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Study of antifungal agent caspofungin adsorption to laboratory materials

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ABSTRACT

Treatment of invasive fungal infections with Caspofungin is used as the first-line antifungal agents. The minimum inhibitory concentration value is a test which indicates the degree of sensitivity of a strain regarding a drug. However, no value of minimum inhibitory concentration for caspofungin is available because very variable value is obtained. In this work, we study the link with the adsorption phenomenon of CSF previously described in literature and the lack of minimum inhibitory concentration value.

A systematic study of the impact of different parameters on CSF adsorption is reported. The effect of the nature of container material, the aqueous solution pH and the organic solvent proportion was studied. In addition, the possibility of using a coating agent to minimize the adsorption was assayed and evaluated.

Results obtained showed the importance of the material used during the manipulation of CSF. The use of acidic pH aqueous solution or the addition of acetonitrile or methanol proportions (50 % and 70 %, respectively) were found efficient to avoid adsorption of CSF on glassware material, which is the relevant strategy for analytical samples of caspofungin. The treatment of HPLC glass vials and 96-well plates with N-(2-aminoethyl)-3-aminopropyltrimethoxysilane reduced the adsorption. The significant adsorption observed in this work especially with plastic materials, questions the results obtained before in different assays and explained the absence of MIC value.

1. Introduction

Invasive fungal infections have risen as one of the most concerning human diseases, especially in hospitalized and immunocompromised population [1–5]. In this context, echinocandins (ECs) have become the first-line therapy for invasive candidiasis in different patient groups [6,7]. However, during the last years, therapeutic failures have been reported attributed to the emerging resistance of different *Candida* spp. to ECs, especially *Candida glabrata*.

Kartsonis *et al.* [8] reported inconsistency among calculated minimum inhibitory concentration (MIC) values and the clinical or microbiological results obtained for patients with invasive candidiasis treated with the EC caspofungin (CSF). MIC reference values are established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or Clinical & Laboratory Standards Institute (CLSI). Nevertheless, the EUCAST does not provide any reference value for CSF MIC in *Candida* spp. The lack of established MIC values is linked to an enormous interlaboratory variability in experimental results obtained [9,10].

One of the reasons of this lack of coherent microbiological test results could be associated to the EC loss during sample handling [8,11–13]. Already at the early beginning of ECs development, Schwartz *et al.* [14] pointed out that adsorption of CSF to glassware was apparently known. However, this study did not provided any evidence of this adsorption and mentioned that they did not observe the same phenomenon on plastic materials. The adsorption of CSF to plastic materials was mentioned during the solid phase extraction procedure of urine samples as an explanation of decrease of recovery. Similarly, Traunmüller *et al.* [15] observed low recovery results for CSF when a sample treatment by protein precipitation was applied. They showed that the addition of organic solvents or the change of pH of the CSF solutions improved the results obtained. Furthermore, difference in determination of the CLSI clinical breakpoints using treated or untreated polystyrene 96-well from a commercial source was highlighted [10].Therefore, this study demonstrates that the nature of the polystyrene may be one factor that

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influences the in vitro potency of caspofungin for Candida species. However, the mechanism of adsorption was not explored. Other factors may be incriminated such as potential differences in media and plastic obtained from different sources.

CSF is a synthetically modified molecule derived from the fermentation of the fungi *Glarea lozoyensis* [16]. The core is a peptidic ring composed by two ornithines, two prolines and two threonines (Fig. 1) which contributes to acid-base properties due to the different pKa values of the several functional groups. In addition, an aliphatic chain is attached to the ring giving the amphipathic nature and the lipophilicity increase of the molecule that could be responsible of the adsorption phenomenon [17,18].

Non-specific adsorption (NSA) of peptides is well known among the scientific community [17–29], but due to the high variability of the peptides chemical structure, no general behavior has been identified yet [27,30]. The influence of the plastic (polypropylene, polyester, polyethylene, …) or glassware material used in peptide analysis has been deeply studied in literature [17,22,28,30]. Additionally, different approaches for the minimization of non-specific adsorption have been proposed [21,26,31]. Among these strategies, the modification of the surface using coating agents or proteins can be found [21,31–34]. Although this strategy has been successfully applied, Suelter *et al.* [31] demonstrated the low effectiveness of modifying the surface compared with the change on the nature of the solvent used for sample dilution or protein precipitation procedures. In this way, some authors studied the effect of the addition of organic solvent or the acidification of the sample [23,26,28,30]. Furthermore, the impact of surfactants and bovine serum albumin addition has been also studied for peptide adsorption minimization [19,26].

The increasing importance of CSF in the therapy against fungal infections encourages scientists to study its adsorption phenomenon due to the possible impact on MIC determination and on the reliability of analytical methods performed for resistance studies. Therefore, the aim of this paper is the study of the different parameters affecting the adsorption of CSF: different materials (sample container, material,…), solvent pH values and organic solvent proportion. For assays where sample manipulation is not possible, for example in vitro assays, a material treatment was investigated to prevent or minimize CSF adsorption. For this approach, different coating agents were tested for glass and plastic materials.

2. Materials and methods

2.1. Reagents

CSF diacetate (CAS – 179463–17-3) was purchased from Finetech (Wuhan, Hubei). Cell culture media used was RPMI 1640 with L-glutamine and MOPS obtained from Capricorn Scientific (Ebsdorfergrund Germany). 3-(N-morpholino)propanesulfonic acid (MOPS) and ammonium formate (for HPLC) were provided by Fluka (Burch, Switzerland). Sodium hydrogen carbonate (pro analysis) and potassium carbonate (reagent grade) were supplied by Merck (Darmstad, Germany). The (N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AATMS), vinyltrimethoxysilane (VTMS) and (3-glycidyloxypropyl)trimethoxysilane (GPTMS) coating agents were purchased from Sigma-Aldrich (St. Louis, USA). Acetonitrile (ACN) (HPLC LC-MS grade), methanol (MeOH) (HPLC-Isocratic grade), hydrochloric acid (37 %), dimethyl sulfoxide (DMSO) and trifluoroacetic acid (LC-MS grade) were obtained from VWR chemicals (Linars del Vallés, Spain). Acetic acid (LC-MS grade) was purchased from Fluka (Burch, Switzerland). Ultrapure analytical water was obtained from a Milli-Q Element A10 system (Millipore, Milford, USA).

2.2. Standard and working solutions

CSF stock solution was prepared at 1600 mg/L weighing CSF powder using an analytical balance Sartorius CP224 and dissolving it in DMSO. It was aliquoted and kept at −20 °C until working solutions preparation.

All the studies were performed at 0.25 mg/L CSF final concentration. The necessary working solutions were prepared in MOPS pH 7 at 0.5 mg/L and 0.83 mg/L. After 30 min of the working solution preparation, samples were prepared directly in vials by the transfer or dilution of the working solution.

2.3. Materials

Snap top normal glass vials (borosilicate clear glass type 1) and the corresponding plugs (with septum PTFE/silicone precut) were purchased from Scharlau (Barcelona, Spain). Commercially silanized glass vials (VWRI548-1366) were obtained from VWR (Fontenay-sous-Bois, France). Polypropylene vials (250 µL) and polyethylene terephthalate blood tubes (Vacutainer, label Z, 7 mL, 13x100mm) were supplied by Thermo Scientific (Waltham, Massachusetts, USA) and BD (Eysins, Switzerland), respectively. Eppendorf polypropylene 1.5 mL tubes were obtained from VWR. Universal pipette tips of 2–200 µL (Cat. No. 612–5755) and 100–1000 µL (Cat. No. 612–5756) of low-density polyethylene were bought from VWR chemicals. Nunclon delta surface 96-well plates (polystyrene) were purchased from Thermo Scientific.

2.4. Material treatment solutions

Plastic and glass materials were treated with AATMS, VTMS and GPTMS coating agents, following the procedure described by Fukazawa *et al.* [21]. In short: a 1 % (v/v) coating agent solution was prepared using 1 % (v/v) acetic acid solution as solvent. Coating solution was stirred for 1 h. Afterwards, the material was filled with this solution. Five

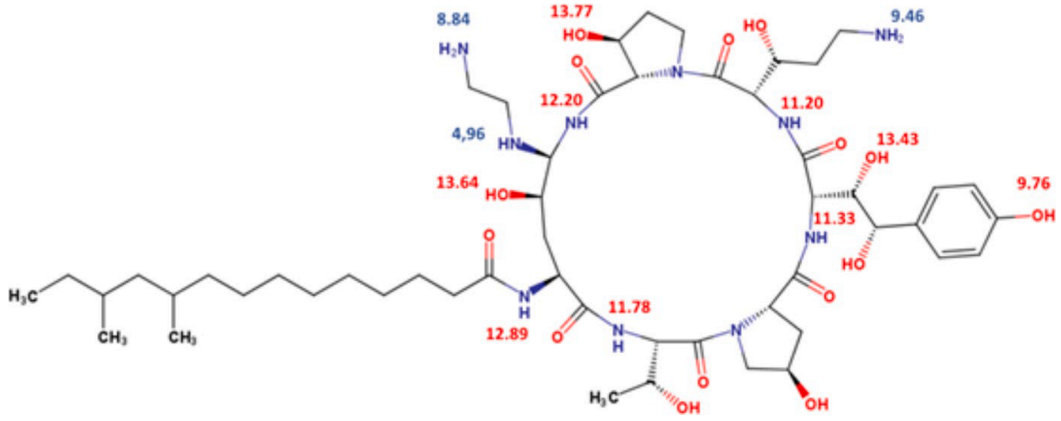

Fig. 1. Chemical structure of caspofungin with pKa values (from 0 to 14 range).

minutes after, the solution was removed and the material was left during 6 h at 50 °C for drying.

2.5. Chromatographic equipment and conditions

Acquity Ultra Performance Liquid Chromatography (UPLC) system (Waters, Milford, USA) coupled to a Fluorescence detector (FLD) was used. The chromatographic column used was an HSS T3 (50 × 2.1 mm, 1.8 μm) from Waters.

Chromatographic conditions consisted of an aqueous mobile phase of pH 2 adjusted with TFA (A) and acetonitrile as organic modifier (B) at a flow rate of 0.5 mL/min. The elution gradient was from 20 to 100 % of B in 6 min and at the end a re-equilibration step of 2 min. 5 μL of sample were injected. During the chromatographic analysis, the column was thermostated at 35 °C and samples were kept at 22 °C in the autosampler. The excitation/emission wavelengths of FLD detector were 229/300 nm.

In preliminary trials [13], a high inter day variability associated with a non-reproducible adsorption to glass material was observed even if using the same working solution during chromatographic injection. The strategy followed in this previous work to prevent this phenomenon was an independent preparation for each sample from the weighting of caspofungin powder until the final dilution using the exact same process. However, due to the high price of the caspofungin raw material, in this present work we use another approach because the main objective was to highlight the variation in the amount of caspofungin. As the exact concentration of caspofungin varies due to its adsorption, for each experimentation the starting concentration was established and then its decrease or increase was measured. To avoid any misinterpretation of the results due to these differences in starting concentration, the mean value of chromatographic peak areas obtained from repeated injections of a same solution were normalized. The normalization step was described in each part.

2.6. Non-specific adsorption to different laboratory materials

The solution of 0.25 mg/L CSF in MOPS buffer (10 mM, pH 7) was prepared in a volumetric flask and transferred to the different containers; glass vials, polypropylene vials, blood tubes (polyethylene terephthalate) and Eppendorf tubes (polypropylene). Five replicates of each one were analyzed and normalized with the data related to the glass vials.

2.7. NSA to glass chromatographic vials

2.7.1. Effect of coating agent treatment

The effect of the use of normal vials, commercially silanized vials, vials coated with AATMS, vials coated with GPTMS and vials coated with VTMS was studied using the same CSF solution (0.25 mg/L CSF in MOPS buffer at pH 7). Injections were performed at time 0 (t0) and after 24 h (t24). Five replicates of each one were analyzed randomly. The maximum percentage was obtained from the mean value of vials treated with AATMS and thus constituted the 100% for the normalization of the other results.

2.7.2. Variation of the NSA with time

0.25 mg/L CSF in MOPS pH 7 solution was placed either in a normal glass vial or in a glass vial coated with AATMS. Vials were left in the autosampler and injected several times during 12 h. Samples were injected each 10 min until 1 h, each 30 min from 1 h to 3 h and each hour until 6 h. Finally, a last injection was done at 12 h. Normalized results were performed for each material. All percentages are expressed related to the mean value of normal and coated vials of each series at t0.

2.7.3. Effect of organic solvent proportion

CSF working solutions (0.5 mg/L CSF and 0.83 mg/L CSF) were prepared and left in the volumetric flask for 30 min. The preparation targeted a final concentration at 0.25 mg/L CSF whatever the dilution solvent used. First the solvent, after the MOPS pH 7 and finally the CSF volume were added.

Different proportions between aqueous and organic phases were prepared.

For ACN, a 500 μL volume of 0.5 mg/L CSF solution was used in each vial. Different ratio of MOPS:ACN (v/v) for a 1 mL total final volume were tested: 1:0, 4:1, 3:2, 2:3, 1:4 and 0:1 corresponding to 0, 10, 20, 30, 40 and 50 % of ACN content, respectively.

For MeOH, a 300 μL volume of 0.83 mg/L CSF solution was used in each vial. Different ratio of MOPS:MeOH (v/v) for a 1 mL total final volume were tested: 1:0, 6:1, 5:2, 4:3, 3:4, 2:5, 1:6 and 0:1 corresponding to 0, 10, 20, 30, 40, 50, 60 and 70 % of MeOH content, respectively.

Samples in vials treated with AATMS and non-treated ones were analyzed. Five vials of each one were injected randomly at t0 and t24, at each time the mean and standard deviation were calculated. Then the data were normalized by selecting as the 100% the highest organic solvent content at t0 in AATMS.

2.7.4. Effect of pH

The effect of pH on the adsorption process was studied. pH values of 4, 7 and 10 were selected in order to cover a wide range of pH where CSF is found in different ionization states (Fig. 1).

A working solution of 0.50 mg/L in MOPS pH 7 (10 mM) was freshly prepared. A volume of 0.5 mL CSF solution was added to each vial already filled with 0.5 mL of the corresponding buffer solution. Solutions were prepared at pH 4, pH 7 and pH 10 using (formic acid/formate), (MOPS) and (carbonate/bicarbonate) buffers at 10 mM, respectively. Five vials of each one were injected randomly. At each pH the mean and standard deviation were calculated. Then, the data were normalized by selecting as the 100%, the pH 4 at t0 in AATMS treated vials.

2.8. Adsorption effect in microbiology 96-well plates and application to EUCAST protocol

Samples were prepared following the broth dilution procedure established by the EUCAST (*35*) and inoculated with *Candida albicans* reference strain 54550. In this technique, a two-fold serial dilution of CSF is made in the liquid medium from one well plate to the next one. Starting at 8 mg/L CSF in RPMI cell culture medium at pH 7 buffered by MOPS, a range from 4 mg/L to 0.015 mg/L was analyzed. Three replicates were injected of each condition after 24 h of incubation (treated and non-treated well plates with AATMS deactivated agent). The data were normalized with the 100% corresponding to the mean value from highest concentration measured in the AATMS treated vial.

2.9. Software and statistics

The chromatographic system control, data collection and data processing were accomplished using Empower 2 software.

For a sequence of experiments in HPLC, normalization data (normalized response %) were obtained by using the mean value of the highest chromatographic area obtained as the maximum (100 %) and the value for the other results from analysis were calculated as a percentage of this maximum value.

Comparison of the averages obtained was carried out using *t* test at 95 % confidence level (Microsoft excel). When $p < 0.05$ the result is significantly different.

Theoretical pKa values were obtained from MarvinSketch v17.22 (Fig. 1).

3. Results and discussion

3.1. NSA to different laboratory materials

In analytical or microbiology laboratories, a lot of different materials are commonly used, consisted of glassware, plastic such as vials, pipette tips, Pasteur pipettes, blood tubes, Eppendorf tubes, …. For that reason, firstly the study of the adsorption phenomenon of CSF was carried out with different types of materials.

In preliminary trials, any effect of adsorption when transferring the CSF solution with a glass pipette (Pasteur) or plastic pipette tips was not observed and this variable was not taken into account for the rest of the experiments. These manipulations being rapid, apparently the phenomenon of adsorption does not have time to take place.

A statistically significant effect ($p > 0.05$, 95 % confidence level) of the sample container was observed (Fig. 2), which is a main issue when using CSF solutions. Polypropylene HPLC vials resulted to be an unsuitable container for the studied analyte due to the low signal obtained. Furthermore, a signal decrease was seen when using blood tubes (polyethylene terephthalate) or Eppendorf tubes (polypropylene).

Based on the results obtained, the impact of using glass or plastic material must be considered when working with CSF solutions. Interestingly, a statistically significant difference ($p > 0.05$, 95 % confidence level) among the signals obtained for the plastic types has been seen. So, even if we can conclude that plastic materials induce statistically significant greater CSF adsorption compared to glassware, it is not enough to consider the type of plastic to foresee the adsorption. For example, HPLC vials and Eppendorf tubes are constituted both with polypropylene, however the adsorption phenomenon is quantitatively very different. A polymer is a complex structure with different variables as the degree of polymerization, the polymorphism or the additives used for its manufacture that have a significant impact in the structural nature of the plastic. Differences in physical properties can be easily observed when using HPLC vials and Eppendorf tubes: while polypropylene HPLC vials were soft and malleable, Eppendorf tubes were so much rigid. The opacity is also a feature between these containers, Eppendorf tubes were transparent whereas the HPLC vials were totally opaque. Probably the crystal structure of them is not the same and the nature of additives, this had a meaningful impact on CSF adsorption. This way, it is difficult to have a perspective without the exact composition of each material and this information is difficult to know.

3.2. NSA to glass chromatographic vials

3.2.1. Effect of coating agent treatment

Different coating agents were tested with the aim of minimizing the adsorption of CSF to material. HPLC glass vials were treated with different coating agents (AATMS, VTMS and GPTMS) and compared with non-treated ones. Commercially silanized glass vials were also assayed in this study. No statistically significant difference ($p > 0.05$, 95 % confidence level) was observed among the signals obtained for the different conditions using at t0. A high variability was observed in the results obtained within each series (Fig. 3). To understand these results we have to keep in mind that the adsorption procedure is time-dependent. In this way, even if using the same solution and the same kind of vials there is a difference in chromatographic response between the first and the last replicate analyzed of each series.

After 24 h, AATMS material treatment showed the weakest adsorption compared with the other conditions. However, we observed that glassware coating is a solution to delay adsorption of CSF, but cannot avoid it.

Acetonitrile was added after 48 h to samples corresponding to the 30 % - concentration. Their analysis resulted in an increase in the signal by two compared to the initial signal measured at t0 in MOPS at pH 7. The increase of the signal showed that ACN reversed the process of adsorption and that it is impossible to avoid adsorption, it was happening from the beginning of the experiments.

Treated plastic and glass materials with specific coating agents, following the procedures described by Fukazawa et al. [21] is a solution to delay adsorption of CSF, but cannot totally avoid it.

3.2.2. Variation of NSA with time

As we observed, the adsorption phenomenon is not totally immediate. In order to know the rate of the adsorption phenomenon, the evolution of CSF concentration with time was studied.

The adsorption profile of CSF 0.25 mg/L in MOPS pH 7 solution obtained during time in normal and treated vial (with AATMS) is shown in Fig. 4. A change in the kinetic profile when treating the glass vial with the coating agent was obtained. In Fig. 4, we could observe that the differences obtained between normal and treated vial were clear. At the second hour, where the adsorption of the normal vial is much more drastic compared with the treated one, CSF concentration decreases until 51 %. Meanwhile, in the treated one, the concentration of CSF was maintained within 90 % of the initial concentration for at least 3 h. This variation confirmed the hypothesis done to explain the reason of the high standard deviation seen in the previous experiment (Section 3.2.1). In this way, if the different conditions are studied during the chromatographic sequence, around 3 h can be used for injection of the five replicates of each series, between first and last replicate.

In the adsorption profile obtained for the normal vial, a plateau was reached after 6 h with a remaining amount corresponding to 10 % of the initial concentration. However, in the vial treated with AATMS, at 12 h the remaining concentration of CSF was 35 %, and the curve is still decreasing, a plateau was not reached. This fact explains the results obtained in a previous work [35], where a decrease in CSF concentration was seen in real samples analysis. In literature, this plateau has been also described for proteins and other molecules chemically related to CSF such as colistin [18]. In protein adsorption studies has been reported

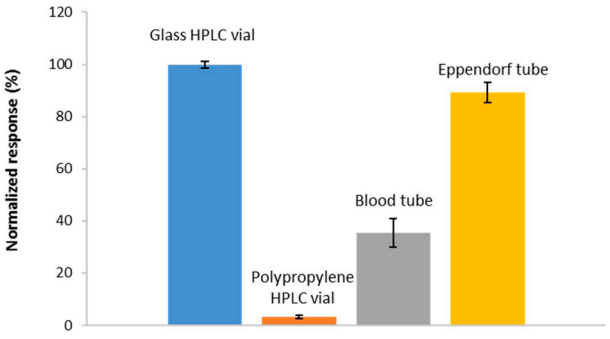

Fig. 2. Effect of different sample containers on CSF response (0.25 mg/L in MOPS pH 7, n = 5).

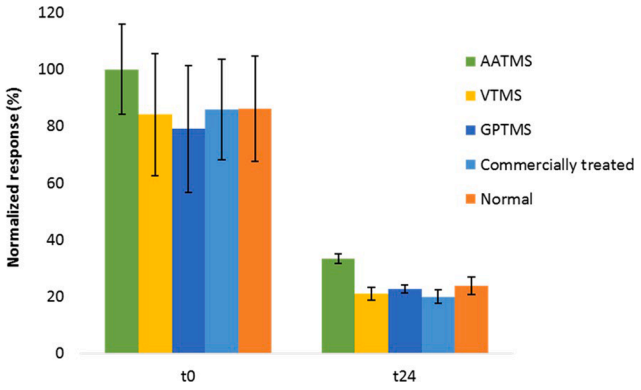

Fig. 3. Effect of different vial treatment on CSF response at t0 and t24 after preparation (n = 5).

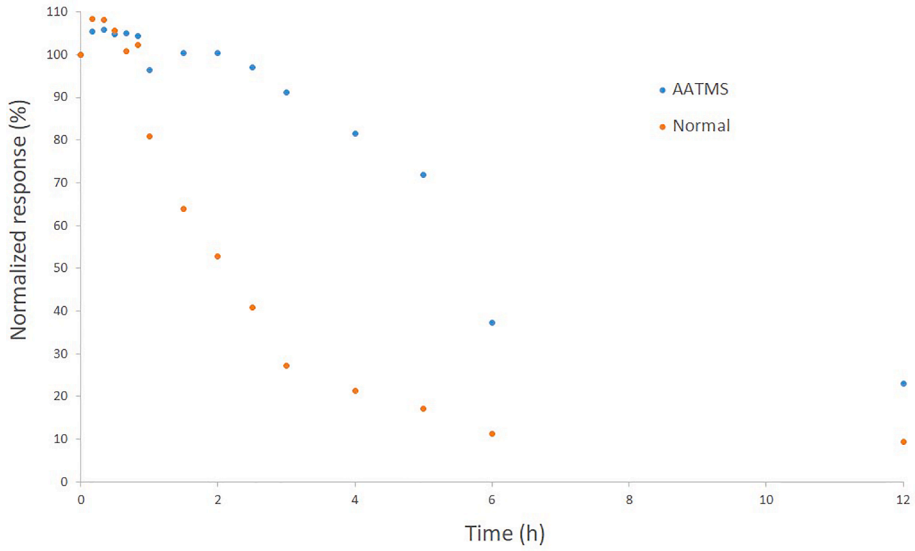

Fig. 4. CSF adsorption profile during time in normal and treated with AATMS glass vials. 0.25 mg/L CSF in MOPS at pH 7.

that a plateau is reached at some point of response-time variation, in some cases faster (60 min) and in others slower (24 h) [30].

As the coating treatment of the glassware having delayed the adsorption of CSF, it has been systematically applied in the following studies in order to give to the authors more time to process the samples.

3.2.3. *Effect of organic solvent proportion*

The effect of MeOH and ACN in the adsorption of CSF to vials was studied in this section, comparing their effect in normal vials and treated vials. Results obtained (Fig. 5) shown that the addition of organic solvent is a valuable way to minimize or avoid CSF adsorption to glass vials. It can be observed that as soon as 10 % of MeOH or ACN is added, a statistically significant increase of the signal is measured, except for MeOH in normal vials where obtained response kept comparable with the result obtained at 0 % MeOH.

The use of at least 30 % of ACN apparently avoids the adsorption at

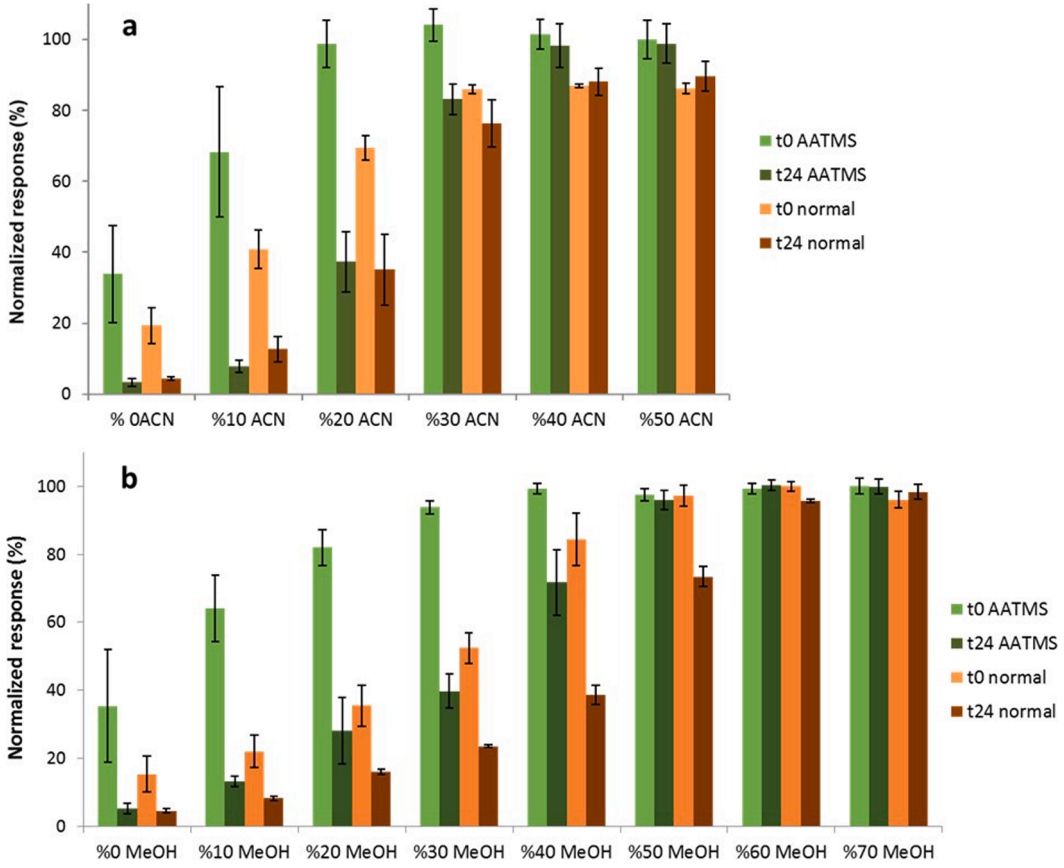

Fig. 5. Effect of ACN (a) and MeOH (b) on the response of CSF MOPS solution (pH 7). Treated with AATMS and normal vials, 0.25 mg/L CSF final concentration (n = 5).

t0, but it is not enough to suppress the adsorption during 24 h (Fig. 5 **a**). 40 % of ACN is required to ensure no effect of adsorption in 0.25 mg/L CSF samples after 24 h. Comparing treated and non-treated vials (exception 20 % ACN), no statistically significant difference has been observed ($p > 0.05$, 95 % confidence level), demonstrating that the use of an organic solvent is more noteworthy to prevent CSF adsorption than material treatment.

MeOH proved to be less effective to avoid adsorption (Fig. 5 **b)**, since a wider range of MeOH percentages (0 to 70 %) than ACN (0 to 50 %) was used for the study of solvent effect. As seen in Fig. 5, the percentage of MeOH required to avoid analyte adsorption is higher than in the case of ACN. At least 60 % of MeOH is needed to avoid the adsorption of CSF in freshly prepared samples in treated vials and to ensure quantification during 24 h. For vials non treated with AATMS, a 70 % of MeOH was found as the necessary minimum proportion to ensure comparable results at t0 and at t24 analyzed samples ($p > 0.05$, 95 % confidence level).

Results obtained in this section are supported by some studies found in literature where other authors proved the effectiveness of organic solvent addition to minimize the adsorption of peptides [23,26,28]. Addition of organic solvent is a valuable way to minimize or avoid CSF adsorption to glass vial: methanol is less effective to avoid adsorption than acetonitrile, as already was suggested by Fukazawa et al.[21] The impact of organic solvent addition on peptide adsorption is particularly noticeable with hydrophobic peptides. Organic solvents improve their solvation and thus improved their solubility.

3.2.4. *Effect of pH*

The effect of the pH in the adsorption of CSF was studied in a pH range providing different ionization states of CSF. From pH 4 to 10, ionization state of four chemical functions can changed (Fig. 1). In Fig. 6, an important difference in normalized response was observed depending on the pH values assayed. Results demonstrated a signal significantly higher at pH 4 whatever a treatment or not of the vials was used. In addition, at pH 4 no time dependence has been seen during 24 h. At pH 7 and pH 10, a significant decrease in signal was observed comparing with acidic conditions. At pH 4 the signals were maximum and no difference occurred between samples prepared in AATMS treated vials compared to non-treated ones, suggesting that at this pH adsorption is suppressed. At pH 7, a high variability at t0 was obtained, suggesting a rapid adsorption process. After 24 h, the signal was very weak and more repeatable, suggesting that an equilibrium was reached. At pH 10, all the results are very low (signals < 20%), in this condition the state of ionization of the molecule favors adsorption. The adsorption phenomenon of CSF seemed increase with the pH increase. pH changes can have several effects: 1) the global charge of the molecules and the glass material, and thus the forces of electrostatic interactions between CSF and the material; 2) the molecule solubility. These effects can occur simultaneously especially at pH 4 where glass material are more neutralized compared to pH 7 and 10.

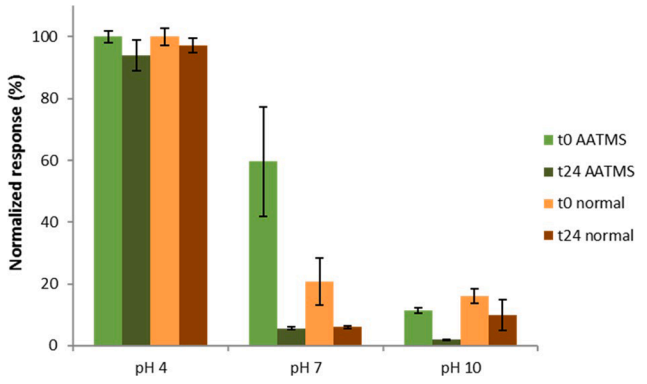

Fig. 6. CSF analysis at different pH values in vials treated and non-treated with AATMS (0.25 mg/L MOPS pH7 , n = 5).

3.3. *Effect of material treatment in EUCAST protocol of MIC determination*

Resistance studies of *candida spp*. are usually carried out by means of MIC calculation and comparison with reference values. As before mentioned, no MIC value is given for CSF in *candida* spp. due to the lack of interlaboratory reproducibility [8]. Taking in consideration the results obtained, the analyte adsorption to materials could explain the differences observed between laboratories. For this reason, the adsorption of CSF in 96-well plates was tested and the possibility of minimizing the adsorption by treating the material with a coating agent (AATMS, VTMS and GPTMS) was evaluated.

As observed for the glass vials, treatment with AATMS as a coating agent was the best option to minimize adsorption of CSF in the 96-well plates. The EUCAST procedure for the determination of MIC was performed using 96-well plates treated with AATMS and untreated plates. Results in Fig. 7 shown the differences in CSF amount obtained. The CSF concentrations in each position of the plates corresponded to successive dilutions from 4 to 0.125 mg/L of CSF. Only concentrations above 0.5 mg/L CSF were quantified, however all the responses were significantly higher with the 96-well plates treated with the coating agent. When adsorption occurs, the recovery is the more challenging for the low concentrations.

Results shown a minimization of CSF adsorption when treating the plates with AATMS. In addition, results indicated less variability in CSF responses with treated plates, which is an indication of repeatability improvement. The decrease in variability should have a large impact on the precision of the MIC determination.

We have demonstrated that surface treatment is a possibility to overcome the adsorption of CSF on plastic as already shown by Fothergill et al. [10]. However, a universal solution cannot be provided due to the great diversity of plastics and their batch-to-batch variability.

4. Conclusions

In this work, differences were found in the non-specific adsorption of CSF when using distinct laboratory materials. Dissimilarities within plastic types as low-density polyethylene, polypropylene and polyethylene terephthalate were noticed. Among the conditions studied, pH 4 was found as the best condition to avoid CSF adsorption in aqueous solution. The addition of MeOH and ACN, shown the ability to decrease adsorption to HPLC glass vials since low proportion (10 %). The addition of 40 % of ACN to CSF aqueous solutions (pH 7) has shown to avoid the adsorption of CSF in chromatographic glass vials at least for 24 h. With MeOH, this occurs using 60 % and 70 % of solvent for treated (with AATMS) and normal vials, respectively. Therefore, addition of ACN should be preferred to MeOH for analytical sample preparation for injection in RP-HPLC, due to ACN allowed the use of lower amount of organic solvent in order to have an efficient effect against adsorption. And if organic solvent should be avoided, acidification of sample is an adequate alternative.

Even though these approaches prevent the adsorption of CSF they are not compatible with conditions used in vitro or *in vivo* experiments performed for antifungal activity studies. Taking this into account, in this work we present a surface coating treatment for the sample container in order to avoid any sample manipulation or modification. The AATMS coating agent was found to be able to minimize and slow down the adsorption process of CSF with glass material for few hours. The treatment appears to reduce the adsorption in freshly prepared samples but is not totally effective. The adsorption still happens during time, but slower. Surface coating procedure with AATMS was also found to be effective in minimizing adsorption of CSF in 96-well plate experiments. Important CSF concentration increase was found when treating the plates suggesting why the MIC value cannot be classically

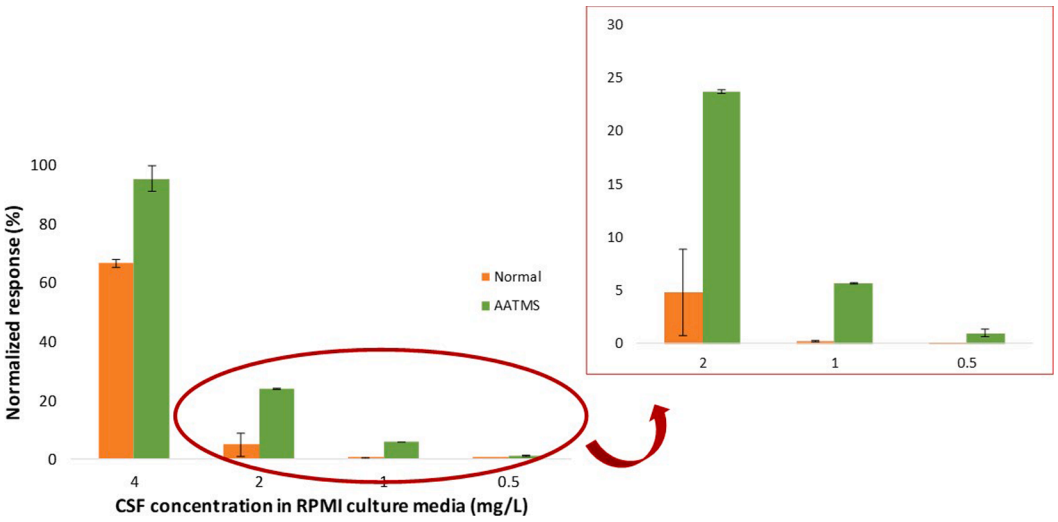

Fig. 7. CSF concentration from real samples obtained following the EUCAST protocol for MIC determination by broth dilution procedure (24 h of incubation). Comparison of normal and AATMS's treated 96-well plates (n = 3).

determined.

CRediT authorship contribution statement

B. Uribe: Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **A. Yaldebere:** Investigation, Formal analysis, Writing – review & editing. **O. González:** Conceptualization, Methodology, Writing – review & editing, Visualization. **X. Guruceaga:** Investigation, Formal analysis, Writing – review & editing. **A. Ramirez-Garcia:** Investigation, Formal analysis, Writing – review & editing. **A. Rementeria:** Conceptualization, Investigation, Formal analysis, Writing – review & editing. **B.B. Ba:** Writing – review & editing. **K. Gaudin:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Resources, Project administration, Funding acquisition. **R.M. Alonso:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Resources, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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