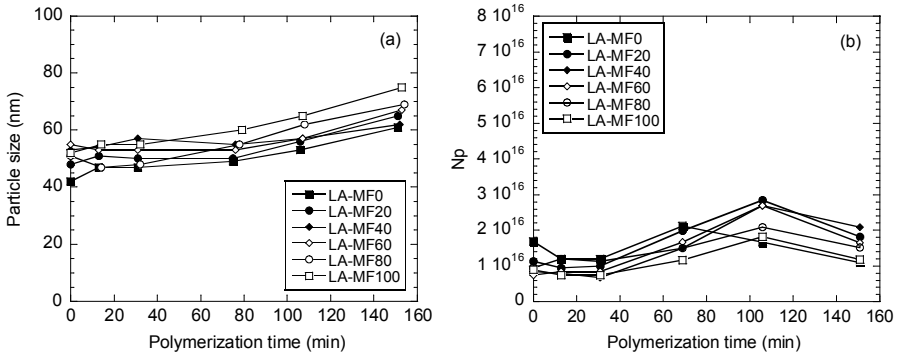


Figure V-10. Product A: (a) polymer particle size and (b) number of particle evolution during the 2nd step of the process

After initiator shot, polymerization occurred so fast that a significant exothermy was observed within the reactors. As shown in Figure V-11, reaction media temperature increased suddenly from 45 °C to 56 °C, independently from monomer types. A second exothermy of about 9 °C happened after the second shot of monomer.

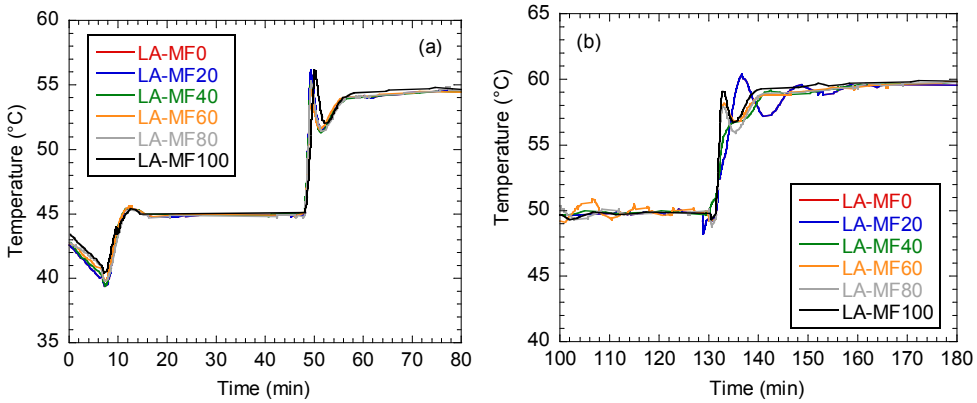


Figure V-11. Evolution of the temperature in reactors during polymerization process. Exothermic peak after (a) initial initiator shot and (b) second monomer shot (right)

As shown in Table V-2, polymerization of the core was very clean in terms of fouling and coagulation: very small amount of filtered residue was obtained and there was no coagulation around the stirrer. Final latex had a basic pH about 8 and a solids content around 38 wt%, as targeted.

Table V-2. Product A: Final latex characteristics

Run	LA-MF0	LA-MF20	LA-MF40	LA-MF60	LA-MF80	LA-MF100
pH	7.8	7.9	7.8	7.8	8.0	8.2
SC (wt%)	38	37	37	36	36	38
Filtered residue (wt%)	0.01	0.01	0.01	0.02	0.02	0.01

In conclusion, the synthesis of partially sugar-based ASR-stabilized latexes via the first technology corresponding to product A was successful. High conversions and low particle sizes were obtained with negligible amount of fouling.

V- 3.2. Product B: Sugar-based ASR-stabilized latex synthesis

ASR stabilized latexes from product B were prepared replacing from 20 to 100 wt% of MMA by MF in the ASR and 100 wt% of MMA in the soft phase. In the reference product B from Allnex, the ASR contains 69 wt% of MMA while the second monomer mixture (soft phase) consists of 19 wt% of MMA. As a result sugar-based latexes containing from 16 to 44 wt% of bio-based monomer with respect to the total monomer amount were produced.

Figure V-12 shows the increase of polymer particle sizes along polymerization time. Particle size evolution is in accordance with a semibatch emulsion polymerization process. Rather low polymer particle sizes are achieved between 80 and 100 nm. Although a slight deviation in particle diameter is observed at the end of the polymerization, it is still interesting to note the good reproducibility of particle nucleation and growth whether MMA is substituted by MF from 0 to 100 wt%. This tendency indicates the bio-based monomer does not apparently modify the reaction kinetics, as exemplified by Figure V-13. Overall conversions followed the same trend for all runs and reactions were performed in starved conditions.

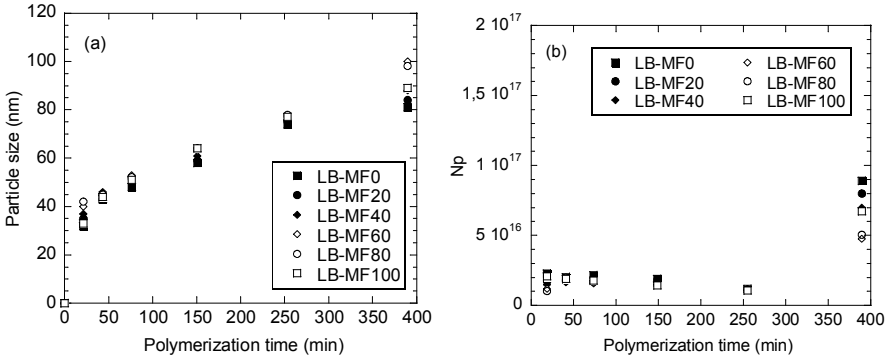


Figure V-12. Product B: (a) particle size and (b) number of particle evolution during polymerization process

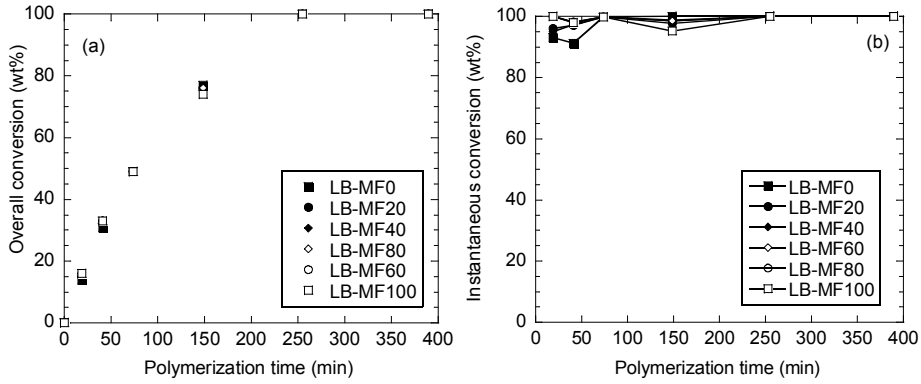


Figure V-13. Evolution of (a) overall conversion and (b) instantaneous conversions along polymerization time

The final characteristics of the latexes are given in Table V-3. High solids content of about 40 wt% were obtained. Accumulation of material around stirrer was observed, suggesting the need of agitation optimization. Further, we believe the observed increase of particle size after six hours of reaction for the runs containing more MF is due to a shear-induced coalescence of particles, also responsible for the increased amount of filtered coagulum when high fractions of MMA were substituted by MF.

Table V-3. Final latex characteristics

Run	LA-MF0	LA-MF20	LA-MF40	LA-MF60	LA-MF80	LA-MF100
pH	8.2	8.4	8.5	8.2	8.0	8.6
SC (wt%)	42	41	41	39	39	39
Filtered residue (wt%)	0.07	0.16	0.14	0.46	0.55	0.20

V- 3.3. Paint formulation and evaluation

V- 3.3.1. Paint formulation for clear coat application

Waterborne paints from Products A and B were prepared by following a standard formulation from Allnex for clear coat application, described in Table V-4. Various common additives, such as defoamer, thickener and cosolvent were used in concentrations ranging from 0.3 to 4.0 wt% with respect to binder amount. Additives were added to the binder one by one following the order of the table from left to right and agitation was carried out at the end using a high speed mixer at 3000 rpm for 5 minutes.

Table V-4. Additives type, name and amount for the coating formulation of products A and B

Additives	**Cosolvent**	**Cosolvent**	**Defoamer**	**Surfactant**	**Surfactant**	**Thickener**	**Water**
	Dibutylglycol	Dowanol PnP	Tego foamex 800 sol	Surfinol 104 DPM	Byk333 sol	Borchi Gel L75N sol	
Amount %wt based on binder	4.0%	1.4%	0.3%	0.5%	0.3%	0.3%	6.8%

V- 3.3.2. Paint evaluation

The resulting paints containing sugar-based latex binders were evaluated in terms of gloss, haze and chemical resistance and compared with the corresponding reference product from Allnex (LA-MF0 and LB-MF0). Formulated dispersions were applied on glass substrates using a barcoater (120 µm wet film thickness) and dried at 22 °C and 50 % of humidity.

Films hardness was measured, showing a typical increase upon drying time. For products of type A (Figure V-14), increasing the amount of methacrylate fructose in the ASR induced an increase in films hardness, likely due to the stiffness of sugar units in the chains. For fractions of 60 wt% and above (of MMA substituted in the ASR) hardness reached a maximum. Still, values of 110 s were achieved for sugar-containing paints whereas the reference paint exhibited a hardness of 70 s.

Films from type B products showed good hardness with values oscillating between 78 and 97 s. However, no direct correlation with MF content could be seen to explain such differences. Most likely, measurements disparity is due to potential morphology differences. In fact since both processes involve the use of two different phases, namely hard and soft, composition heterogeneity is expected within the polymer particles. Although morphology was not studied here, the contrast between products A and B in terms of hardness evolution with respect to MF content, suggests that polymer microstructure of products A and B was significantly different.

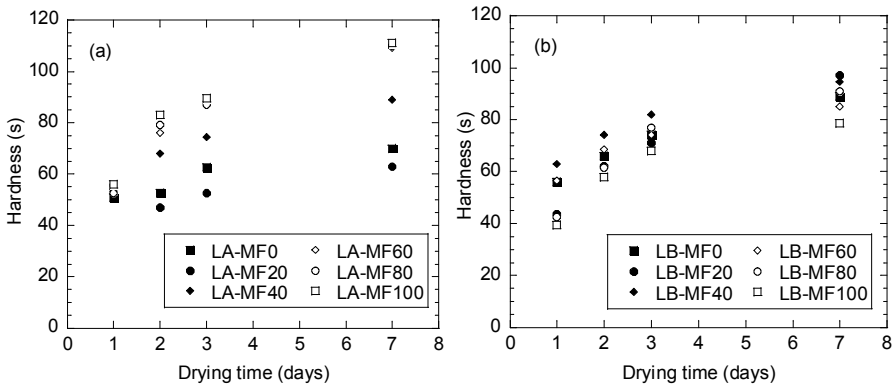


Figure V-14. Evolution of thin film hardness along drying time for products of (a) type A and (b) type B

Films were casted on black and white sealed opacity chart from Leneta.[27] Gloss measurements were performed at a 20° angle, which is the common angle of incidence for highly glossy films. Average values were calculated from five repetitions per sample. Figure V-15 shows the results obtained. The incorporation of 100 wt% of MF with respect to MMA in product A induced a decrease of gloss from 69 to 62 together with an increase in haziness. In fact haziness can be a typical indication of incompatibilities between the binder and foreign matter or a sign of bad film formation. Here the suspected shear sensitivity of sugar-containing latexes mentioned earlier could explain their lower performance. Indeed paint agitation for additive incorporation could generate microcoagulums, i.e. aggregated particles responsible for film defects.

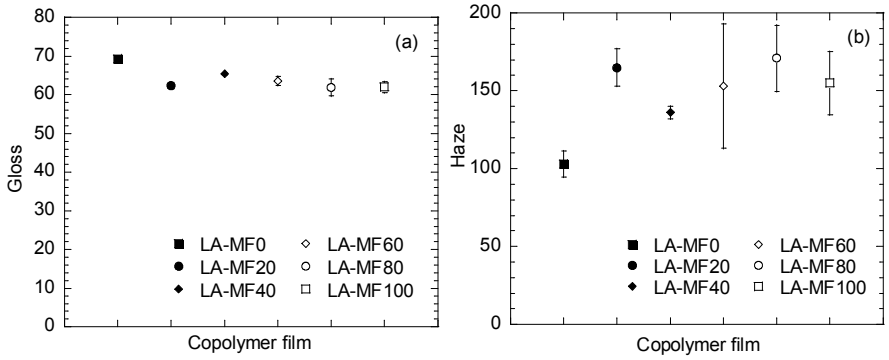


Figure V-15. (a) Gloss (20°) and (b) Haze measured on thin films from products A containing from 0 to 100 wt% of MF instead of MMA in the hard phase

Gloss and haze measured from products B paints are plotted in Figure V-16. As opposed to products A where a clear trend was observed regarding the effect of MF incorporation, the high gloss of product B is maintained in all cases. Besides bio-based films haziness are in a comparable range with the reference.

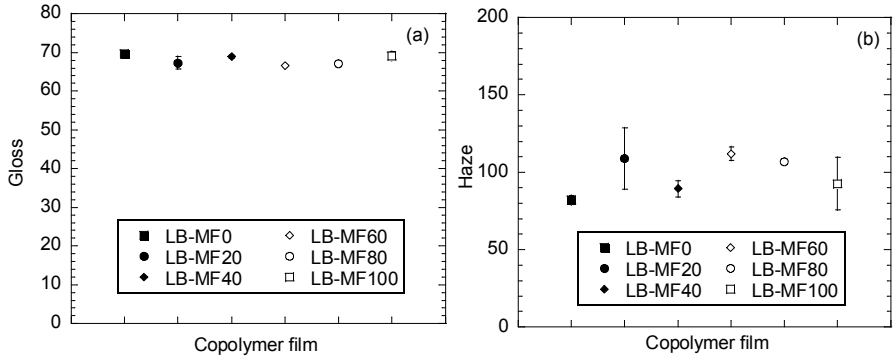


Figure V-16. Gloss (20°) and Haze measured on thin films from products B containing from 0 to 100 wt% of MF instead of MMA in the hard phase

Chemical resistance of the films against ethanol, water and coffee was also tested (See details in Chaper IV). Films were prepared on Leneta test cards and dried during 7 days. The "spot test" was carried out by depositing drops of liquids on top of the film.

In general very good chemical resistance of paints from type A products was obtained from spot test independently of bio-based content as can be seen on Figure V-17. Although a minor decline of ethanol resistance was obtained when MMA was fully substituted, MF content does not significantly affect coffee or water resistance.

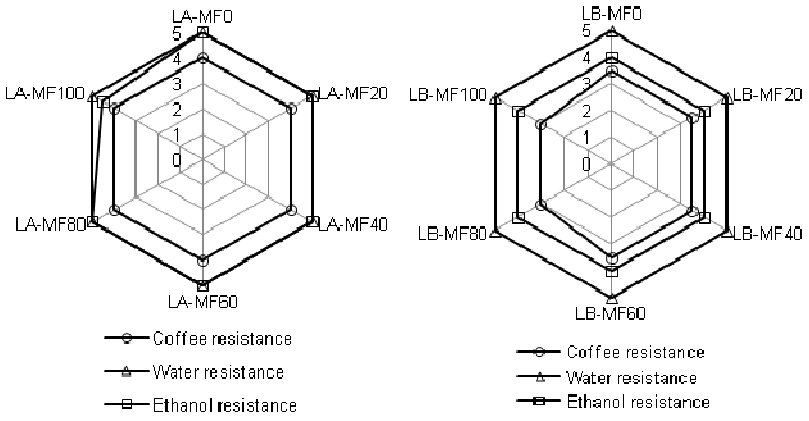


Figure V-17. Chemical resistance of clear coats from products of type A (up) and B (down)

Adhesion tests on wood samples were also carried out following a standard method.[28] Two layer coats were applied on an oaks wood panel. Smooth sanding was performed between the two applications. The cross-cut test is a method for determining the resistance of paints and coatings to separation from substrates by utilizing a tool to cut a right angle lattice pattern into the coating, penetrating all the way to the substrate. An X-cut is made through the film with a carbide tip tool to the substrate. Pressure-sensitive tape is applied over the cut. Tape is smoothed into place by using a pencil eraser over the area of the incisions. Tape is removed by pulling it off rapidly back over itself as close to an angle of 180°. Adhesion is assessed on a 0 to 5 scale (0- Greater than 65% area removed & 5 is 0% area removed).

Results are given Figure V-18 in terms of marking and visual appearance of the paints is presented in Figure V-19. Although glossy clear coats are difficult to evaluate because of their transparency, it can be seen that paint integrity was not altered.

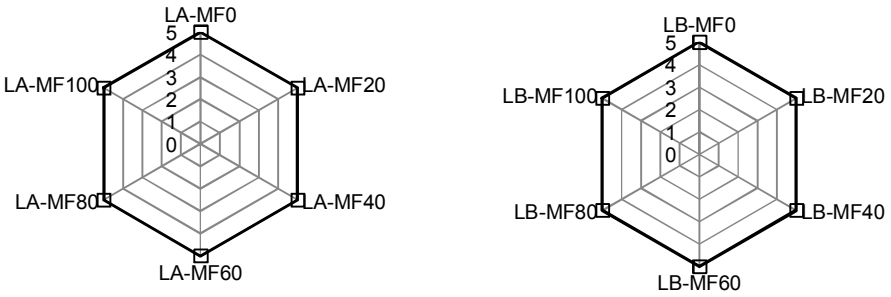


Figure V-18. Adhesion of clear coats on Wood panels from products of type A (left) and B (right)

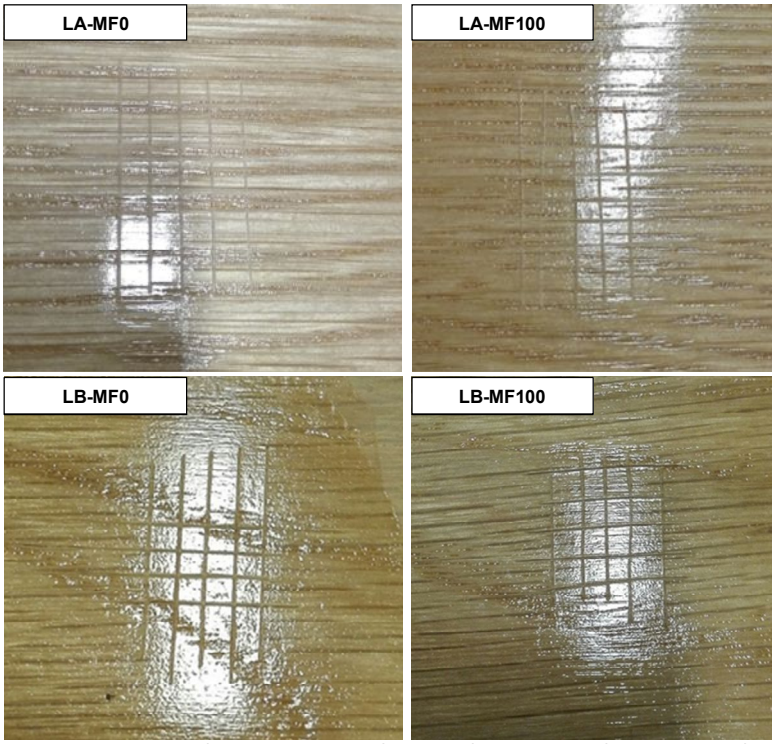


Figure V-19. Film appearence after adhesion test for the reference paint from Allnex (left) and a paint where MMA was fully substituted by MF (right). Products of type A.

V- 4. ASR STABILIZED LATEXES CONTAINING METHACRYLATE GLUCONIC ACID (MGA) AND METHACRYLATE FRUCTOSE (MF).

A second sugar-based methacrylate monomer, based on gluconic acid was used as comonomer in the synthesis of sugar-based ASR-stabilized latexes. Products of type A were prepared following the formulation given in section V-2.2. The structure of MGA is given in Figure V-20. MGA is a linear hydrophobic sugar-based monomer with acetyl protecting groups, making the molecule hydrophobic enough to be considered in emulsion polymerization. Homopolymer from MGA exhibited a glass transition temperature around 30ºC (cf Chapter II) which is relatively close to BMA homopolymer Tg (around 20 °C). Therefore, in this section the substitution of BMA by MGA in ASR will be investigated. The nomenclature used to reference synthesis runs will follow the same code given in section V-3.

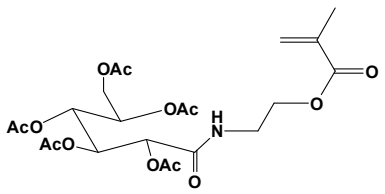


Figure V-20. Methacrylate gluconic acid monomer structure

V- 4.1. ASR synthesis

Two different ASRs were synthesized. In one case 50 wt% of BMA was replaced by MGA and in the other case MGA was incorporated in combination with MF to respectively substitute 100 wt% of BMA and 40 wt% of MMA. In addition, a reference run, using the conventional

monomers was carried out. It is important to note here that considering the states of MGA and MF, that is to say a caramel-like solid and a solid respectively, it was difficult to incorporate more than the amounts described above in the monomer mixture.

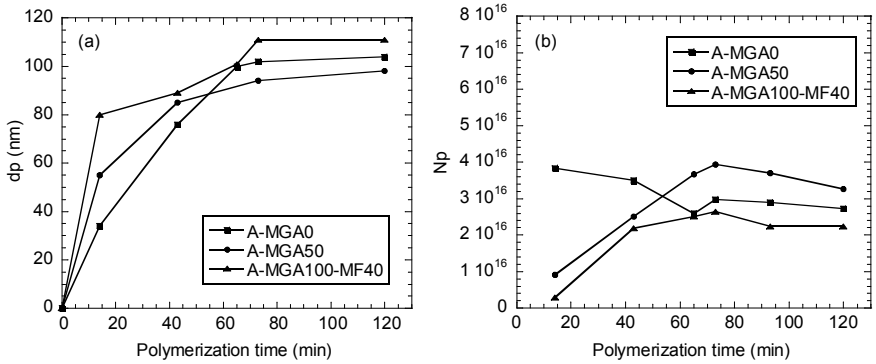


Figure V-21. Synthesis of ASR, product A: Particle size evolution

Figure V-21 shows the evolution of polymer particle sizes along the polymerization of ASR containing sugar-based monomer. The run performed with conventional monomers was also represented as reference (A-MGA0). Particle diameter was essentially larger when incorporating bio-based monomers, in particular at the beginning of the polymerization. This was mainly due to the more difficult dispersion and homogenization of high viscosity monomers (here MGA and MF). Indeed, at the beginning of the reaction, the number of particles was lower for the runs performed with bio-based monomers for the same reasons. Still, final particles sizes are in the same range of values around 100 nm. High conversions were achieved in all cases (Figure V-22), independently from monomer type.

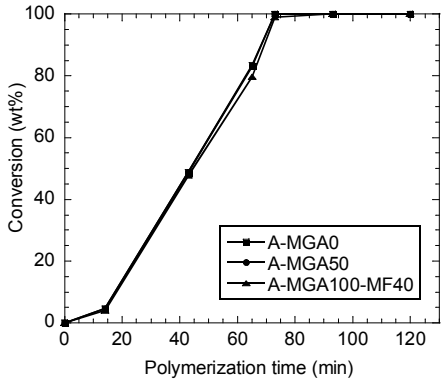


Figure V-22. Overall conversion of ASR synthesis vs polymerization time

Quite significant amounts of material were accumulated around the stirrer in this particular study, and this coagulation led to a decrease in final solids content from 30 as targeted to 28 wt% (Table V-5). Resulting ASR molecular weights were measured and values were in reasonable agreement with the results reported in paragraph V-5.1. Similarly, lower molecular weights are resulting from sugar-based monomer incorporation as explained by the variation in CTA/monomer molar ratios.

Table V-5. Product A: ASR solution characteristics

Run	A-MGA0	A-MGA50	A-MGA100-MF40
Mw (g/mol)	17000	9900	11200
Đ	2.0	1.9	2.0
SC (wt%)	30	27	28

V- 4.1.1. Preparation of ASR-stabilized latexes

In a second step, the polymerization of a soft phase using the resulting ASRs as polymeric stabilizers was carried out. Monomers consisting of a soft phase were added to the neutralized ASR solutions and left to stabilize under agitation while swollen aggregates would be formed. Figure V-23 reports the evolution of polymer particle sizes after polymerization was initiated. The substitution of 50 wt% of BMA by MGA did not affect particle size nucleation and growth along polymerization time as compared with the reference (LA-MGA0). In contrast, the incorporation of a high amount of MGA combined with the use of 40 wt% of MF instead of MMA shows a substantial effect on aggregate size and thus final particle diameter. As mentioned above, since the size of polymeric aggregates is closely related to their intrinsic hydrophobicity/hydrophilicity, it was expected that a significant variation in composition as it is the case in run LA-MGA100-MF40 where polar sugar units are incorporated, involved particle size changes. Nevertheless, the overall evolution of particle diameter over polymerization time remains in agreement with the tendency observed for the reference. Besides, in all the cases, the number of particles was kept relatively constant, indicating polymerization was occurring as wanted in initial polymer aggregates.

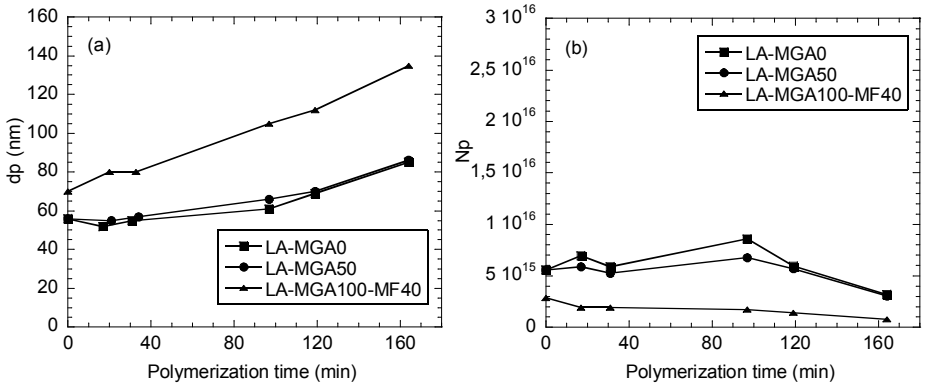


Figure V-23. Product A: (a) polymer particle size and (b) number of particle evolution during the 2nd step of the process

V- 4.2. Paint formulation and evaluation

The latexes obtained from these three runs were formulated for clear-coat application, following a common formulation from Allnex, given in Table V-4. Coating properties including hardness, chemical resistance, gloss, haze and adhesion were tested.

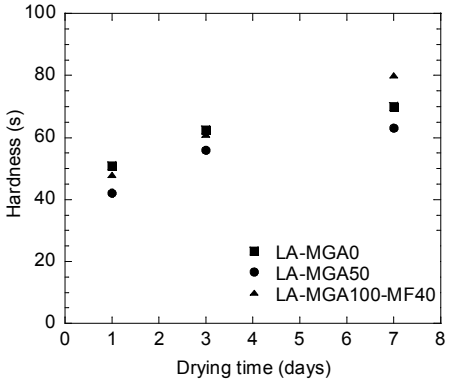


Figure V-24. Evolution of thin film hardness along drying time

The incorporation of 50 wt% of MGA instead of BMA in the ASR implies lower film hardness with respect to the reference (Figure V-24). Glass transition temperature is one of the main factors potentially affecting film hardness. However poly(MGA) has a slightly higher Tg than poly(BMA), 30 and 20 °C respectively. Given that partial coagulation occurred during reaction, real composition of the copolymers cannot be ensured and as a result theoretical Tg cannot be speculated. Moreover, particles morphology is meant to play a crucial role in film properties, especially hardness and mechanical strength. Herein no information could be given about particle morphology but we believe that the nature of the sugar-based monomers may affect ASR behaviour, in corroboration with aggregates sizes evolution seen above. This phenomenon could result in morphology variations but no conclusion could be made.

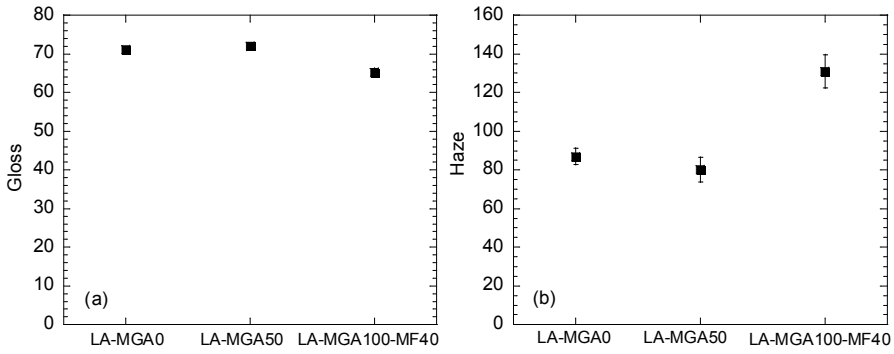


Figure V-25. (a) Gloss and (b) haze measurement from product A, where MGA and/or MF were incorporated

Gloss and haze of the formulated clear coat are shown in Figure V-25. Good properties of the ASR-stabilized latexes are maintained when only 50 wt% of BMA is substituted by the sugar-based monomer. In fact the incorporation of MGA as half fraction of BMA leads to a

slight decrease of film haze. However the combined use of MGA instead of BMA and MF (40 wt% of MMA) negatively affect the paint gloss and its haziness.

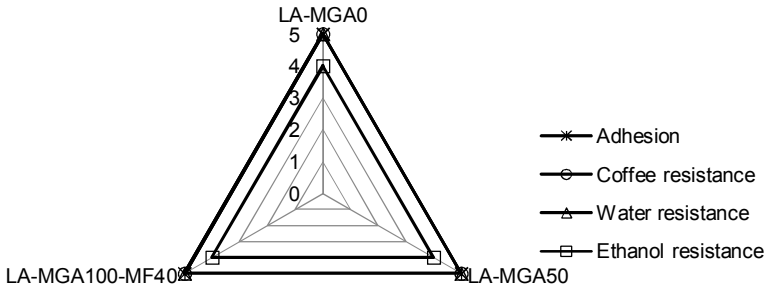


Figure V-26. Chemical resistance and adhesion performance of paint films from products A

Interestingly, paints containing bio-based monomer exhibited good chemical resistance and adhesion. Performance of Allnex product A reference are maintained (Figure V-26).

V- 5. CONCLUSIONS

In this chapter the use of two different bio-based monomers coming from carbohydrate resources and designed to be able to polymerize in emulsion polymerization was investigated. High value-added binders from Allnex Company were selected as reference to carry out synthesis where petroleum based methyl methacrylate was incrementally substituted by methacrylate fructose. Up to 100 wt% of MMA was replaced in the formulation of two types of ASR-stabilized products. Additionally, BMA was replaced by methacrylate gluconic acid. Final binders with bio-contents of about 45 wt% based on total monomer content were produced. As

an overall, the reaction performance of the ASR stabilized latexes containing the sugar-based monomers was similar to the petroleum-based counterparts. Some polymer accumulation around stirrer were observed, suggesting the sensitivity of the sugar-containing systems to shear. Indeed, sugar-based monomers are more viscous than their conventional homologue and as a result agitation during polymerization is an important aspect to consider.

The new waterborne bio-based polymers were incorporated as binders into paint formulations. Their performance properties in terms of hardness, gloss, haze and chemical resistance were observed to be similar to a commercial clear-coat waterborne paint. Therefore, the present results showed again the potential applications for sugar-based feedstocks.

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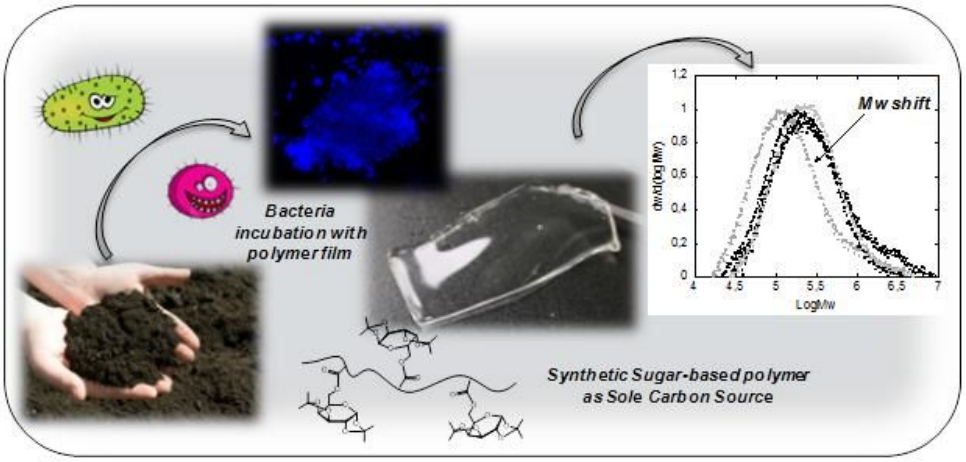
Chapter VI- Biodegradability Assessment of Sugar-based Polymers by Bacterial Strains isolated from Soil and Activated Sludge



VI- 1. INTRODUCTION

Chemically synthesized polymeric materials have become an important part of our human society, since they meet the need of a great variety of applications that enhance the quality of the life, such as in the field of agriculture, electric and electronic, automotive, building and construction or packaging. Indeed, approximately 310 million tonnes of synthetic polymers are produced worldwide each year.[1] However, they present disposal problems when their usefulness ceases.[2] This is the case of most commodity polymers. For example, in Europe, around 25 million tonnes of post-consumer polymer waste was ended up in the waste upstream, from which 30.8% went to the landfill. This announces a potentially huge environmental accumulation and pollution problem. On the other hand, many polymeric materials are widely used because they have long persistence times, and usually well beyond the intended practical lifespan of the material. For instance, in outdoor applications, polymers are selected, among other properties, for their resistance to the attack by microorganisms. So, industrial polymers are now source of problems related to their biostability in connection with the concept of time-limited applications after which material become waste.[3,4]

In parallel, newly developed polymeric materials are emerging each year, and some of them jump from R&D to industrial scales and commercialization relatively rapidly. It appears thus of crucial importance to start giving more attention to the degradable capabilities of materials at the very early stage of their design. In this regard, polymer biodegradation appears as a very interesting aspect. The international organization for standardization (ISO) defined biodegradable plastic as a degradable polymer in which the degradation results from the action of naturally-occurring microorganisms such as bacteria, fungi and algae.[5] More recently,

following IUPAC recommendation, the concept of biodegradable was rephrased as a qualifier for polymeric substances susceptible to degradation by biological activity by lowering of the molar masses of macromolecules that form the substances.[6]

Biodegradation of polymers involves the attachment of microorganisms to the surface of the material, followed by their growth utilizing the polymer as carbon source. Primary degradation of the polymer and its ultimate degeneration until complete mineralization can be achieved. This process occurs either under aerobic (1) or anaerobic (2) conditions, being the end products CO_2, H_2O or CH_4.

$$C_{polymer} + O_2 \rightarrow CO_2 + H_2O + C_{residue} + salts \quad (1)$$

$$C_{polymer} \rightarrow CO_2 + CH_4 + H_2O + C_{biomass} + C_{residue} + salts \quad (2)$$

Further, macromolecules biodegradation is governed by many variables, being the main factors polymer characteristics, type of organisms and nature of pre-treatment.[7] A general rule is that the closer the similarity of a polymeric structure to a natural one, the easier it is to be degraded and mineralized by microorganisms. Therefore, sugar-based polymers appear as potential biodegradable resources. A. Takasu and coworkers demonstrated that the inclusion of glycosylated monomers accelerated the biodegradability of poly(vinyl acetate)-based emulsions[8] and poly(vynil alcohol).[9] Also, Barros et al. showed partial biodegradability of copolymers based on sucrose derivatives and styrene by using a microbial fungi (Aspergius niger) culture method.[10]

On the other hand, soil and wastewater are commonly used as inoculum source in biodegradation standard test methods.[11,12] In many cases, however, biodegradation assessment result problematical because of low growth development processes and large time scales. In order to fasten the process, the microflora associated with the candidate polymers

must be isolated. Besides, the specificity of each bacterial specie in terms of growth conditions (temperature, pH, oxygen level, salt concentration, etc.) is a crucial parameter to consider when conducting biodegradation studies. As a result, all these aspects imply the requisite of studies on a case by case basis. Indeed literature displays a broad collection of investigations dealing with synthetic polymers, including polyurethanes,[13–15] polyethylene,[16–20] poly(vinyl-chloride),[21,22] poly(vinyl-alcohol),[23–25] but also natural ones, mostly poly(3-hydroxybutyrate) derivatives,[26–29] poly(hydroxyalkanoates),[30–32] polycaprolactame,[33] or poly(lactic-acid)[34–36] as well as blends from polysaccharides and synthetic polymers.[37–39] Each case involves the isolation, identification and incubation of selected microorganisms or yeast.

As a result, polymers biodegradability definitely appears as a multifaceted subject whose evaluation requires long-term studies. Difficulty lies on finding the appropriate bacterial strains as well as their optimum growth conditions together with consistent polymer chemical analysis and good relevance to practise relationship.

In that context, the aim of this chapter was to explore the capability of some randomly picked bacteria (present in the environment) to degrade given sugar-based synthetic polymers. To do so, polymer samples were inoculated with municipal sewage sludge as well as soil extracts. Biodegradation was assessed if microorganisms were able to develop and grow using the polymer as sole carbon resource. This work was carried out in collaboration with the group EEM (Equipe Environnement et Microbiologie) from the University of Pau (Université de Pau et des Pays de l'Adour - UPPA), in France.

VI- 2. METHODS

Biodegradability assessment requires the following steps: (i) choice of the polymer; (ii) selection of a minimal microbial growth medium; (iii) preparation of inoculums; (iv) culture preparation and incubation; (v) characterization. These steps are individually described below.

VI- 2.1. Synthetic polymers and medium selection

VI- 2.1.1. Polymer material selection

Among all polymers and copolymers presented in this thesis, a selection of four materials with distinct structures and properties was carried out. The homopolymer from methacrylate galactose (P, as a hydrophobic powder) and its hydrophilic deprotected homologue (D, as a hydrophilic powder) obtained by acid hydrolysis, were selected because of their close similarity to natural products. Still, synthetic sugar-based polymers differ from natural polysaccharides by the position of the sugar units as pendant side groups (Figure VI-1). This configuration can be expected to have an influence on the mechanism of degradation, although galactose units are meant to be good carbon resource for microorganisms. P was obtained from latex precipitation in alcohol while D was prepared from latex P by hydrolysis in THF with concentrated formic acid.

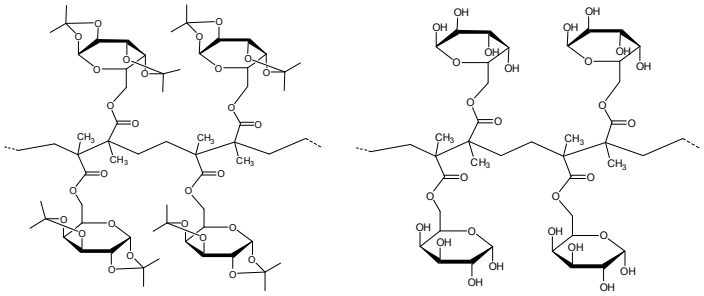


Figure VI-1. Molecular structure of poly(MG) P (left) and its deprotected form D (right).

In addition, a copolymer from methacrylate galactose and butyl acrylate, referred as F1 throughout this chapter, was tested as well as a MMA/BA conventional system (F2), which does not contain any renewable resource. The latter was considered as a reference. Both copolymers were evaluated as films, dried at ambient temperature. Table VI-1 summarizes their names and types.

Table VI-1. Polymer samples names and characteristics

Sample name	**P**	**D**	**F1**	**F2**
Composition	poly(MG)	deprotected poly(MG)	poly(MG-co-BA) 40/60	poly(MMA-co-BA) 50/50
Form	powder	powder	film	film

VI- 2.1.2. Culture media characteristics

A liquid minimal microbial growth medium, referred as M9 and carbon-free was used and contained Na_2HPO_4 12 g/L, KH_2PO_4 3 g/L, NH_4Cl 1.7 g/L, NaCl 0.8 g/L, $MgSO_4$ 2 mM and $CaCl_2$ 0.1 mM.

In addition agar powder (LB Agar, Lennox-cat N°1083) from Conda Lab was employed as solid culture media under the form of a gel swollen by distilled water. The standard formulation contained: Tryptone 10 g/L, NaCl 5 g/L, yeast extract 5 g/L, bacteriological agar 15 g/L.

VI- 2.2. Inocula preparation

Two inocula resources were used in this study, a soil sample and a municipal wastewater, also referred as activated sludge. The soil sample was purchased from a supermarket and presented the following characteristics: Solids content: 36 %; Organic content: 64 %; pH: 6.4; Conductivity: 45mS/m; Nitrogen: 280 g/m^3; phosphoric anhydride: 170 g/m^3; potassium oxide: 45 g/m^3. Isolation of its containing biodiversity was carried out by mixing 10 g of soil with 40 mL of M9 medium. Microorganisms were allowed to be extracted in solution and gentle centrifugation allowed the separation of soil particles. The resulting supernatant was considered as inoculum “S”.

Activated sludge used as inoculum resource was collected from San Sebastian sewage disposal plant. Sampling was done from re-circulating water (aeration tank), after oil-water separation step and before decanting period. A brief centrifugation was done to eliminate particles and supernatant was withdrawn to be used as inoculum, called “E”.

Figure VI-2 and Figure VI-3 show the microscopy images of inocula solutions from activated sludge and soil respectively. Presence of microorganisms was confirmed in both cases. Concentration of microorganisms can be achieved in terms of number of cells per field of vision (per image). Wastewater sample contained a much higher concentration of microorganisms. Indeed, although the soil sample contained quite a lot of soil particles (black

spots), an average of $3.9x10^6$ microorganisms per image was counted, while $1.3x10^7$ cells per image were seen for inoculums E. Further, most of the bacteria present in the wastewater sample are of bacillus type, whereas the soil sample showed more diverse morphologies.

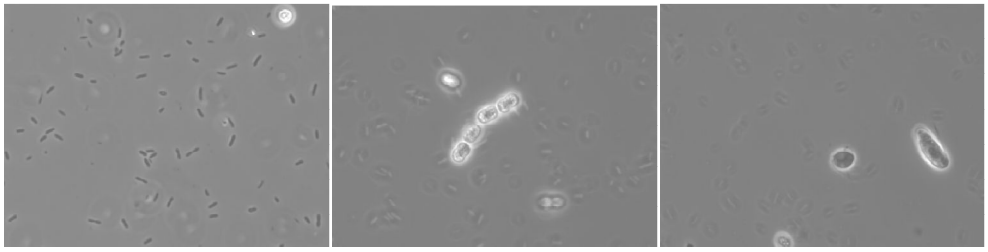


Figure VI-2. Optical microscopy images of a wastewater inoculum sample E (x 10 000)

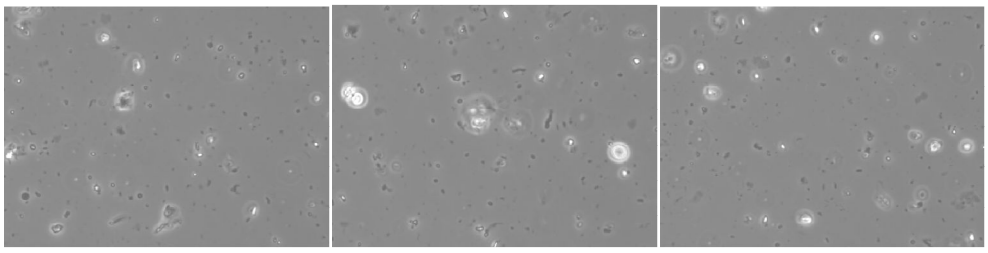


Figure VI-3. Optical microscopy images of a soil inoculum sample S (x 10 000)

Interestingly, natural contamination of the sugar-based freshly synthesized polymers was observed by TEM, as shown in Figure VI-4. Based on the experience of our laboratories, such significant presence of cells is very rarely observed in conventional monomer based dispersions. This result suggested a higher microbial sensitivity of sugar-containing polymers and greatly emphasized the need of biodegradation assessment in this work. From this point of view, it appeared interesting to also explore the route of natural biodegradation by incubating

samples devoid of specific inocula. Thus naturally-occurring microorganisms were given the chance to grow up in an extra culture, annotated with the suffix T.

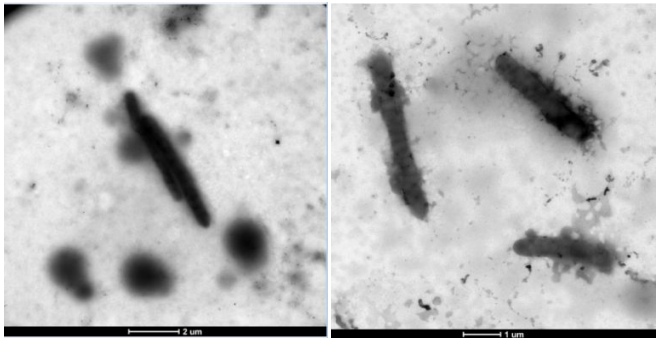


Figure VI-4. Transmission Electronic Microscopy (TEM) images of microorganisms observed casually in a latex of poly(MG) (left) and in a latex of poly(MG-co-BA) (right)

VI- 2.3. Incubation

Cultures were prepared from samples and inocula. Polymeric materials were sterilized prior to their use, except for the particular case of cultures T where natural contamination was considered as inoculum. Materials and bacterial suspensions (inocula) were added to tube containing M9 and were incubated for several days in aerobic conditions at given temperatures and agitation rates. Sampling was done at different time intervals. To facilitate this operation and to increase the surface contact area, the films were cut in small pieces (F1 & F2). D and P (deprotected and protected poly(MG) respectively) had a different behaviour in solution: D was a gel that tend to dissolve/swell or sink in the vial, whereas P is a floating hydrophobic powder.

Table VI-2. Cultures preparation

Inoculum	Wastewater (E)				Soil (S)			Natural contamination (T)			
Sample	D	P	F1	F2	P	F1	F2	D	P	F1	F2
Culture Name	**DE**	**PE**	**F1E**	**F2E**	**PS**	**F1S**	**F2S**	**DT**	**PT**	**F1T**	**F2T**
Sample weight (g)	0.25	0.50	0.53	0.52	0.50	0.51	0.52	0.26	0.50	0.50	0.48
M9 (mL)	17	31.5	31.5	31.5	32	32	32	17	33	33	33
Inoculum (mL)	0.75	1.5	1.5	1.5	1	1	1	0	0	0	0
Total volume (mL)	17.75	33	33	33	33	33	33	17	33	33	33
Sample concentration (mg/mL)	14.1	15.2	16.1	15.7	15.2	15.4	15.7	15.2	15.0	15.1	14.7

Table VI-2 reports the preparation of 11 cultures. Herein culture name will follow the following code where the first letter corresponds to the polymer sample (i.e. D, P, F1 or F2) and the second letter refers to the inocula (E, S, T).

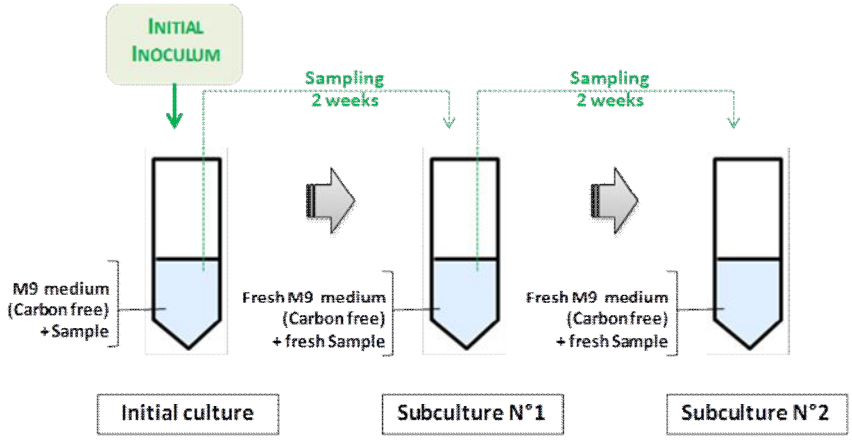


Figure VI-5. Enrichment culture method and serial dilution

As mentioned in the introduction, biodegradation is assessed by development of microorganisms with polymer materials as sole resource of carbon. In microbiology, to proof experimentally that a given product is the only carbon resource in a culture allowing the microorganisms growth, sequential inoculations are carried out. A subculture is made by transferring some cells from an initial culture to a fresh growth medium, containing fresh polymer (carbon source) as illustrated in Figure VI-5. This process ensures that microorganisms development is not due to the presence of impurities accidently introduced in the initial culture. It is important to note that the duration between subculture sampling strongly depends on the rate of microorganism growth and can be very different from one sample to another (2 weeks in the example given above).

VI- 2.4. Polymer characterization

After a given duration of incubation, polymer samples were withdrawn from culture media and analyzed by various techniques. Gel permeation chromatography in THF was performed for hydrophobic samples such as P, F1 and F2, using an Agilent chromatograph equipped with 3 columns from Shodex. Chains soluble in THF were extracted by soxhlet. Molecular weights from D samples were measured in water using a Agilent chromatograph.

The glass transition temperature (Tg) of the sugar-based polymers were measured by means of a differential scanning calorimeter, series DSC Q1000 (TA Instruments). The measurements were carried out in a range from -70 to 200°C at a heating rate of 20 °C/min.

Fourier Transform Infrared Spectroscopy (FTIR) measurements were performed on a Brüker Alpha-p FTIR spectrometer. Spectra were collected from 4000 to 250 cm^{-1} with the following settings: 42 scans per sample and spectral resolution: 4 cm^{-1}.

VI- 2.5. Methods for bacterial growth observation

Cultures were observed with an Olympus BX60 optical microscope equipped with a Qimaging (QI Click) camera. A drop of 10 µL withdrawn from culture medium was deposited. A factor specifically determined for this equipment was used to relate the number of cells per field image to the number of cells per volume unit of sample.

VI- 3. RESULTS: PRELIMINARY SCREENING

The interest of using soil and activated sludge for biodegradation assessment lies in a better mimicking of environmental conditions. In fact waste of polymers used as binders may end up in landfill or domestic wastewater systems as a result of a wrong disposal. Thus the choice of these inocula is relevant here. However the main drawback of it is the lack of information on the biodiversity composing the inocula, because consequently the conditions required to promote microbial growth are unknown. This is the reason why preliminary screening was carried out to identify, if possible, the better conditions to favour cell development. Given this, the absence of bacterial growth in any case can be exclusively correlated to the carbon resource inadequation.

VI- 3.1. Incubation at 20 °C

A first set of cultures was prepared according to the conditions given in section VI-2.3 using all 4 polymers, and were incubated at 20ºC without agitation. Spectrophotometry is commonly used in microbiology to follow optical density of liquid culture upon time.[40,41] In fact turbidity at 600 nnm wavelength is meant to highlight growth and metabolic activity of cells. Unfortunately here this technique was not suitable since the presence of polymer disturbed the measurement. Therefore, bacterial growth evaluation was followed by optical microscope. It is worthy to note that for a given inocula, culture preparation was rigorously identical, assuming the amount of microorganisms introduced in the cultures at time zero was equivalent for every material. Initial concentration of cells in the distinct inocula was different though. Cultures with different inocula may not be compared in terms of number of microorganisms.

Figure VI-6 shows the concentration of microorganisms per volume unit of culture observed in the samples withdrawn after 7 and 32 days of incubation at 20 °C. Interestingly after 7 days almost no microorganisms were remaining in cultures carrying MMA/BA films. After one month, the concentration of cells was found to have significantly decreased in all cases, concluding bacterial growth failure. This result indicated that either the bacterial strains present in the given inocula were not able to use the polymers as carbon resource or incubation conditions were not adequate, or eventually both.

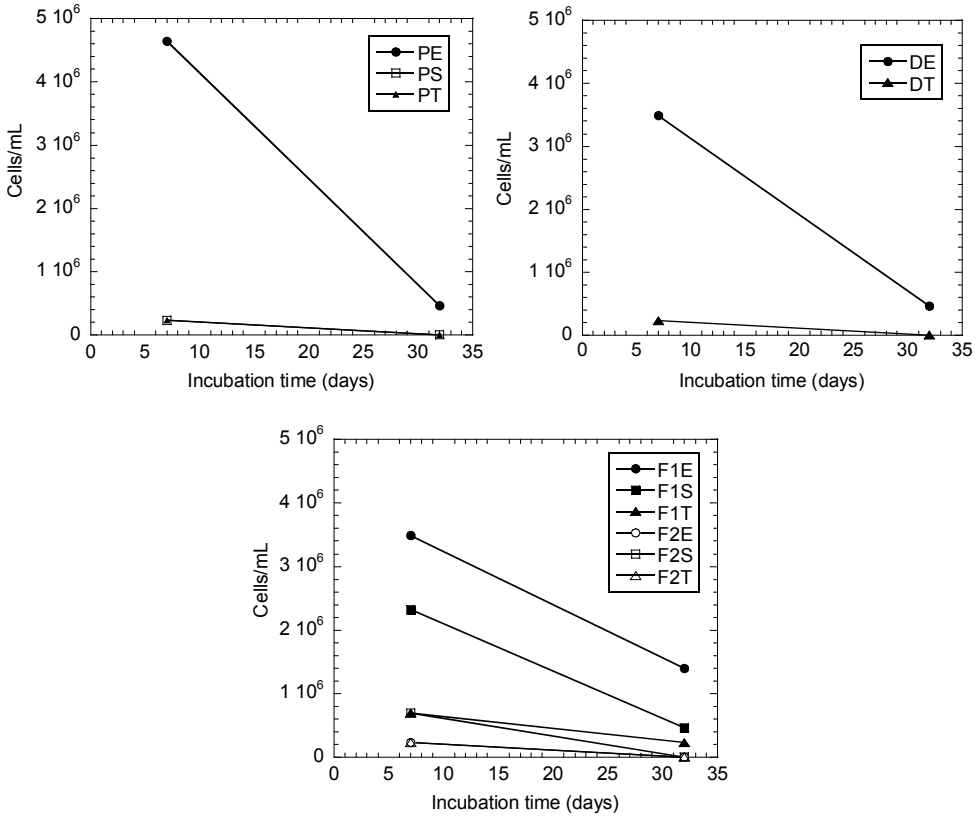


Figure VI-6. Observation of microorganism density by optical microscopy after 7 and 32 days of incubation for the initial cultures at 20°C.

VI- 3.1.1. Polymer analysis

Even though no obvious bacterial growth was observed in the culture, sampling and polymer analysis were carried out. ATR-FTIR spectroscopy was employed to track any change in polymers functional groups. As observed in Figure VI-7, the four incubated polymers showed the same functionalities before and after incubation at 20 °C for 7 days.

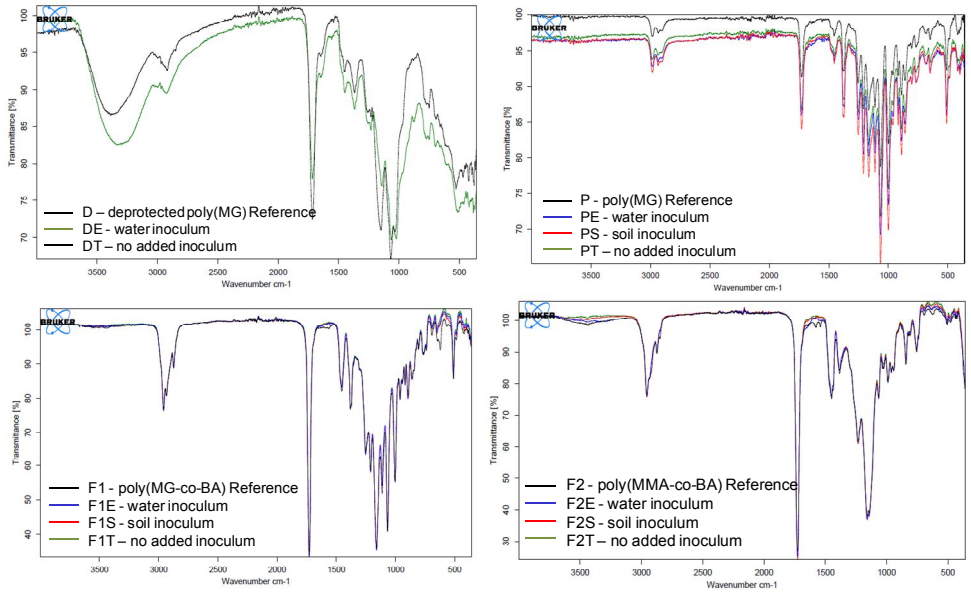


Figure VI-7. ATR-FTIR spectra of four polymers (D, P, F1 and F2), incubated at 20 °C for 7 days, with different inocula.

Polymer analyses are in accordance with culture observation. Indeed, incubation at 20 °C of the four type of polymers selected did not lead to bacterial growth neither to polymer degradation in terms of functionality modification.

VI- 3.2. Incubation at 30 °C, with agitation

Aiming at screening the potential conditions in which bacteria from inocula would develop, another set of cultures was prepared, using a temperature of 30 °C in combination with agitation of the tubes.

Samples D, P, F1 and F2 were incubated at 30 °C in aerobic conditions following the formulation described in Table VI-2. After 15 days of incubation, samples were withdrawn and observed by optical microscopy. Comparisons were made in terms of microorganism concentration.

As opposed to cultures at 20 °C, and based on the images given Figure VI-8, a quite important amount of microorganisms was observed in several cultures at 30 °C after 15 days, as revealed by Figure VI-9. Concentration of cells can exclusively be compared between samples for a given inocula, and not between inocula, given that initial bacterial density in soil and wastewater extracts were different. Still, it can be seen that microorganisms from inocula E developed in sample D in a much important extent than in sample F1. No growth of this inocula was observed in cultures carrying P though. Same trend was revealed for the inocula coming from soil. It is worthy to note that for the cultures containing P, cells concentration remained significant after 7 days of incubation in culture PT exclusively. Interestingly, this particular culture was the one containing natural contaminants. Furthermore, the species observed were of bacillus type and showed a peculiar tendency to “stick” together longwise, just like the species previously observed by chance by TEM (Figure VI-4). Finally, bacterial growth appeared to fail in cultures containing the fully petroleum-based copolymer film F2, independently of inocula type.

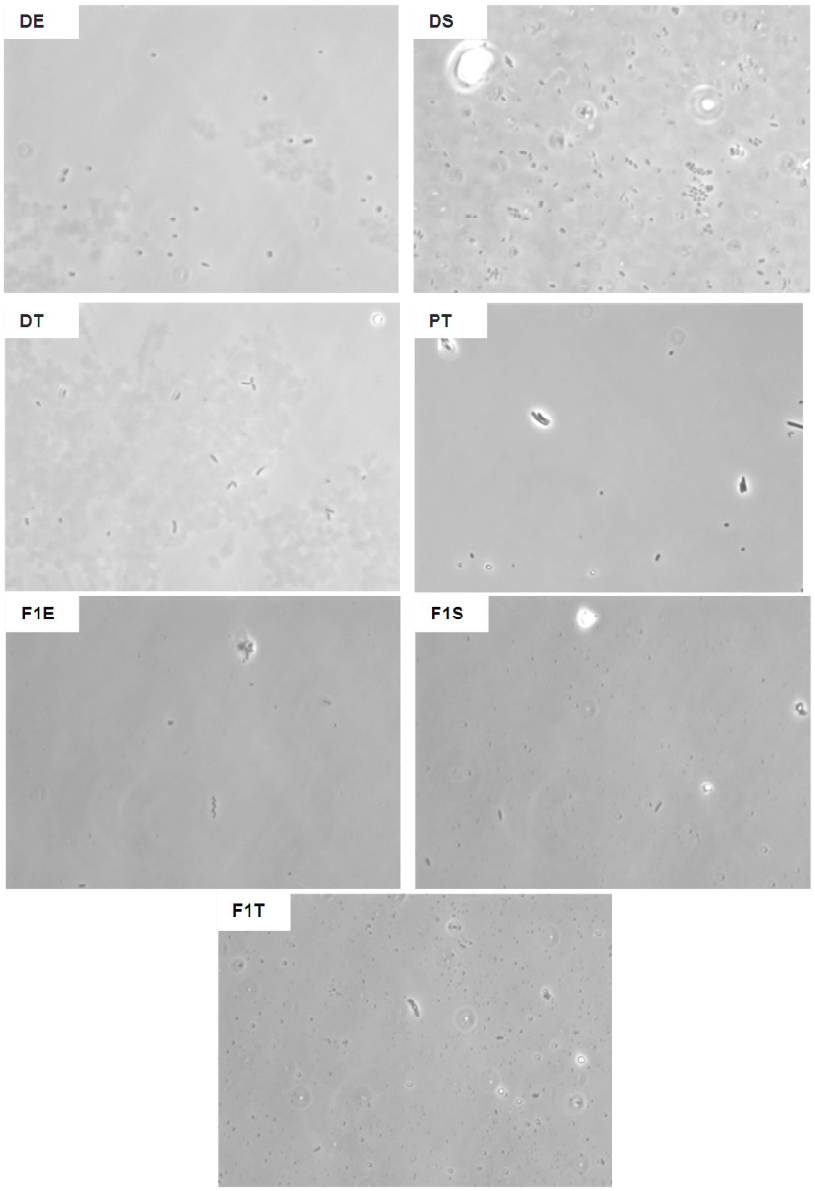


Figure VI-8. Optical microscope images from cultures incubated at 30 °C for 15 days.

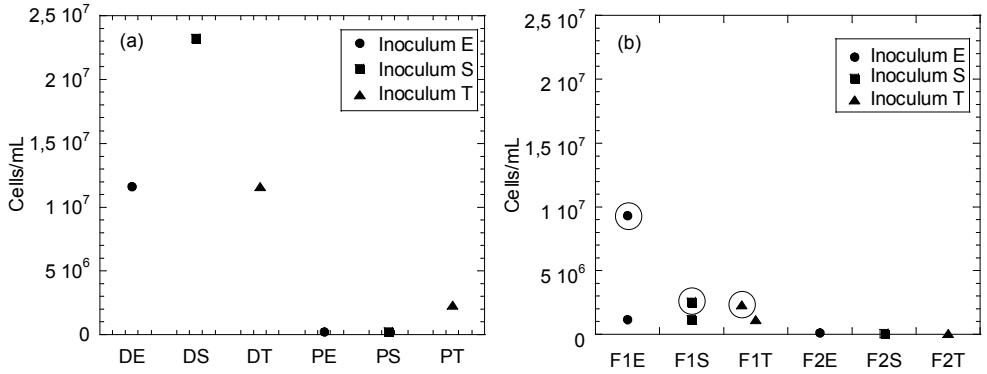


Figure VI-9. Cells concentration at 30 °C for (a) cultures containing D and P after 15 days of incubation, and (b) cultures containing F1 and F2 after 15 days and after 30 days (circled data points) of incubation.

Based on these preliminary observations, subcultures were incubated from DE, DS, DT and PT to confirm that bacterial growth was possible thanks to sugar-based polymer assimilation as sole carbon resource.

As mentioned before, each type of bacteria needs specific growth conditions and this can imply differences in growth rates. Given the lower amount of microorganisms in cultures containing F1, its incubation was extended to 30 days. Figure VI-10 shows the bacterial density observed after 30 days of incubation at 30 °C. A clear increase in microorganism number was observed, indicating bacterial multiplication within the time delay of observation. Subculturing was thus carried out also for F1.

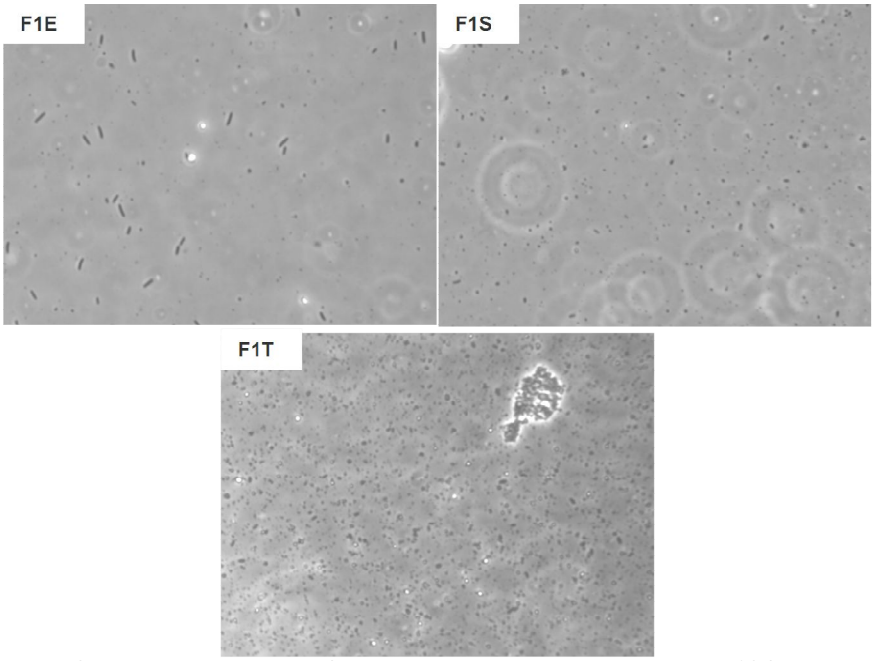


Figure VI-10. Optical microscope images from cultures containing F1, incubated at 30 °C for 30 days.

Figure VI-11 compiles the evolution of cells concentration per volume of culture determined by optical microscopy (Figure 12 and Figure VI-13) for the incubation of subcultures N°1 (S1). The outcome difference between cultures carried out at 30 and 20 °C is obvious. At 30 °C, a clear growth was concluded by observing a significant increase of number of cells during incubation.

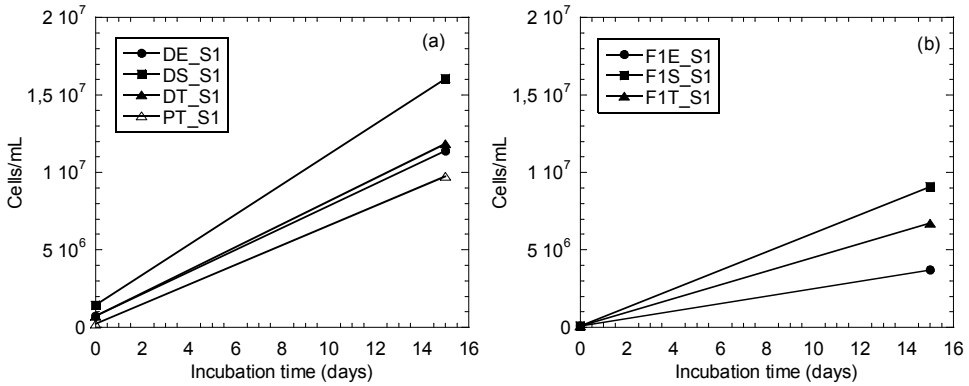


Figure VI-11. Evolution of cells concentration in subcultures S1 incubated at 30 °C for 15 days, containing (a) protected and deprotected MG homopolymer and (b) MG/BA copolymer

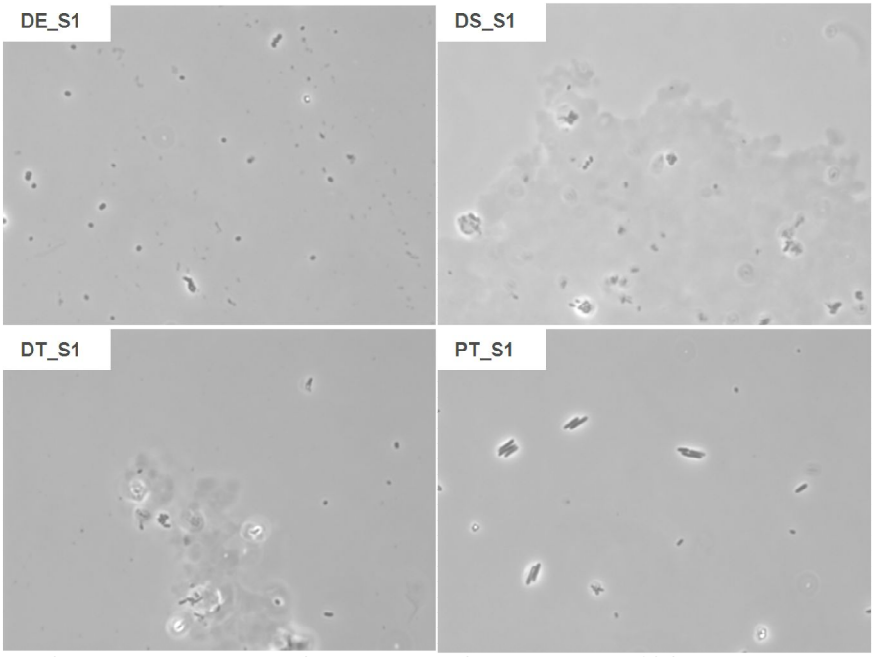


Figure 12. Optical microscope images from subcultures S1 incubated at 30 °C for 15 days.

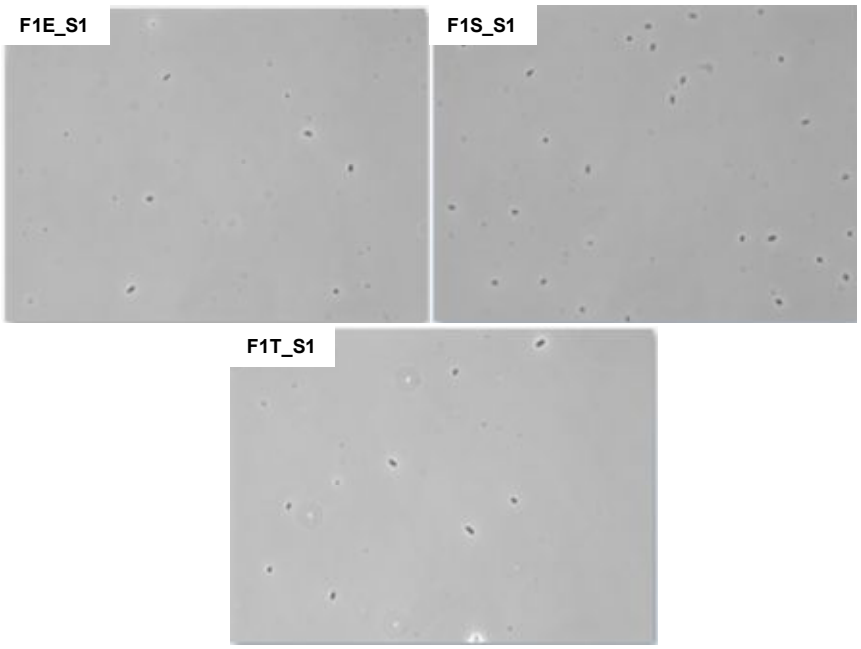


Figure VI-13. Optical microscope images from subcultures S1 incubated at 30 °C for 30 days.

To pursue the enrichment culture process, another dilution was carried out, resulting in subcultures S2 and incubated for 15 days, after which microscope observation was performed (Figure VI-14). Results are summarized in Figure VI-15. Again, clear evidence of bacterial growth was observed in samples DE, DS, DT and PT. Because of accidental technical issues, subculture N°2 for F1 could not be observed.

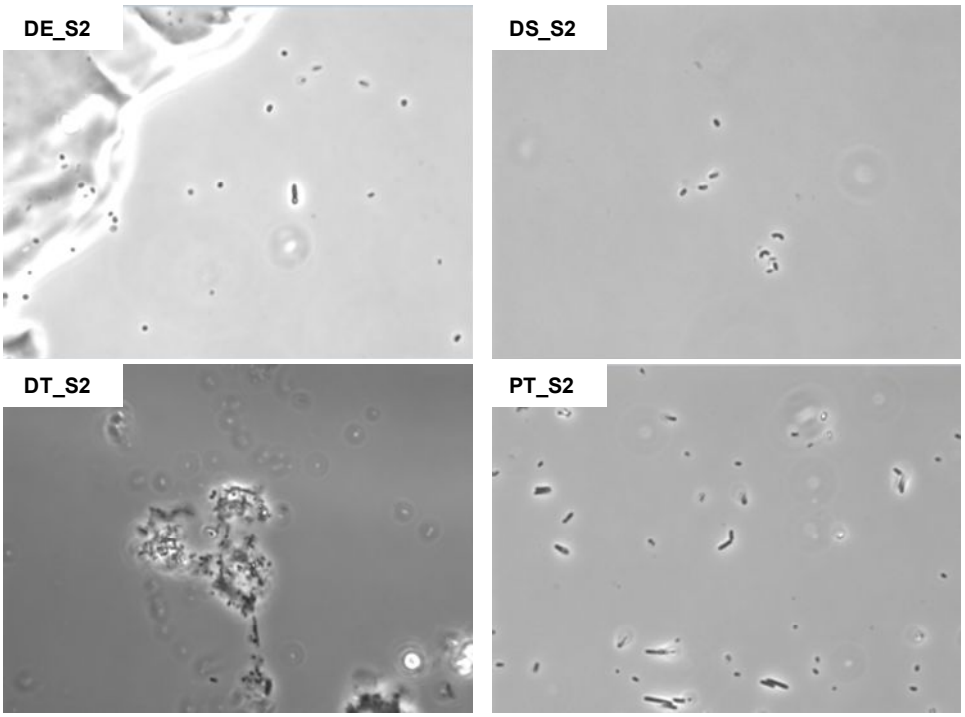


Figure VI-14. Optical microscope images from subcultures N°2 incubated at 30 °C for 15 days.

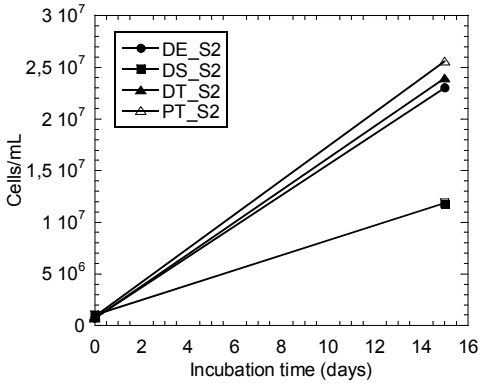


Figure VI-15. Evolution of cells concentration in subcultures S2 incubated at 30 °C for 15 days, containing protected and deprotected MG homopolymer

As first insight, these experiments showed that the bacterial populations present in some of the samples (i.e. DE, DS, DT, PT and F1) were actually able to use sugar-based polymers as sole carbon resource to grow up. Furthermore the type of microorganisms present in the samples were diverse: DE presented more spherical shapes, whereas DS, DT and PT consisted of bacillus type microorganisms. Generally the samples containing the highest amount of microorganisms both at 20 and 30 °C were the culture containing the hydrophilic deprotected galactose-based homopolymer D. This tendency was expected since this sample is the most similar in terms of molecular structure to natural polysaccharides.

VI- 3.2.1. Polymers analysis

Systematic polymer characterization remained difficult along the study, mainly due to material solubility issues in complement with limited amounts coming from small scale culture preparation. This is why in some cases, characterization could not be done.

Figure VI-16 shows the molecular weight distribution of samples incubated with different inocula at 30 °C for 15 days. A shift in mass was observed for samples DT (hydrophilic homopolymer with natural contaminants) and F1S (galactose-based copolymer films with soil inoculum), indicating the chemical degradation of polymer chains. The protected homopolymer from galactose did not show any change in molar mass after 15 days of incubation.

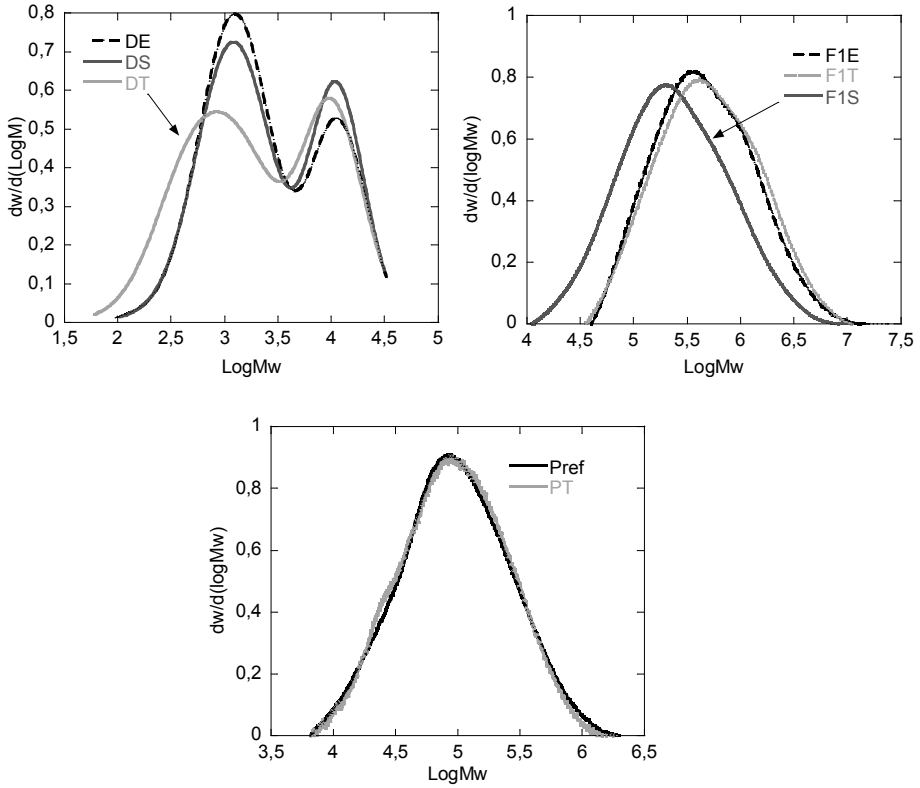


Figure VI-16. Molecular weight distribution of polymers incubated at 30 °C for 15 days

FTIR spectroscopy was employed to monitor any modification in polymers functional groups for P a(Figure VI-17). No clear evolution was observed. It is worthy to note that broadening of signals at 3500 cm^{-1} is most likely due to humidity in the samples.

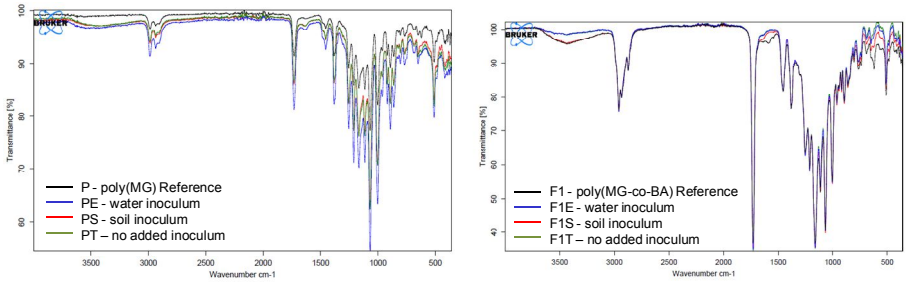


Figure VI-17. ATR-FTIR spectra of four polymers (P, F1), incubated at 30 °C for 16 days, with different inocula

VI- 4. ISOLATION OF BACTERIAL STRAINS, IDENTIFICATION OF ISOLATES AND INCUBATION

It is very important to point out that co-cultured microorganisms can create an ecosystem. A culture containing different species of bacteria showing growth does not necessarily indicate that all of the bacterial strains present in the medium are able to degrade the polymer. Indeed, some of them may be able to break down polymer chains (by enzymatic release eventually) and others could simply benefit of degradation sub-products. This effect was observed by Benedict et al. for the biodegradation of poly(caprolactone) when mixed cultures completely metabolized polymer breakdown products while in some cases pure cultures did not.[33] This is the reason why, isolating the cells present in subcultures is essential. Therefore, in this part of the work pure bacterial strain were isolated and then individually incubated with polymer.

VI- 4.1. Isolation of bacterial strains

Isolation of bacterial strains from subculture S2 (for D and P) and S1 (for F1) was carried out following the streak-plate technique of isolation, illustrated in Figure VI-18.

First, solid media were prepared by adding 3.5 g of commercial LB agar rich of carbon resources (tryptone and yeast extracts) to 100 mL of distilled water. The resulting mixture was sterilized by heating at 150 °C for a couple of hours. Hot mixtures were then poured in petri-dishes and left to cool down to form jelly uniform sterile media. Inoculation was carried out by adding 30 µL of culture medium from previously cited subcultures. Petri-dishes were then incubated at 30 °C. Streaking was carried out at different time intervals based on visual evaluation of bacterial growth.

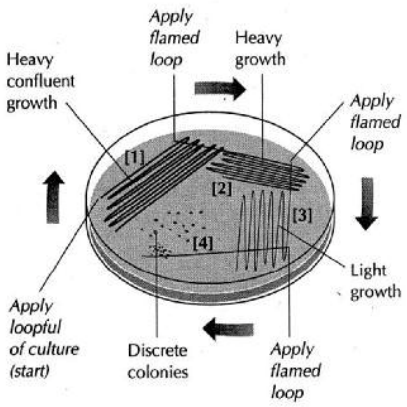


Figure VI-18. Streak-plate technique of isolation[42]

Pictures of the resutting petri-dishes are shown Figure VI-19. Eight bacterial strains were successfully isolated, namely two strains from DS, and one from each following culture DE, DT,

PT, F1E, F1S and F1T. The corresponding pure isolates were referred as LB- followed by the original culture name, as for instance LB-F1E for the pure strain isolated from culture F1E (containing sugar-based copolymer and wastewater).

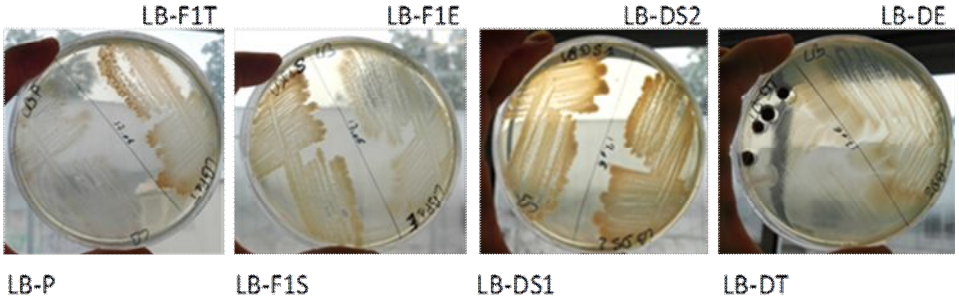


Figure VI-19. Bacterial strain isolation on LB agar solid media by streak-plate technique

VI- 4.2. Identification of isolates

DNA sequencing and phylogenetic analysis were performed by GATC-biotech Company, France. Basic local alignment search tool[43] was used in combination with reference sequence databases[44,45] to allow the identification of isolates. More details are given in Appendix 3.

Table VI-3. Identification of isolates

Isolate name	Identification	Phylum
LB P	*Pseudomonas veronii*	γ-*Proteobacteria*
LB F1S	*Alcaligenes aquatilis*	β-*Proteobacteria*
LB F1E	*Achromobacter aegrifaciens*	β-*Proteobacteria*
LB F1T	*Alcaligenes aquatilis*	β-*Proteobacteria*
LB DE	*Alcaligenes faecalis* subsp. *phenolicus*	β-*Proteobacteria*
LB DS1	*Brucella abortus*	α-*Proteobacteria*
LB DS2	*Ochrobactrum anthropi*	α-*Proteobacteria*

Isolates types and names are given in Table VI-3. All isolates belong to the *Proteobacteria phylum* (cf Appendix 3), a group including a wide variety of pathogens. Note that most of the works reported in the introduction involving both natural and synthetic polymers degradation also deal with *Proteobacteria* phylum species.

Three strains were isolated from soil: LB-DS1, LBDS2 and LB-F1S. Stem called LB-DS1, consisted of *Brucella abortus*. *Brucella abortus* is an intracellular bacterium that enters phagocytes, such as macrophages, in humans and in cows. This bacterium can remain alive outside the host without replicating depending on the exact conditions. This bacteria is classified in the group 3 from the 1994's decree published in France,[46] therefore this strain had to be destroyed and no further study could be carried out.

O. anthropi was isolated from culture DS. It is a highly versatile α-proteobacterium with ability to colonize an exceptionally wide variety of habitats, from hostile environments such as polluted soil[47], insects[48], animals and human.[49,50] *O. anthropi* strains are rod shaped, aerobic ; they can produce acid from several carbohydrates, and reduce both nitrate and nitrite. Holmes et al[51] reported the differentiation of *0.anthropi* from closely related and phenotypically similar genera: 56 strains were tested positive for the utilization of galactose.

The last strain isolated from commercial soil (from F1S culture) was *Alcaligenes aquatilis,* previously reported as sediments habitant in Germany.[52] Interestingly, the same bacterial specie was found in the non-inoculated culture containing F1 referred as culture F1T, suggesting that both populations could come from natural contamination.

Moreover, an additional strain of same genus (*Alcaligenes*) was isolated from culture containing D and inoculated with activated sludge (LB-DE). *Alcaligenes faecalis* subsp.

phenolicus were reported to belong to phenol hydroxylase and nitrite reductase groups.[53] They are well known to degrade poly(hydroxybutyrate) natural polymers.

Achromobacter aegrifaciens[54], isolated from F1E culture, belongs to the same family (*Alcaligenaceae*). Exemples of *Achromobacter aegrifaciens* strains, such as strain LMG 26852 was used in a microbial consortium, for the degradation of pesticides (such as carbofuran) from agricultural wastewater.[55]

Pseudomonas veronii was isolated from the non-inoculated culture containing P (poly(MG)). A broad range of strains coming from this species were reported to degrade several organic molecules, as for instance dioxin derivatives, salicylic acid, catechol,[56] pentachlorophenol (PCP)[57], and long chain alkylphenols.[58]

It is very important to underline that different strains coming from the same species can exhibit different metabolic capacities. In that sense, information found in literature has to be considered with caution.

VI- 4.3. Incubation of pure isolates

To verify the capability of isolated strains to show growth with polymer as sole carbon resource, their incubation was carried out, following the experiments set up in the previous section. An addition control culture was set up, devoid of any inocula. All polymer samples were sterilized before incubation, except for F1T, where natural contaminants were considered as inocula. Herein, more attention was focused on the sample consisting of MG/BA copolymer film.

VI- 4.3.1. Incubation at 30 °C, with agitation

Incubation at 30 °C, with agitation was repeated but using pure isolated strains instead of biodiverse inocula. Sampling and observation were performed at different time intervals of incubation.

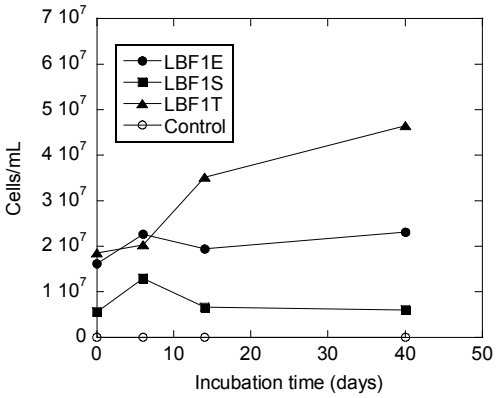


Figure VI-20. Planktonic cell concentration evolution along incubation time for the copolymer MG/BA.

Figure VI-20 shows the evolution of cells concentration in the cultures containing the copolymer film. On one hand, cells concentration from the culture carrying natural contaminants increased along incubation time. On the other hand, in the case of soil and wastewater inocula, microorganisms concentration increased rapidly within the first 7 days of incubation and kept constant during 1 month. This tendency could be related to two phenomena, either the absence of growth or the development of a parallel growth mechanism implying more aspects. Indeed, it is important to remind that sampling and observation carried out from cultures reflects the state of the liquid medium. A distinction has to me made between

planktonic cells (namely motion-free microorganisms in suspension in culture media) and attached cells, eventually consisting of a biofilm. A biofilm is defined as a layer of agglomerated cells showing adhesion to a given substrate. In fact, since the polymer substrate used in this work is not water soluble, most likely the stagnation of planktonic cells concentration is explained by the parallel development of microorganisms adhesion and accumulation on copolymer film surface, as represented in Figure VI-21.

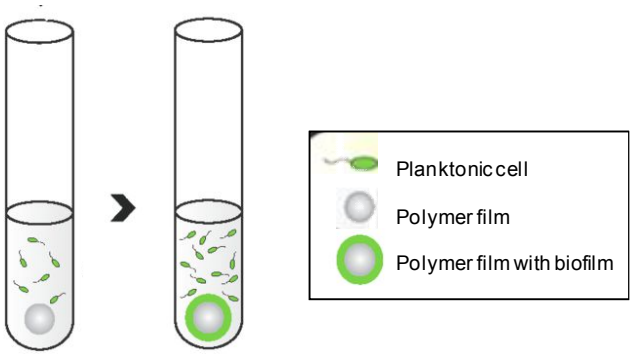


Figure VI-21. Schematic representation of cells concentration evolution during biofilm formation

Still, large amounts of planktonic microorganisms were observed in cultures, as shown for instance in samples withdrawn after 2 weeks of incubation, in Figure VI-22. Microorganisms were very mobile (sign of healthiness) and thus microscope focus to get neat image was difficult, hence the poor quality of the images.

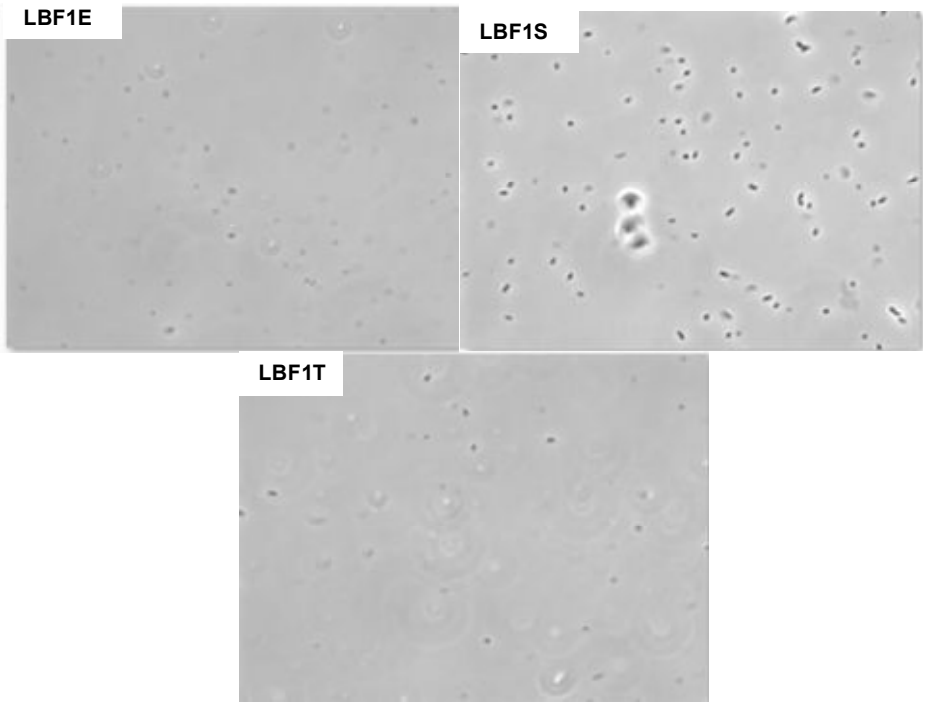


Figure VI-22. Microscope images of cultures inoculated from pure isolates at 30 °C with during 16 days.

VI- 4.3.2. Polymer analysis

Soxhlet and molecular weight of incubated films was carried out after 15 days. While no significant differences were observed in gel fractions, a clear shift of soluble polymer Mw distribution towards lower molecular weight was measurement for LBF1S. This observation is very important and corroborates the result obtained in the previous section. It is worthy to note that incubation time/degradation percentage ratio cannot be compared with the cultures previously prepared since the initial concentration of cells is different because cultures were prepared from different inocula. Still in both cases, the chemical degradation of sugar-based

copolymers was highlighted for isolate coming from soil. Average molar mass (in weight) was shifted from 313 000 to 282 000 g/mol, corresponding to a reduction of approximately 10 wt%.

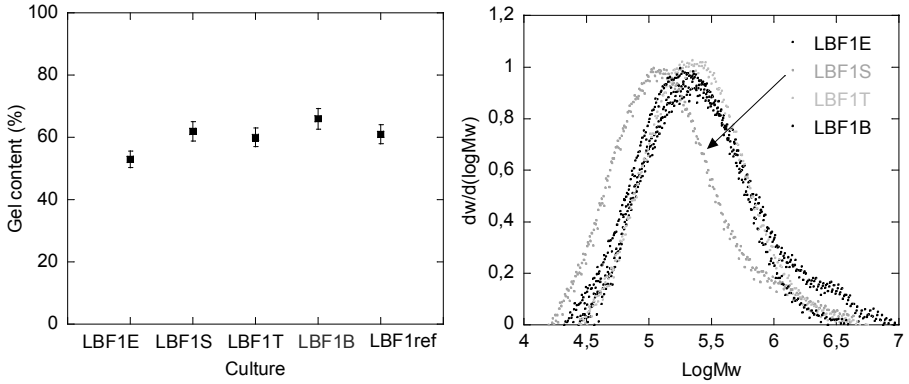


Figure VI-23. Molecular weight distribution of sugar-based copolymer films incubated with pure isolates at 30 °C for 16 days

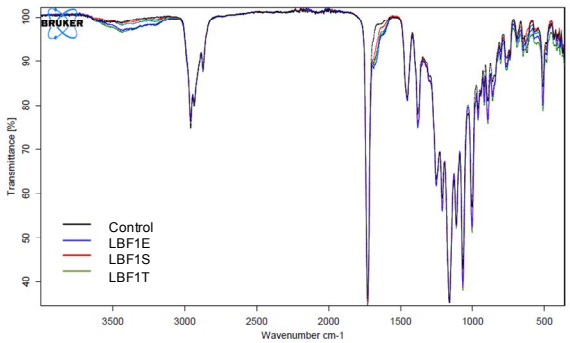


Figure VI-24. ATR-FTIR spectra of F1, incubated with pure isolates at 30 °C for 16 days

Additional characterization was performed, including FTIR spectroscopy (Figure VI-24). The appearance of a small carbonyl signal at 1750 cm^{-1} was observed in all the samples excep in the control cultures. In fact, shifting of carbonyl signal indicates a modification in the near environment of group, most likely coming from degradation mechanism. Carbonyl groups

generally result in high intensity peaks in FTIR, thus small modification of it in the sample are relatively easier to detect than any other. Further, it could be speculated that, modification of a small portion of carbonyl group environment happened in all the samples (except the control) but it was important enough to be quantified by GPC only in the case of soil inoculum. Slight evolution of the 3500 cm^{-1} spectra region was also observed in lower extent and was attributed to the presence of humidity in the sample.

No obvious changes could be noticed from the differential scanning calorimetry analysis between the different samples from cultures (Figure VI-25).

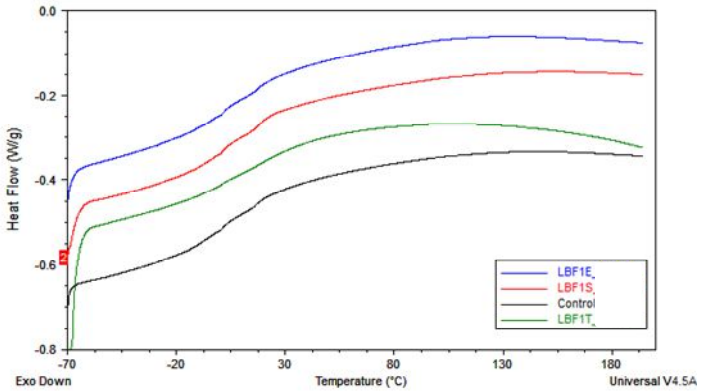


Figure VI-25. Differential scanning calorimetry of films incubated with pure isolates at 30 °C for 16 days.

The elucidation of the mechanism of degradation was not the purpose of this study. Still, it can be speculated that most likely sugar pendant units were detached from polymer backbone, leading small molecular weight carbohydrates as intermediate product. There exist various families of enzymes capable of specifically breaking down polymer chains.[59,60] Among them, esterases are a very wide class of enzymes, typically causing the cleavage of ester bondage.

They are involved in the biodegradation of poly(lactic acid) and polyhydroxyalkanoate for instance.[61]

The postulate according to which detachment of sugar pendant units by action of esterases enzyme occurred is greatly supported by the data obtained from GPC. Indeed, molecular weight changes implied a complete distribution plot shift, suggesting a global decrease of monomer units mass. In fact the alteration of polymer chain length, that is to say the cleavage of C-C boundaries would be expected to cause polymer molar mass distribution shouldering. The latter mechanism would involve the action of enzymes of laccase type.[62]

VI- 5. COLONIZATION AND BIOFILM FORMATION ASSAY

Bacteria require a variety of nutrients to develop, and among them carbon resources. The latter can take diverse forms in nature such as liquid or solid, dissolved or dispersed in a media as well as static surface. Metabolisms of bacteria can differ significantly between species but typically biological assimilation of nutriments is needed for the cells to function. Thus the use of long chain polymeric molecules as sole carbon source by microorganisms requests extra efforts. A general mechanism proposed in literature is the release of extracellular enzymes acting to break down polymer chains into digestible smaller units, eventually with improved solubility (Figure VI-26).[25,63,64]

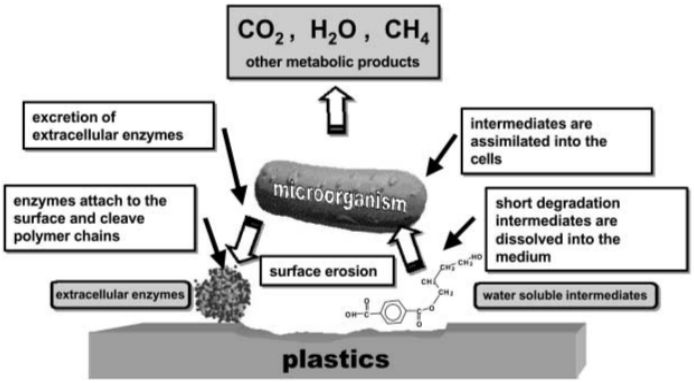


Figure VI-26. General mechanism of plastic biodegradation under aerobic conditions.[64]

An additional aspect to consider arises from the particular case of solid non-water soluble polymers. Indeed, to achieve the above described biodegradation process, bacteria often switch from a free-living lifestyle to a surface-adapted multicellular organization known as biofilm. In fact, in most habitats microbial populations tend to form biofilm on solid surfaces,[65,66] especially on polymeric materials.[64,67] The biofilm formation process can be divided into distinct phases, represented on Figure VI-27. First planktonic bacteria (cells in solution) attach to a surface. Next, the bacteria multiply, forming microcolonies and producing an extracellular matrix which consists mainly of extracellular polymeric substances. This phase is called the biofilm maturation. In the last stage, bacteria detach from the biofilm, spread and can cause new infections.

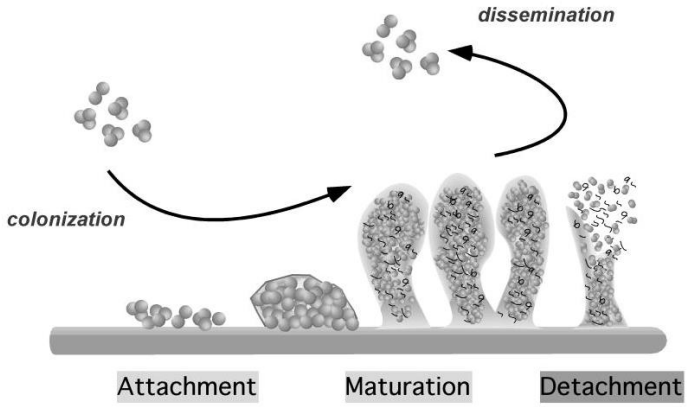


Figure VI-27. Schematic representation of biofilm formation steps[68]

These informations suggest that most likely sugar-based copolymer film biodegradations follow the route of biofilm formation for microbial growth. As a result, in this section colonization of the film surface was attempted in order to evaluate the capability of the isolated species to adhere on the polymer surface. Then, development of the biofilm was monitored by bacterial staining and observation in fluorescence microscopy.

VI- 5.1. First strategy: Visual monitoring of biofilm formation by Crystal Violet (CV) coloration

A common method to follow biofilm formation consists of inoculating polymer films under pre-determined conditions and staining the material surface at different time intervals with crystal violet(CV), also known as hexamethyl pararosaniline chloride. CV is a dye having a blue-violet colour with an absorbance maximum at 590 nm under regular pH conditions (here

slightly basic). Its strong coloration power results in an easy following of biofilm formation by simple naked-eye observation.

Sugar-based copolymer latexes were dried at ambient temperature directly in a 24-well plate. This process allowed obtaining an immobilized polymer film at the bottom of the wells with smooth, regular and comparable surface topography. Films were inoculated with the pure isolates LBF1S and incubated at 30 °C.

At different incubation times, coloration of the films was carried out using a protocol described in Appendix 3. It is important to mention that prior to coloration, the liquid medium is removed from the well and films are gently rinsed with distilled water to eliminate any planktonic cell. Figure VI-28 shows the resulting plate, where the first lines (up) represent triplicates of F1 incubation and coloring at different intervals. The bottom line consists of a blank experiment where no inoculum was added.

An increase in film color intensity was observed in triplicate cultures from 1 to 3 days of incubation. However no clear tendency was distinguished after these given times. Further, sugar-based polymer films were not inert against CV coloration. Indeed, polymer films got partially stained by CV, as notable in blank cultures, making the results more difficult to interpret.

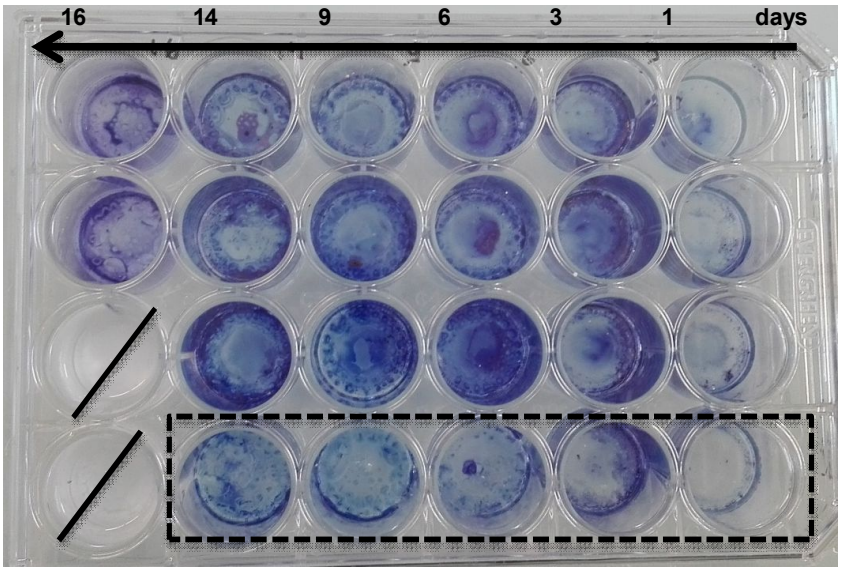


Figure VI-29. Biofilm formation assay with LBF1S pure isolate on fresh F1 samples in a 24-well plate. Picture after coloration by cristal violet dye.

VI- 5.2. Second strategy: Biofilm formation monitoring by Hoescht coloration

Sugar-based copolymer latexes were dried at ambient temperature in disc-shape silicon moulds of one centimetre diameter to result in 24 films of similar thickness, diameter and surface topography. Films were placed in a 24-well plate. The main difference with the previously described experiment is that films are not attached to the support. Still, they were allowed to drown in solution and stay static at the bottom of the wells. Inoculation was carried out with LBF1S strain and cultures were incubated at 30 °C.

Removal of planktonic bateria and staining of the attached ones was performed at different incubation time intervals. Several common dyes were tested, including Stylo 9,

propidium iodide, DAPI and Hoescht. Hoescht appeared to be the only stain allowing a selective coloration of bacteria in the presence of the film. All others were colouring the polymer surface, limiting microorganisms differentiation.

A commercial aqueous solution of Hoechst (10 mg/mL - Aldrich) was diluted to 10 μg/ml in M9. Culture media were first withdrawn from wells and the latter were gently washed with a M9 sterile solution to remove any planktonic bacteria. Dye solution was added in the wells (500 µL) and left to act for 15 to 20 minutes in the dark to avoid any dye photodegradation. Films were then removed from wells and carefully rinsed with M9 to remove any excess of dye solution. A very small piece of film was cut and placed between slide and slide cover for fluorescent microscope observation. Handling of the sample was delicate since polymer films were thin and not stiff. Besides, immersion of polymer films in aqueous media is well-known to cause material swelling and whitening. Since microscope observation was carried out from wet films,the quality of resulting images was rather poor.

Still, the clear presence of attached bacteria on sugar-based copolymer films was highlighted. Triplicate experiments were set up and all of them resulted in comparable observations. A qualitative increase of microorganism concentration on top of polymer films was observed after 3 days of incubation, indicating a rapid adherence of bacteria on material surface in the given conditions. Evolution of bacteria density after 6 days appeared insignificant. However, images recorded after 15 days of incubation revealed partial agglomeration of cells, suggesting the beginning of a biofilm formation and maturation (Figure VI-30). After 42 days of incubation, agglomerates were still present at films surfaces but no significant increase of their apparent area was noticed (Figure VI-31).

Indeed, the spacial structuration and organization of microorganisms on a particular surface are generally materials and microorganisms specific.[69,70] Many factors can affect the rate of biofilm formation, including material intrinsic characteristics,[71] surface properties, organism features,[72] or ambient environmental conditions.[73]

As such, biofilm formation is a complex phenomenon and in-depth investigation of bacteria/film interaction is not the purpose of this work. Nevertheless, experiments carried out definitely indicate a colonization of *Alcaligenes aquatilis* (LBF1) on galactose-based material. Adhesion of cells on polymer surface was demonstrated. Furthermore, the formation of biofilm can be speculated from the presence of cell agglomerates at the film surface after a couple of weeks of incubation.

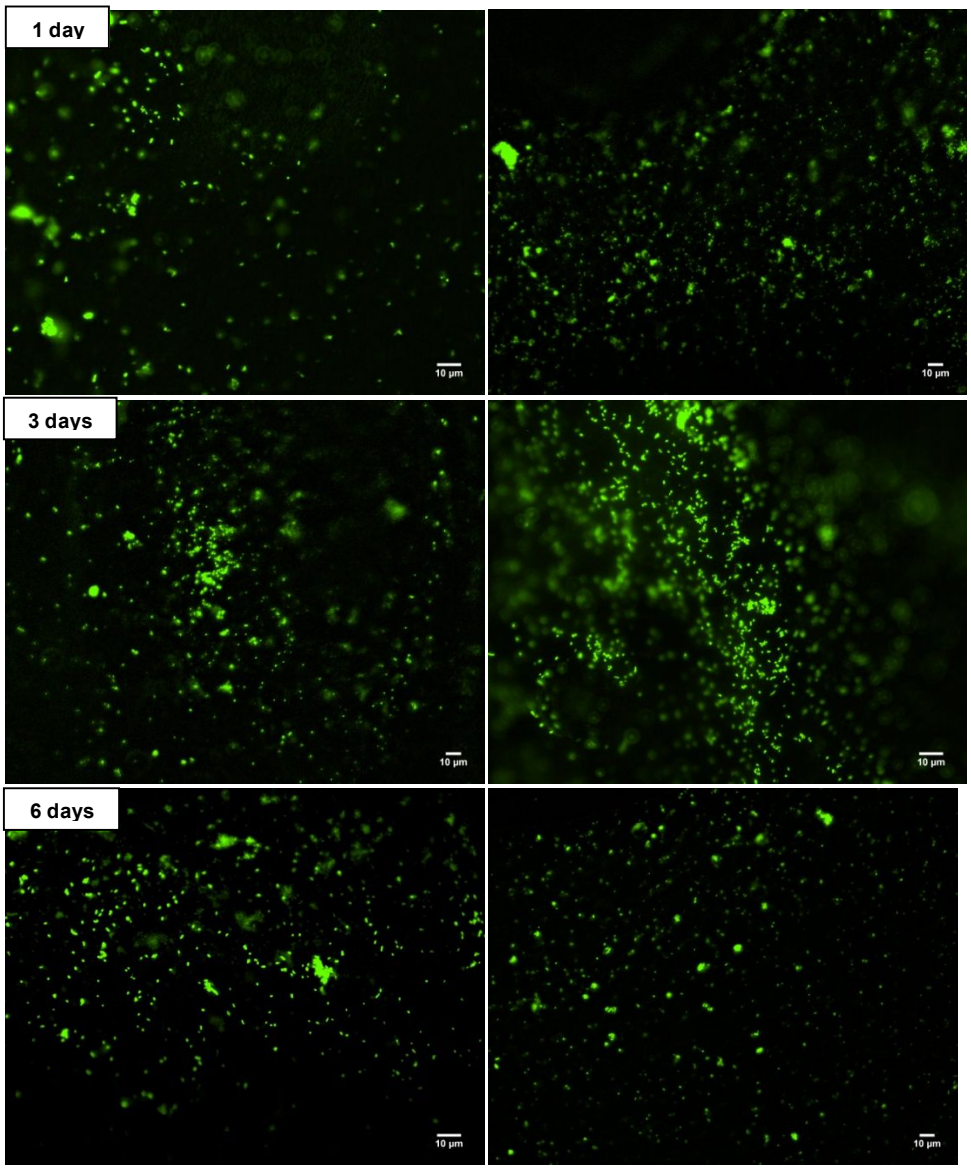


Figure VI-30. Fluorescence microscope images of stained microorganisms attached to the sugar-based polymer film. Observation after 1, 3 and 6 days of incubation with pure isolates at 30 °C.

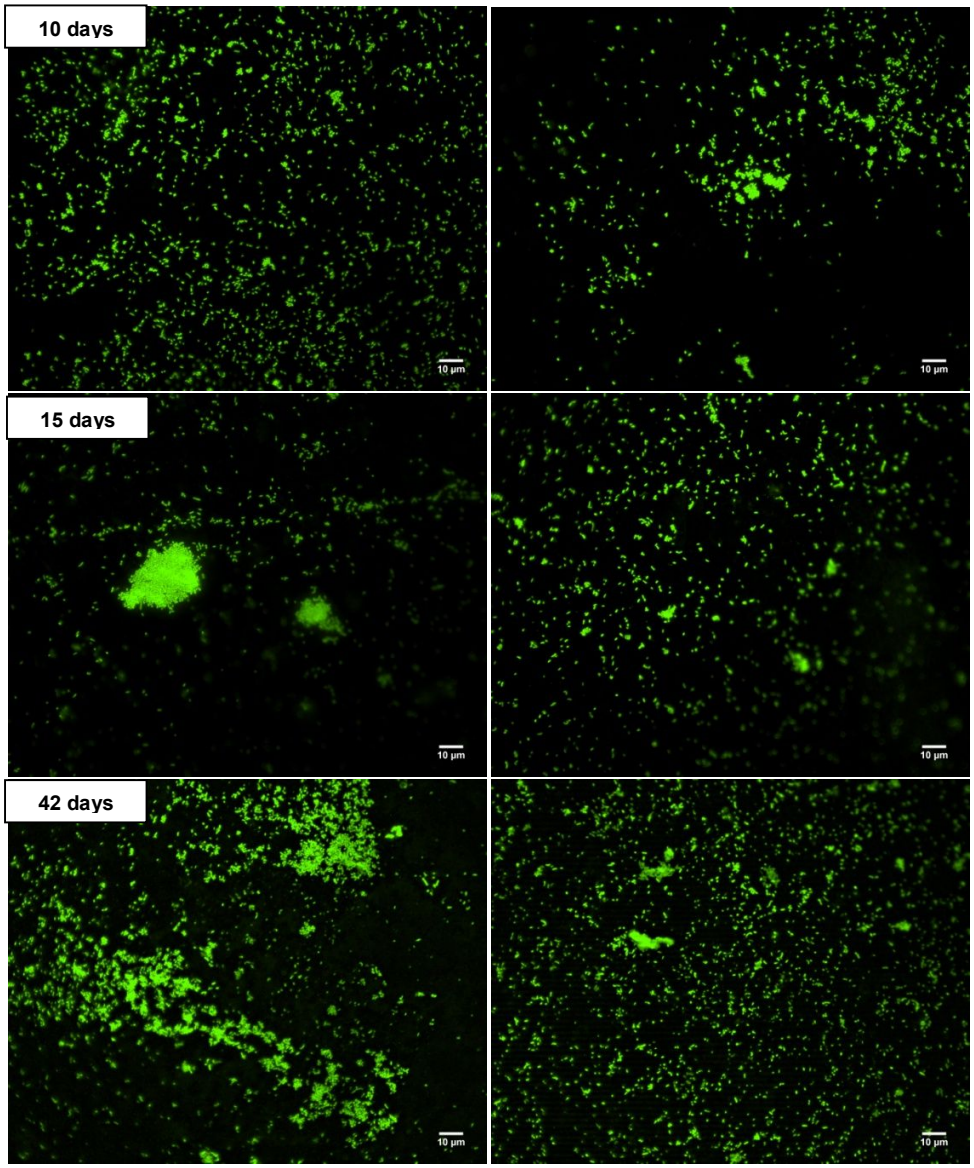


Figure VI-31. Fluorescence microscope images of stained microorganisms attached to the sugar-based polymer film. Observation after 10, 15 and 42 days of incubation with pure isolates at 30 °C.

VI- 6. CONCLUSIONS

During the past years new plastic materials have gradually replaced the traditional metal, wood or leather materials. Because of their time-limited application and wrong disposal, accumulation of polymeric materials in the environment has dramatically increased. Ironically, the most preferred property of plastics, i.e. durability, exerts the major environmental threat. As a result, efforts must be made in two directions, to enhance the potential biodegradability of most used polymers and in parallel to provide with more knowledge on the mechanisms, capabilities and outcomes of materials microbial attack in nature.

In this work, a novel type of copolymers was synthesized from renewable resources and the consequence of the use of sugar feedstock on the biodegradation features of the resulting material was assessed. Aiming at mimicking environmental conditions, inocula were selected from soil and activated sludge, providing a large biodiversity of organisms. Enrichment culture methods were effective for isolating bacteria capable of utilizing polymers as the sole carbon and energy source. More precisely, *Alcaligenes aquatilis* was found to degrade copolymers partially supplied from galactose, and a reduction of approximately 10 % of chains average molecular weight was measured after two weeks of incubation at 30 °C. Since copolymers were studied in the form of dried films, a colonization assay of polymer surface by the isolate was carried out. It was found that isolated *Alcaligenes aquatilis* adheres to polymer film surface, forming an apparent nascent biofilm, by means of cells agglomeration. Biofilm matrixes are reported to act as an external digestive system by keeping extracellular enzymes close to the cells, enabling them to indirectly metabolize solid polymers. Mechanisms of degradation were not the purpose of this work, but given the observations made in terms of

chains molecular weight distribution shift, speculation was made. Pendant sugar units were thought to be removed from polymer backbone through ester bond cleavage as to provide intermediate assimilable products.

VI- 7. PERSPECTIVE

This chapter deals with complex concepts which in fact open the discussion to more practical and essential concerns. In spite of the recent progress in biodegradation assessment methods and the efficient collaborations between chemists and microbiologists, there is always a gap between laboratory experiments and real environment conditions. It is rather complex to put biodegradation studies into perspective and straightforward conclusions cannot be made in that sense. Distinction must be made between observations done under defined controlled conditions and potential behaviour expected within the environment. Figure VI-32 illustrates this idea, so important to be conscious of.

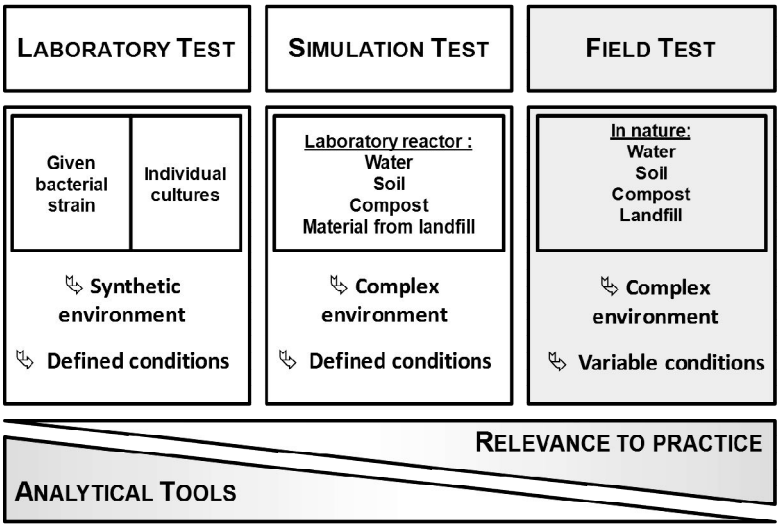


Figure VI-32. Schematic overview on evaluation of polymers biodegradation

Moreover and unfortunately, biodegradation of polymers involves conflicting aspects, which are products durability upon application time and smart disposal, ideally leading to biodegradation. For that matter, additional delicate questions will need to be answered in the future, including: *"What is the time frame in which biodegradation should occur in order to be of practical value?" and "How to control and balance product sustainability/disposal relationship?"*

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Chapter VII- Conclusions

In this thesis renewable feedstocks were used to prepared waterborne copolymers for coating application. Among the wide range of naturally occurring materials, carbohydrate resources were selected for their wide availability, in particular coming from agro-industrial, retailing market and domestic food wastage. Sugars are versatile molecules of diverse molecular weight, carrying several functional hydroxyl groups available for chemical functionalization.

Herein the exploration of a selection of monomers synthesized from low molar mass sugars for the preparation of waterborne copolymers was conducted. Because of their lack of reactivity against radically activated molecules, functionalization of sugars with a reactive polymerizable vinyl function was essential. In addition, protection of their hydroxyl functions to ensure obtaining non-water soluble molecules was a crucial aspect of this synthesis work. Firstly monosaccharide galactose was studied as valuable precursor for the synthesis of various monomer structures. Its isopropylidene equivalent was functionalized with a methacrylate group, eventually separated from the sugar scaffold by a spacer. The latter was shown to be of great influence on the resuting homopolymer thermal properties, inclunding Tg, ranging from 36 to 115 °C. Seeking for a simple and effective route to prepare monomers at rather high scales, a strategy involving straightforward esterification reaction of methacrylates was preferred to obtain spacer-free methacrylate protected galactose (MG). This synthesis pathway was extended to the functionalization of protected fructose, resulting MF. Both galactose and fructose based monomers were shown to be able to homopolymerize by free radical polymerization in emulsion at 10 % solids content, leading to hard materials exhibiting a Tg of about 110 – 115 °C.

On another hand, methacrylate structures were prepared from open sugars, including gluconic and lactobionic acids. The resulting monomers were homopolymerized in solution resulting in macromolecules of lower Tg, respectively 26 and 51 °C.

Since high Tg monomers are of particular interest, especially in formulations where the petroleum-based monomer MMA is used in rather high proportions, both methacrylate galactose and fructose were selected for further investigation. Copolymerization of the latter in emulsion was carried out with butyl acrylate to produce waterborne copolymers of various compositions. 30 % solids content latexes were prepared in batch and no effect of the renewable material was observed in terms of colloidal stability or thermal behavior. Copolymers exhibited rather low solubility in THF, with gel fractions of about 85 % in all the cases. Moreover, THF insolubility was increasing with time as a matter of further reactions occurring upon storage. Most likely, partial deprotection of sugar units was the reason for the creation of hydrogen bonding and thus physically insoluble entangled chains. Nonetheless, the potential of MG and MF for the production of waterborne copolymers was greatly valued by the successful increase of formulation solids content to achieve 45 % solids content latexes in good yields.

Given these promising results, the investigation of MF/BA high solids content binders for clear-coats application was carried out by means of design of experiments methodology. The performance of MF-based binder resulted dependent on the copolymer composition and process used. In fact, a 40/60 copolymer from MF/BA exhibited not high enough hardness to be considered as proper binder. Nevertheless, the substitution of MMA in an industrial formulation from Allnex permitted obtaining a sugar-based binder with equivalent gloss, lower haze, and better water resistance and anti-blocking properties than its commercial homologue containing MMA.

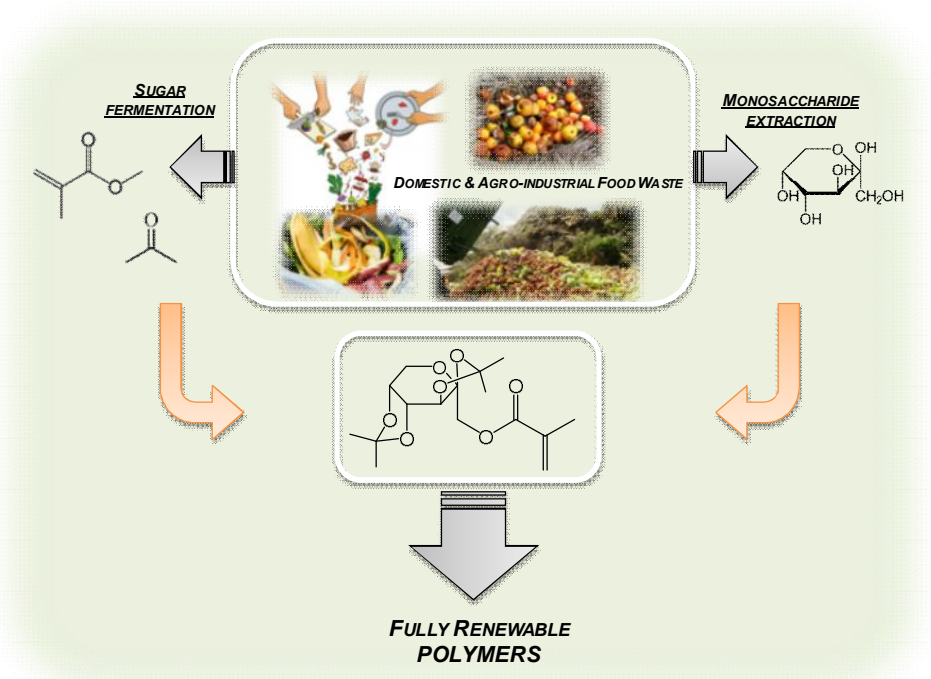
The exploration of renewable resources was extended to the additional use of a commercial monomer, mainly based on coconut natural oil (Viosiomer C13-MA). Miniemulsion copolymerization were carried out at 30 % with different ratios of sugar-based methacrylates to achieve latexes with major reduction of volatile organic compound content. The resulting waterborne dispersions open the window towards the possible development of fully renewable polymeric materials.

Additionally, the use of methacrylate sugars in more complex industrial processes was evaluated. ASR-stabilized waterborne dispersions were prepared following two different strategies, both used at industrial scale by Allnex to prepare binders for commercialization. Partial to full substitution of MMA in the formulations was performed and high solids content dispersions were obtained in good yield in all the cases. Since sugar-based monomers are high density, viscous substances, their dispersion in aqueous media is delicate and shear sensitivity during polymerization was noticeable. Still, waterborne dispersions were formulated for clear-coat paint application and their performance was evaluated in terms of hardness, gloss, haze, chemical resistance, blocking and adhesion. Sugar-based ASR-containing paints exhibited good performances as compared with the commercial reference from Allnex.

In the last stage of this work, the exploration of sugar units presence in synthetic polymers was studied in terms of biodegradation capabilities. Indeed, natural resources are fully biodegradable as a matter of naturally occurring biological processes building a powerful ecosystem. The use of renewable feedstock for the production of synthetic polymeric materials is expected to imply product lifetime. Yet, very little is known on the impact of natural product chemical modification on biodegradability, most likely because of the specific outcome of each particular case and the large range of possibilities to assess it. In that context, material

sensitivity towards microbial attack is of high importance and providing with more knowledge towards this topic appears essential. As a matter of fact, a study aiming at screening the potential influence of sugar units in polymer backbones on microbial degradation was carried out. A biodiverse population of micro-organisms was extracted from soil and activated sludge and incubated with sugar-based homo and co-polymers. Eight bacterial strains were found to be able to use polymers as sole carbon resource for growth. Besides, colonization of sugar-based copolymer film was highlighted by fluorescence microscopy, showing the capacity of bacteria to adhere on the bio-based material. As such, this observation consisted of a preliminary proof of biofilm formation. Besides, evidence of average copolymer molecular weight decrease was given. Consequently, the microbial development in cultures incubated with sugar-based macromolecules as sole carbon resource was directly related to polymer biodegradation. Still this result must be considered with caution since extrapolation to closer environmental conditions is difficult.

Chapter VIII- Perspectives: Development of future-oriented polymers



Designing tomorrow's products, application and processes is a challenge that scientists are willing to overcome. Widening the scope of raw materials as well as their recovering methods are crucial aspects of this objective.

The present chapter aims at briefly showing the technological feasibility of producing high-value monomers and materials using carbohydrates.

The production of food waste covers all the food life cycle: from agriculture, up to industrial manufacturing and processing, retail and household consumption.[1–3] This said phenomenon raises ethical concerns as well as economic and environmental problems.

While common sense and wisdom suggest that any edible material left should definitely be distributed to country's starving population, there are still many type of food waste sources that could be valorised by the chemical industry. Non-comestible food residues such as fruit peels, nutshell, and eventually putrefied fruits and vegetables are sources of sugars.

One of the monomer explored in this thesis, referred as methacrylate fructose (MF), showed great interest for the production of bio-based industrial waterborne polymers. It was prepared from a monosaccharide and its modification involved the use of both solvent such as acetone for its protection, ethanol and water for its purification and functionalizing agents, mostly methacrylate-based. In fact, most of the chemicals needed to produce sugar-based valuable methacrylate monomers can be supplied by nature in a sustainable approach.

Indeed, a large collection of building blocks produced from biomass fermentation is available, including (meth)acrylate reagents[4,5] opening the window to monomer preparation in accordance with the green chemistry principles.[6] For instance, methacrylic acid can be

produced by decarboxylation of itaconic acid or by oxidation of isobutylene, a building block for synthetic rubbers that is being produced at pilot scale from sugars.

It is noteworthy that the production of acetone by bacteria is one of the largest known biotechnological processes.[7,8] This technique was developed more than 100 years ago, in 1915 by Dr. Chaim Weizmann during World War I, for the manufacturing of gunpowder. Nowadays, some companies look back to this method and industrial scale up are being set up.[9–11] In addition to this, bio-ethanol is undeniably considered as renewable solvent and various technological methods are being investigated to achieve more sustainable processes.[12–17]

Furthermore, the direct use of mono or di-saccharide as valuable chemical is an interesting side aspect of food waste and plant sugar recovery. Monosaccharides can actually be successfully extracted and isolated from food waste. Grap'Sud group, through its subsidiary NUTRITIS in France, is offering a large range of sugars from fruits that are being removed from the food market chain because of their high ripeness.[18,19] As a result, fructose can be obtained at industrial scale from fruit waste and further used as chemical precursor for any kind of valuable chemistry.

By suggesting the use of agricultural and food wastes to prepare value-added monomers in combination with water-based polymerization processes and biodegradability-induced features, this thesis wishes to emphasize the interest of "sweet solutions" to novel bio-based materials.

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Resumen y Conclusiones

En esta tesis se utilizaron materias primas renovables para preparar copolímeros en base acuosa para su aplicación en recubrimientos. Entre la amplia gama de materiales de origen natural, en este trabajo se seleccionaron los recursos de hidratos de carbono. Una de las razones es su gran disponibilidad, bien como derivados de la agroindustria, el mercado minorista y el desperdicio de alimentos en el hogar. Por otra parte, los azúcares son moléculas versátiles de diverso peso molecular, que presentan varios grupos hidroxilo funcionales disponibles para la posterior funcionalización química.

En este trabajo se llevó a cabo la exploración de una selección de monómeros sintetizados a partir de azúcares de bajo peso molecular para la preparación de copolímeros dispersos en medio acuoso, vía polimerización radicalaria. Para ello, fue necesario la funcionalización de los azúcares con una función vinílica polimerizable reactiva. Además, la protección de los grupos hidroxilo para asegurar la obtención de moléculas no solubles en agua fue un aspecto crucial de este trabajo de síntesis. En primer lugar se estudió la galactosa monosacárida como precursor valioso para la síntesis de diversas estructuras monoméricas. Su equivalente de isopropilideno se funcionalizó con un grupo metacrilato, eventualmente separado de la estructura por un espaciador. Se demostró que este último era de gran influencia en las propiedades térmicas del homopolímero con Tg que oscilaba entre 36 y

115ºC. Buscando una ruta simple y eficaz para preparar monómeros a “gran” escala, se prefirió una estrategia que implicaba una reacción de esterificación sencilla de metacrilatos para obtener galactosa (MG) protegida con metacrilato libre de espaciador. Esta síntesis se extendió a la funcionalización de fructosa protegida, resultando el monómero denominado MF. Se demostró que ambos monómeros basados en galactosa y fructosa eran capaces de homopolimerizar mediante polimerización de radicales libres en emulsión, dando lugar a materiales duros que exhibían una Tg de aproximadamente 110 - 115ºC.

Por otro lado, se prepararon estructuras de metacrilato a partir de azúcares abiertos, incluyendo los ácidos glucónico y lactobiónico. Los monómeros resultantes se homopolimerizaron en solución dando como resultado macromoléculas de Tg inferior, 26 y 51ºC respectivamente.

Puesto que los monómeros de Tg alta son de particular interés, especialmente en formulaciones en las que el monómero de base de petróleo MMA se utiliza en proporciones bastante altas, se seleccionaron tanto el metacrilato galactosa (MG) como la fructosa (MF) para investigación adicional. Se estudió la copolimerización en emulsión de ambos monómeros con acrilato de butilo, para diferentes composiciones. Se prepararon látex de contenido en sólidos al 30% en discontinuo, obteniéndose copolímeros en un amplio rango de Tg. Por otra parte, no se observó efecto del tipo de material renovable en términos de estabilidad coloidal ni comportamiento térmico. Los copolímeros mostraron una solubilidad bastante baja en THF, con fracciones de gel de aproximadamente 85% en todos los casos. Además, se observó que la insolubilidad del látex en THF aumentaba con el tiempo. La hipótesis considerada en este trabajo es que las unidades de azúcar sufran una desprotección parcial durante el almacenamiento, que den lugar a puentes de hidrógeno, y por tanto, cadenas entrecruzadas físicamente insolubles. Sin embargo, la posible hidrólisis no pudo

detectarse por técnicas de infrarrojo ni RMN. Hay que mencionar que unos pocos grupos OH son suficientes para dar lugar dichos enlaces, y su concentración puede estar por debajo del umbral de detección de dichos equipos. No obstante, el potencial de MG y MF para la producción de copolímeros en base acuosa se valoró en gran medida por el aumento exitoso del contenido de sólidos de la formulación al conseguir látex estables con un 45 % de contenido en sólidos.

Dado estos resultados prometedores, se llevó a cabo la investigación del desempeño del látex con 45% de contenido en sólidos MF / BA (40/60) como ligante para la aplicación como recubrimiento transparente. Se observó que el látex no formulado formaba un muy buen film, pero su dureza no era lo suficientemente alta. Por ello, como siguiente pasó se evaluó la incorporación de aditivos que mejoren la dureza. Este estudio se realizó en base a un diseño de experimentos y "high-throughput experimentation" (HTE). La incorporación de un 30% de ASR permitió mejorar la dureza del recubrimiento de manera importante, sin perjudicar el resto de propiedades. Como estudio posterior, y a fin de poder evaluar la viabilidad de utilizar el MF procedente de fuente renovable, se procedió a una nueva síntesis donde se llevó a cabo la sustitución de MMA por MF en una formulación industrial de Allnex. Los films preparados con dichos látexes presentaron un brillo equivalente, menor turbidez y mejor resistencia al agua y propiedades anti-bloqueo que su homólogo comercial que contenía MMA.

La exploración de recursos renovables se extendió al uso de un monómero comercial, proveniente fundamentalmente de aceite natural de coco (Visiomer C13-MA). Dada la muy alta hidrofobicidad de este monómero, la polimerización se llevó a cabo en miniemulsión, donde la polimerización ocurre fundamentalmente en las gotas de monómero y se evita la necesidad de difusión del monómero a través de la fase acuosa, como es el caso de la polimerización en

emulsión convencional. Se realizaron reacciones de copolimerización en discontinuo con diferentes relaciones de metacrilatos basados en azúcar y 30% de contenido en sólidos. Las dispersiones acuosas resultantes mostraron buenas propiedades coloidales, films relativamente homogéneos y con buena estabilidad térmica. Estos resultados muestran la posibilidad de desarrollar materiales poliméricos basados completamente en fuentes renovables.

Además, se evaluó el uso de azúcares de metacrilato en procesos industriales más complejos. Las dispersiones acuosas estabilizadas con ASR se prepararon siguiendo dos estrategias diferentes, ambas usadas a escala industrial por Allnex para preparar ligantes para uso comercial. En ambos casos, se sustituyó el MMA por MF en las formulaciones, obteniéndose dispersiones hasta un contenido bio-based del 45% en peso respecto al monómero total, alto contenido en sólidos y buen rendimiento en todos los casos. Las dispersiones acuosas se incluyeron en la formulación para su aplicación en recubrimientos transparentes y su desempeño se evaluó en términos de dureza, brillo, neblina, resistencia química, bloqueo y adhesión. Las pinturas que contienen ASR en base de azúcar exhibieron buenos resultados en comparación con la referencia comercial de Allnex.

En la última etapa de este trabajo se exploró el efecto de la presencia de unidades de azúcar en polímeros sintéticos en términos de capacidad de biodegradación. De hecho, los recursos naturales son totalmente biodegradables como consecuencia de procesos biológicos naturales que constituyen un ecosistema poderoso. Por ello, se espera que el uso de materia prima renovable para la producción de materiales poliméricos sintéticos incida en la vida útil del producto. Sin embargo, se sabe muy poco sobre el impacto de la modificación química del producto natural sobre la biodegradabilidad, probablemente debido al resultado específico de cada caso particular y al amplio rango de posibilidades para evaluarlo. En este contexto, la

sensibilidad del material hacia el ataque microbiano es de gran importancia y es esencial disponer de más conocimiento sobre este tema. Así, se llevó a cabo un estudio con el objetivo de investigar el efecto de la presencia de las unidades de azúcar en las cadenas poliméricas ante la degradación microbiana. Se extrajo una población de biodiversidad de microorganismos del suelo y lodos activados y se incubó con homo y copolímeros a base de azúcar. Se descubrió que ocho cepas bacterianas eran capaces de usar polímeros como único recurso de carbono para el crecimiento. Además, la colonización de la película de copolímero a base de azúcar se caracterizó por microscopía de fluorescencia, mostrando que las bacterias tienen capacidad para adherirse en el material de base biológica. Esta observación supuso una prueba preliminar de la formación de biofilm. Además, se obtuvo evidencia de la disminución del peso molecular promedio del copolímero. Estos resultados son indicativos de biodegradación del polímero.

Como conclusión final, indicar que el uso de residuos agrícolas y alimentarios para preparar monómeros de valor añadido en combinación con procesos de polimerización en base de agua y su posible biodegradabilidad demostrado en esta tesis se puede considerar como *"soluciones dulces" para la producción de nuevos materiales*.

Acronyms list

Đ	Polydispersity index
DAF	Diacetone-D-Fructose (2,3:4,5-Di-O-isopropylidene-b-D-fructopyranose)
DAGA	Diacetone-D-Galactose (1,2:3,4-Di-O-isopropylidene-D-galactopyranose)
DCE	Dichloroethane
DCM	Dichloromethane
DLS	Dynamic Light Scattering
DMF	Dimethyl Formamide
Dp	Particle size (nm)
DSC	Differential Scanning Calorimetry
EtOAc	Ethyl Acetate
GC	Gas Chromatography
GMA	Glycidyl Methacrylate
GPC	Gel Permeation Chromatography
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Heteronuclear Multiple Quantum Correlation
KPS	Potassium Persulfate
MAA	Methacrylic acid
MG	Methacrylate Galactose
MF	Methacrylate Fructose
MGA	Methacrylate Gluconic Acid
MLA	Methacrylate Lactobionic Acid (2-lactobionamidoethyl methacrylate)
Mw	Molecular weight
NEt_3	Triethylamine
NMR	Nuclear Magnetic Resonance
SDS	Sodium Dodecyl Sulfate
TBHP	Ter-Butyl Hydropexoxide
Tg	Glass transition temperature

TGA Thermogravimetric analysis

TLC Thin Layer Chromatography

TTIP Titanium isopropoxide

Appendix 1 – Characterization methods

A1-1. SUGAR-BASED MONOMERS CHARACTERIZATION

The heteronuclear single quantum correlation experiment, abbreviated as HSQC, is used frequently in NMR spectroscopy of organic molecules to correctly assign signals. The resulting spectrum is two-dimensional (2D) with one horizontal axis for protons ^{1}H and one vertical axis for carbons ^{13}C. For each carbon signal being considered, the spectrum gives information about the protons being directly attached to it. For instance in Figure 1, corresponding to HSQC-NMR of monomer **2**, sugar ring protons at the 3.5 – 4.5 ppm region can be assigned to sugar ring carbons, being the signals at 70 ppm. Similarly in Figure 2 sugar ring protons (4.0 – 4.5 ppm) are correlated to sugar ring carbons (65 - 75 ppm region).

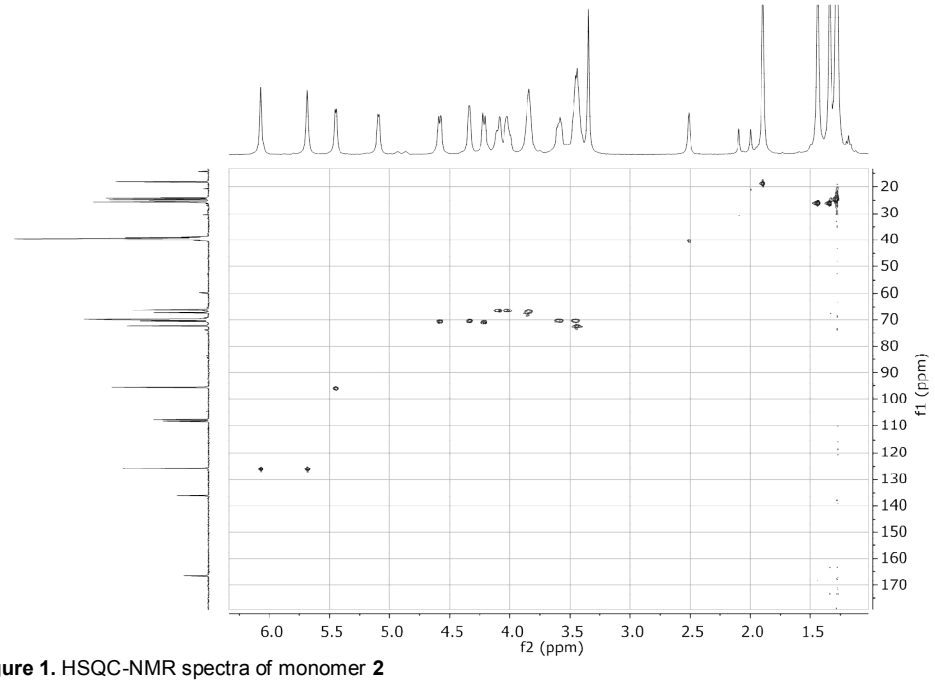

Figure 1. HSQC-NMR spectra of monomer **2**

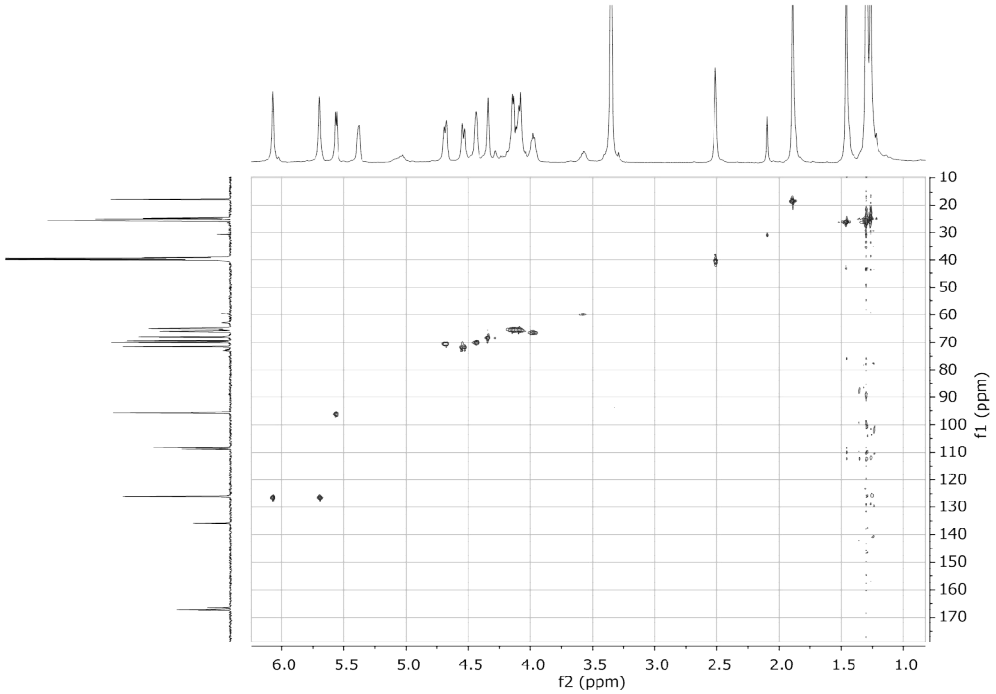

Figure 2. HSQC-NMR spectra of monomer **4**

Figure 3 presents the differentiation of various types of isomers. It is noteworthy that in the case of the present study, only stereoisomers, that is to say molecules that present distinct arrangements in space, were seen. Stereoisomers, including diastereoisomers usually lead to similar signals in NMR spectroscopy. However, in some cases, increasing the temperature of acquisition allows a better separation of diastereoisomers signals, in particular peaks coming from the stereocenter and its close environment. Herein this experiment was performed for monomers **2** and **5** and the resulting spectra are given Figure 4 (zone of interest exclusively).

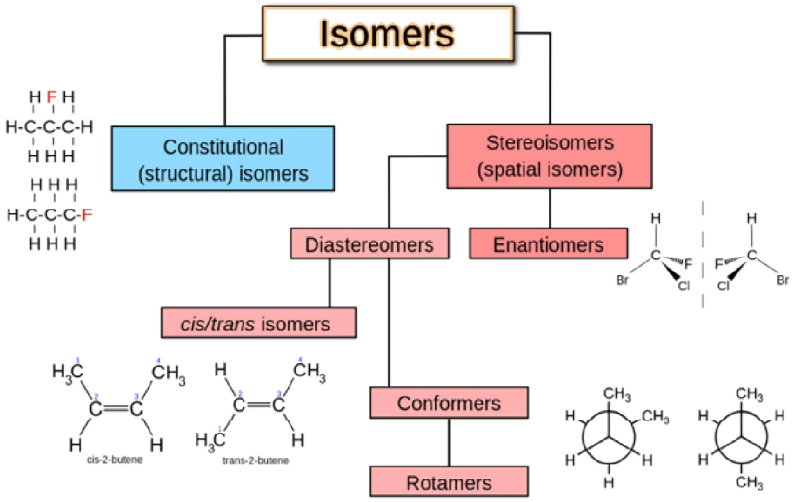

Figure 3. Definition and differentiation of isomers in organic chemistry

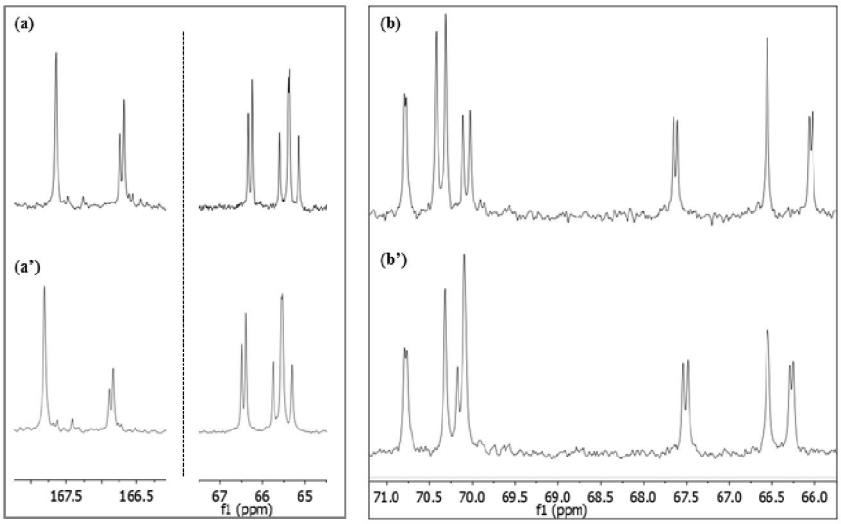

Figure 4. ^{13}C-NMR spectra of monomer **4** (left) and **2** (right) recorded at ambient temperature (a' and b') and at 80 °C (a and b) in DMSO-d_6.

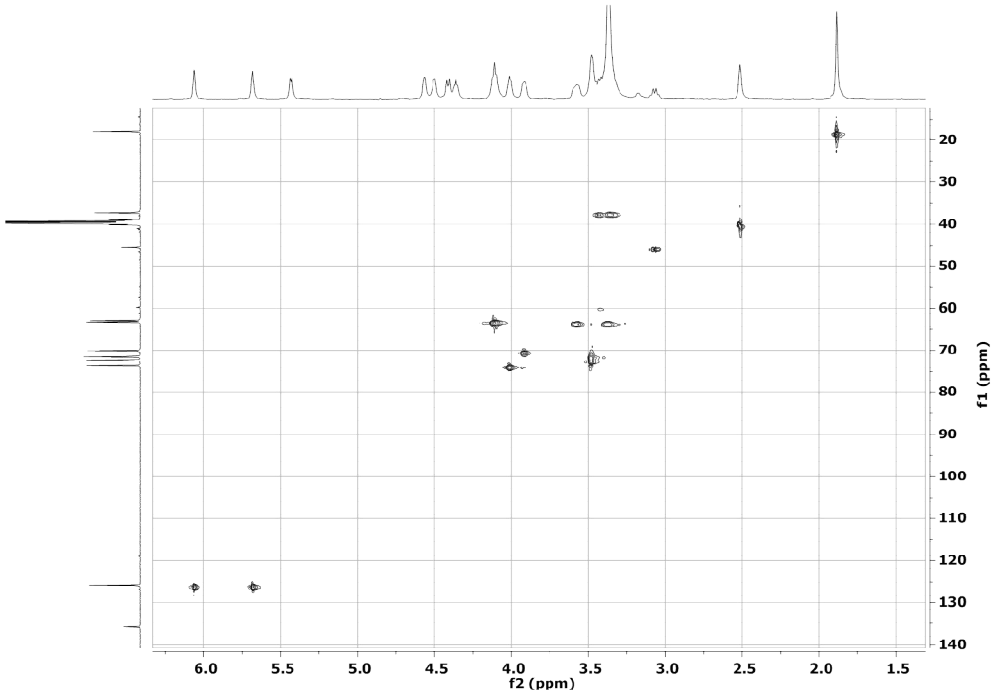

Figure 5. HSQC-NMR spectra of MGA monomer before protection

In the case of open sugars functionalization, including hydrophilic gluconic and lactobionic acids, bi-dimensional NMR was of high importance since it helped confirming the assignation of the various -OH groups, detectable in DMSO-d_6. Indeed, protons from hydroxyl groups are exclusively linked to oxygen atoms and not to carbons. As a result proton signals coming from –OH do not give any correlation with the carbon spectrum in HSQC, as observed for signals around 4.5 ppm for unprotected MGA (Figure 5) and from 4.3 to 5.3 ppm for unprotected MLA (Figure 6).

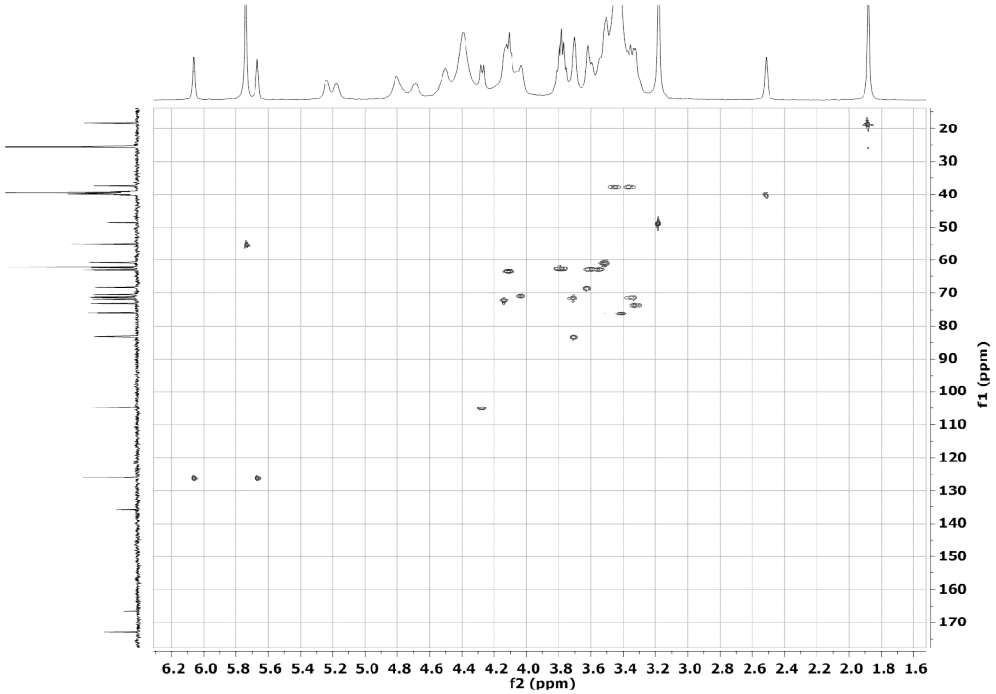

Figure 6. HSQC-NMR spectra of MLA monomer before protection

A1-2. DETERMINATION OF CONVERSIONS BY ^{1}H-NMR SPECTROSCOPY

Samples were withdrawn during polymerization reaction and ^{1}H-NMR spectroscopy was used to monitor monomer conversion. In such a systematic method of characterization, it is very important to well define the conditions of the analysis, namely here the parameters used for the spectra acquisition. An adequate number of scans (NS) and receiver gain (RG) were determined by recording the spectrum of a blank sample prepared from a latex and looking for the best signal to noise ratio. In addition, the relaxation time of the protons of interest from the

monomer, i.e. vinyl protons was measured. NMR relaxation is the process by which an excited magnetic state returns to its equilibrium distribution. This phenomenon is of particular importance since it is directly related to the value obtained from peak integration. Indeed, if the relaxation decay set up in the acquisition parameters is not long enough to allow full relaxation of the vinyl proton, the integral obtained after peak integration will be underestimated and as a result conversion calculation will be negatively affected. Given the fact that in this work, novel monomers from renewable resources are being used, the determination of monomer's vinyl proton specific relaxation delay appeared necessary. MG was the monomer used in that study.

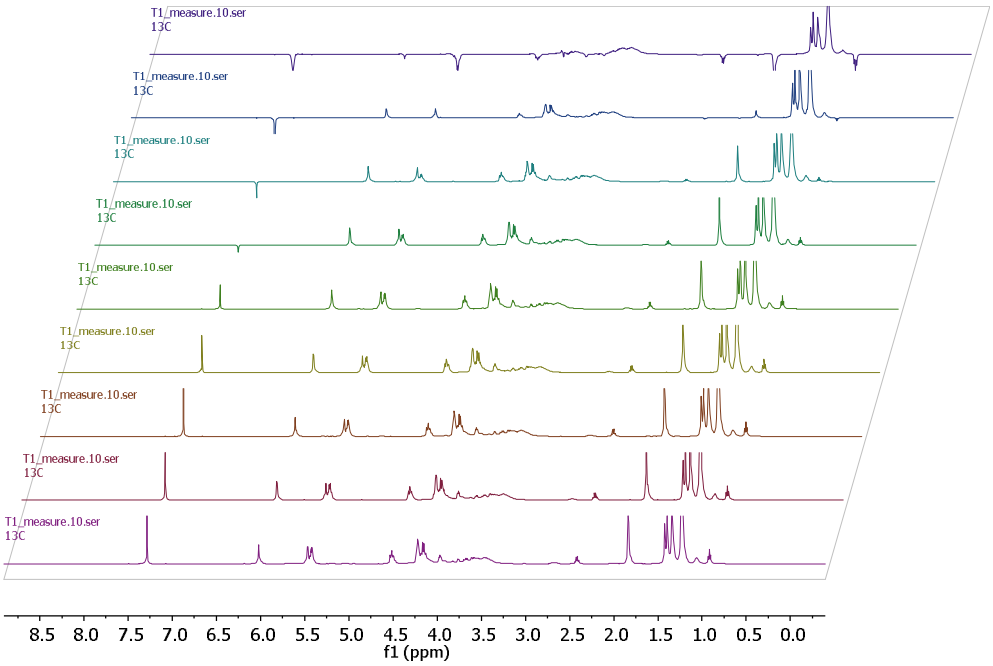

Figure 7. Spectra sequence for the determination of relaxation times proper to MG in $CDCl_3$

Figure 7 shows the 9 points used to determine t1 corresponding to the vinyl protons of MG monomer and Table 1 gives the value obtained. Both vinyl protons exhibit relaxation times around 1 second. Therefore, in order to allow obtaining a reliable integral when monitoring vinyl proton signal disappearance during polymerization, NMR acquisition conditions were adapted as follow: NS = 32, t1 = 10 seconds, RG = 128 (for low conversions) and 1024 (for high conversions). The relaxation delay was set 10 times higher than the actual vinyl proton.

Table 1. Values of t1 for both vinyl protons signals

Integral region	t1
6.198 to 5.894 ppm	977.0 ± 5.7e-03 ms
5.571 to 5.286 ppm	1.4 ± 8.3e-03 s

NMR sample preparation was carried out with 50 µL of latex and 450 µL of a solution containing benzene-1,3,5-tricarboxylic acid (BTC) as reference at a concentration of 1 g/L. ^{1}H-NMR spectra were recorded in the above mentioned conditions, using WATERGATE method to suppress the signal of water, and integration of the vinyl proton signals and reference signal was performed.

A1-3. Nd AND Np CALCULATIONS

Miniemulsion droplets and polymer particle sizes were measured by dynamic light scattering (DLS, Zetasizer Nano Z, Malvern Instruments). The equipment determines the particle size by measuring the rate of fluctuations in light intensity scattered by particles as they

diffuse through a fluid. Samples were prepared by diluting a fraction of the latex or miniemulsion with deionized water. Results obtained from DLS were used to determine the number of droplets (Nd) and number of particles (Np).

$$N_d = \frac{V_t}{V_d} = \frac{6(W_{mon}/\rho_{mon})}{\pi d_d^3} \quad (1)$$

$$N_d = \frac{V_t}{V_p} = \frac{6(W_{mon}/\rho_{mon})}{\pi d_d^3} + \frac{6(W_{polym}/\rho_{polym})}{\pi d_p^3} \quad (2)$$

Nd was calculated using Equation 1, where W_{mon} was the amount of monomer, ρ_{mon} the monomer density (density of MG and MF being 1.16) and d_d the average droplet size. Np was determined following Equation 2. In this case, W_{polym} corresponds to the amount of polymer, ρ_{polym} to the polymer density (1.1 as an average) and d_p to the average particle size.

A1-4. GEL CONTENT

The gel content by definition is the fraction of polymer that is not soluble is a good common solvent such as tetrahydrofuran (THF). The gel fraction was measured by Soxhlet extraction (Figure 8).

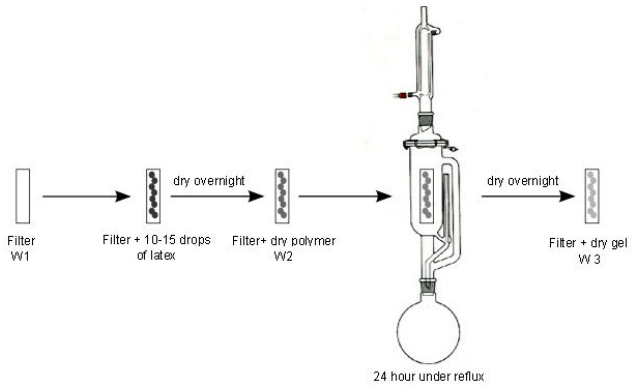

Figure 8. Scheme of soxhet extraction method for gel content measurements.

To measure the gel content glass fiber square pads (CEM) were used as backing. A few drops of latex were placed on the filter (weight=W1) and dried at 60 °C for a couple of hours. The filter together with the dried polymer was weighed (W2) and a continuous extraction with THF under reflux in the Soxhlet for 24 hours was done. After this period of time, the wet filter was weighed (W3) and dried overnight. Finally the weight of the dry sample was taken (W4). Gel content was calculated as the ratio between the weight of the insoluble polymer fraction and that of the initial sample, as shown below.

$$\text{Gel content}\,(\%) = \frac{W_4 - W_1}{W_2 - W_1} \times 100$$

Appendix 2- Paint Formulation.

Methods and Basic Principles.

A2-1. ANALYSIS OF VARIANCE ANOVA PRINCIPLE

Results obtained from each paint testing can be treated as responses from the DoE and fitted into a mathematical model describing the interaction between the formulation variables and the performance measured.

To do so, analysis of variance (ANOVA) was used. It determines if the responses obtained from each experiment can be used to describe a tendency. Responses are exploited to determine the variation caused by a factor, which can be related to the sum of squares.

Figure 9 represents the ANOVA key principle through which different sum of squares is being calculated.

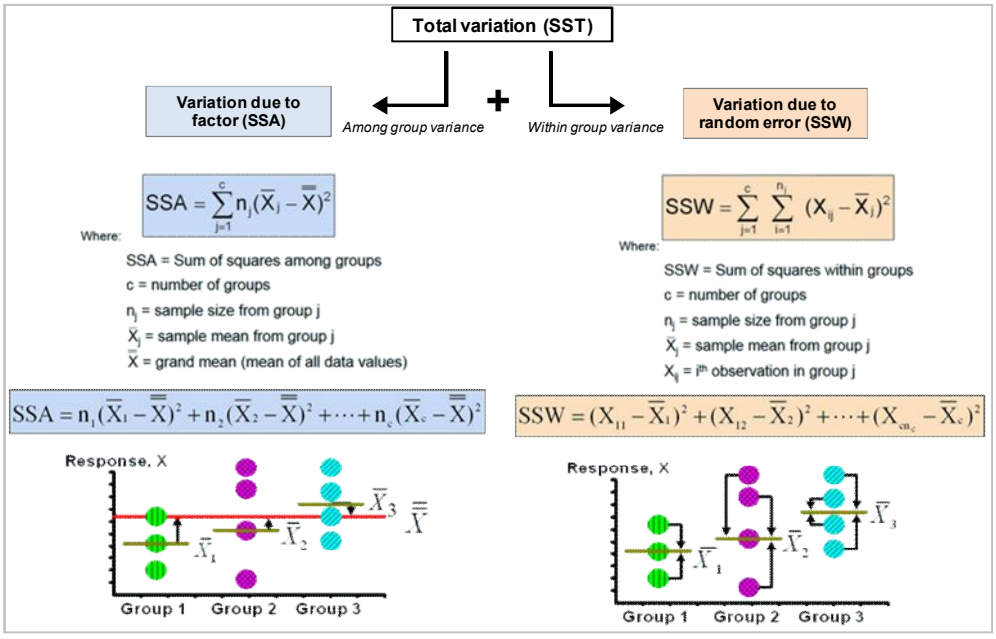

Figure 9. Analysis of variance method (ANOVA): representation of variance partitioning

The mean squares are obtained by dividing the various sums of squares by their associated degrees of freedom, as described below:

Mean square among: MSA = $\frac{\mathrm{SSA}}{\mathrm{c}-1}$

Mean square within: MSW = $\frac{\mathrm{SSW}}{\mathrm{n}-\mathrm{c}}$

Mean square total: MST = $\frac{\mathrm{SST}}{\mathrm{n}-1}$

c = number of groups ; n = sum of the sample sizes from all groups

The mean squares obtained allow the calculation of F-value, basically giving a signal to noise ratio value and thus information on the consistency of the model: F = $\frac{\mathrm{MSA}}{\mathrm{MSW}}$.The basic logic behind ANOVA is that within-group variance is due only to random error. Thus if the variability between groups is similar to that within groups, then the means are likely to differ only because of random error. Thus, high F-value is needed to reject the null hypothesis. F-value from a given study helps determining how consistent the results are with the null hypothesis and to calculate probabilities. That probability allows us to verify how common or rare our F-value is under the assumption that the null hypothesis is true. If the probability is low enough, we can conclude that our data is inconsistent with the null hypothesis. In addition to this, the probability of having a high F-value due to noise is calculated and referred as p-value. In brief, a high F-value and a low p-value are evidence of a good model fitting.

Still, the fitting of predicted vs experimental responses needs to be ensured by verification of additional parameters. Model validation is possibly the most important step in the model building sequence. Residuals are estimates of experimental error obtained by subtracting the observed responses from the predicted responses. Examining residuals is a key part of all

statistical modeling, including DoE's. They represent elements of variation unexplained by fitted model. Overfitting needs to be double-checked as well. It occurs when a statistical model tends to fit random errors or noise instead of the underlying relationship. A model which has been overfit will generally have poor predictive performance. A couple of additional parameters may be checked in complementation.

Finally, equations can be extracted from the model to predict behaviors, following the scheme given Figure 10. Response Y is controlled by setting the factors (A, B, etc.). The effects (α, β, etc.) give information on how much weight each factor has.

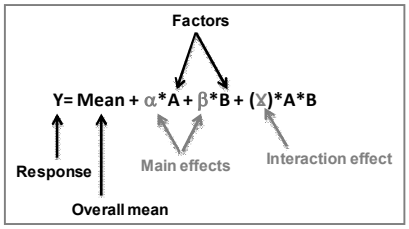

Figure 10. Model equation representation

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Appendix 3 – Microbiology.
Terms Definition and Methods.

A3-1. IDENTIFICATION OF ISOLATES

DNA sequencing and phylogenetic analysis were carried out by GATC-biotech Company, France. DNA amplification was carried out by Polymerase Chain Reaction (PCR).[37] The PCR is a biochemical technology in molecular biology used to amplify a DNA fragment across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. It was performed using DNA primers 8F (AGAGTTTGATCCTGGCTCAG)[38] and 1492R (ACCTTGTTACGACTT)[39]. Isolates were identified from the partial DNA sequence encoding 16S rRNA Basic local alignment search tool[40] was used in combination with reference sequence databases[41,42] to allow the identification of isolates.

Table 2. Isolates identification

Isolate name	Identification	Phylum	% sequence identity	Size of the used sequence
LB P	*Pseudomonas veronii*	*γ-Proteobacteria*	100	644 b
LB F1S	*Alcaligenes aquatilis*	*β-Proteobacteria*	100	566 b
LB F1E	*Achromobacter aegrifaciens*	*β-Proteobacteria*	99.9	714 b
LB F1T	*Alcaligenes aquatilis*	*β-Proteobacteria*	100	640 b
LB DS1	*Brucella abortus*	*α-Proteobacteria*	99.4	493 b
LB DE	*Alcaligenes faecalis* subsp. *phenolicus*	*β-Proteobacteria*	99.7	643 b
LB DS2	*Ochrobactrum anthropi*	*α-Proteobacteria*	100	555

A3-2. TAXONOMY IN MICROBIOLOGY

Taxonomy is an established classification method for assigning isolates name and origin. It is very important to underline that different strains coming from same species can exhibit

different metabolic capacities. Therefore it is difficult to conclude on the potential ability of the isolated strains because strict comparison with literature cannot be done.

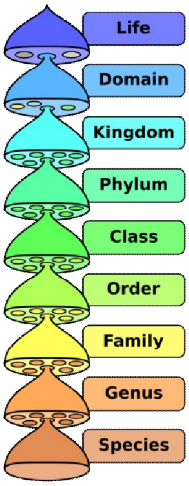

Figure 11. A rank based classification: Taxonomy

A3-3. BACTERIA COLORATION PROTOCOL

A3-3.1 Staining with Crystal Violet (CV)

Culture medium was withdrawn from the plate and wells were rinsed off with a sterile M9 solution to remove planktonic cell, i.e. non-attached to the film substrate. A solution of crystal violet (1 % (w/v)) was added in the well (400 µL). After 3 minutes, the excess of dye solution

was removed and wells were gently washed with distilled water. Visual observation of substrate coloration by CV could be achieved straightforward.

A3-3.2 Staining with Hoechst

A commercial aqueous solution of Hoechst (10 mg/mL - Aldrich) was diluted to 10 μg/ml in M9. Culture media were first withdrawn from well and the latter was gently washed with a M9 sterile solution to remove any planktonic bacteria. Dye solution was added in the wells (500 µL) and left to act for 15 to 20 minutes in the dark to avoid any dye photodegradation. Films were then removed from wells and carefully rinsed with M9 to remove any excess of dye solution. A very small piece of film was cut and placed between slide and slide cover for microscope observation.

A3-4. REFERENCES

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