Impact of Biocompatible Poly(ethylene glycol)-*block*-Poly(ε-caprolactone) Nano-Micelles on the Antifungal Efficacy of Voriconazole

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Abstract: This study aimed to encapsulate voriconazole into nano-micelles of poly(ethylene glycol)-*block*-poly(ε-caprolactone) to enhance its antifungal activity and reduce the required doses. The nano-micelles were prepared at various drug/polymer ratios, and their various physicochemical properties were studied. The nano-micelles had a small particle size in the range of ~50-60 nm and homogenous size distribution. The nano-micelles had high encapsulation efficiency and loading capacity in the range of ~40-95% and ~20-27%, respectively. Both encapsulation efficiency and loading capacity could be modulated by changing the drug/polymer ratio. Voriconazole release from the nano-micelles was much slower than the drug solution. The drug release pattern was biphasic, with a relatively faster initial phase followed by a sustained release. The antifungal efficacy was evaluated in vitro against Aspergillus flavus and Candida albicans using the drug solution in dimethyl sulfoxide/water as a control. The inhibition zone diameters of the fungi increased with increasing the drug concentration. The diameter of the inhibition zones against Aspergillus flavus was comparable for the nano-micelles and control. In contrast, the nano-micelles had significantly wider inhibition zones against Candida albicans than the control. These results show that poly(ethylene glycol)-*block*-poly(ε-caprolactone) nano-micelles could be used as a promising delivery system to enhance voriconazole antifungal efficacy.

Keywords: voriconazole; fungal infections; polymeric micelles; topical delivery.

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1. Introduction

Superficial fungal infections affect a quite appreciable percentage of the population worldwide. They affect the skin, hair, or nails, and their incidence rises. They are caused mainly by dermatophytes which are different based on the geographical location [1]. Dermatophytes are a group of similar mold fungi, and their infection is rarely life-threatening. However, they cause significant morbidity worldwide. Dermatophytes have the highest prevalence among fungal infections and affect about 25% of the human population [2]. Superficial fungal infections by dermatophytes are more spread in tropical countries since these fungi need humid
and warm climates to flourish [2]. In some other cases, the fungal infection involves internal organs or systems, leading to more serious and life-threatening systemic diseases. Systemic fungal infections are particularly alarming in critically ill or immunocompromised patients for whom they cause severe morbidity and mortality [3]. This is reflected in a high mortality rate for invasive fungal infections; in the range of 30%, and some cases, it could reach 90%, such as those in AIDS patients [4]. It was also found that invasive fungal infections such as mucormycosis result in remarkably high mortality in COVID-19 patients, especially if the infection occurs in patients having diabetes mellitus, malignancy, or other immunocompromised patients [5,6]. Whether superficial or systemic, the type of infection is influenced by several factors, such as the causative fungi, the site of infection in the body, the fungal load causing the infection, and the patient immunological status [4].

There are several efficacious antifungal drug groups, including azoles, echinocandins, polyenes, allylamines, polyketides, and others. Despite this availability, there is an ongoing endeavor to introduce new agents [7-9]. This is due to the limited spectrum of activity of the available drugs, drug resistance, adverse drug reactions, drug interactions, limited aqueous solubility, and insufficient drug concentrations at the site of action [8]. For instance, the azoles (imidazole and triazole) groups which contain the largest number of antifungal drugs, are notorious for hydrophobicity and limited aqueous solubility resulting in poor bioavailability, challenging formulation, and limited choices for administration routes [10,11].

Voriconazole is a second-generation triazole antifungal agent derived from fluconazole [12]. It has remarkably enhanced in vitro antifungal activities against many Candida species and Aspergillus fumigatus compared with fluconazole [13]. It was approved for human use in the United States in 2002 [14]. Its mechanism of action involves inhibition of ergosterol biosynthesis through the inhibition of fungal cytochrome P450-dependent 14-α-sterol demethylase leading to potent antifungal efficacy against a number of fungal species less common molds [15]. However, oral and intravenous administrations of voriconazole cause several side effects such as those on the gastrointestinal tract (nausea, vomiting), liver (high hepatic enzyme levels, cholestasis, hepatocytolysis, or their combination), and skin rashes [16,17]. Its adverse effects profile is even more troublesome, especially for long-term use. Thus, long-term use of voriconazole is associated with increased risk of cutaneous malignancies, particularly squamous cell carcinoma, phototoxicity, periostitis, hallucinations and encephalopathy, peripheral neuropathy, alopecia, nail changes, and hyponatremia. These limitations often result in poor patient compliance. To overcome these shortcomings, voriconazole was incorporated into various nanoparticle-based drug delivery systems. For instance, self-nanoemulsifying drug delivery systems, cationic nanostructured lipid carriers, solid lipid nanoparticles, nanospray dried powder, and nanosuspensions were used to improve the transcorneal permeability voriconazole [18-21]. Liposomes improved voriconazole tissue distribution and enhanced its antifungal activity [22]. In the same vein, ethosomes were employed to improve voriconazole antifungal efficacy and facilitate its delivery through deeper skin layers [23]. Other nanoparticle systems used included human serum albumin-based nanoparticles, polylactic-co-glycolic acid nanoparticles, and chitosan-coated poly lactic-co-glycolic acid nanoparticles [24-28]. These nanocarriers provided sustained drug release and enhanced antifungal efficacy.

Despite the wide spectrum of the nanocarriers employed as delivery systems for voriconazole, a careful review of the literature showed that polymeric micelles were not exploited as delivery systems. These micelles could bring an interesting perspective to drug
delivery nanocarriers due to their intriguing attributes. They are formed from amphiphilic biocompatible and/or biodegradable copolymers, ensuring minimal or no harm to humans [29]. Their amphiphilic nature allows their self-assembly in aqueous solutions in a way that facilitates hydrophobic drug incorporation into the micelle core. This improves the aqueous solubility of hydrophobic drugs, sustaining their release and increasing their permeability through biological barriers [30–31]. The hydrophobic block of the copolymers used in micelle formulation is usually aliphatic polyester-based polymers such as polylactic-co-glycolic acid, poly(caprolactone), and poly(lactide) due to their biocompatibility and biodegradability [32,33]. The most common hydrophilic block is poly(ethylene glycol) due to its water solubility, biocompatibility, and ability to increase the micelle longevity in the blood [33,34].

In light of the above, this study aimed to prepare and characterize poly(ethylene glycol)-block-poly(ε-caprolactone) nano-micelles as delivery systems for voriconazole to improve its aqueous solubility, sustain its release and enhance its antifungal efficacy. The nano-micelles were prepared by the co-solvent evaporation method and evaluated using various techniques. The in vitro antifungal activity was tested against Candida albicans and Aspergillus flavus.

2. Materials and Methods

2.1. Materials

Voriconazole was purchased from Cerilliant, Round Rock, TX, USA. Dialysis membranes (Spectra/por, MWCO: 3.5–5 kDa, unless otherwise indicated) were purchased from Fisher Scientific (Rancho Dominguez, CA). Sabouraud's dextrose agar was purchased from Sigma Aldrich, St. Louis, MO, USA. Acetone and acetonitrile were purchased from Adwic, EL-Naser Pharmaceutical Co., Cairo, Egypt. The block copolymer poly(ethylene glycol)-block-poly(ε-caprolactone) (PEG2-b-PCL5) was purchased from Polymer Source (Dorval, Canada). The molecular weight of PEG was 2 kDa (degree of polymerization ~ 33), while that of PCL was 5 kDa (degree of polymerization ~ 88). All other chemicals were of reagent grade.

2.2. Methods

2.2.1. Preparation of empty and voriconazole-loaded polymeric nano-micelles

Voriconazole-loaded nano-micelles were prepared by the co-solvent evaporation method [35-37]. Certain weights of voriconazole and polymer (drug/polymer weight ratio of 0–50%) were solubilized in 2.5 mL of acetone. The resulting solution was added dropwise (1 drop/10 s) to 5 mL of deionized water under magnetic stirring in an open glass vial. The mixtures were kept under magnetic stirring in open vials for 24 h to remove acetone and trigger the formation of drug-loaded micelles. Unloaded (free) voriconazole was separated from the drug-loaded micelles by filtration using a 0.45 μm PVDF filter. Empty polymeric micelles were prepared the same way and used as a control.

2.2.2. Determination of voriconazole encapsulation efficiency and loading capacity

A certain volume of filtered voriconazole nano-micelles was diluted 10 times with acetonitrile to break the micellar structure. The absorbance of the resulting solution was measured spectrophotometrically at λ max of 256 nm after proper dilution (UV-spectrophotometer, Schimidzu-50–02, Schmidzu Corp, Kyoto, Japan). Drug concentration was
determined using a calibration curve ($R^2 > 0.998$). Voriconazole encapsulation efficiency and loading capacity were calculated using equations 1 and 2:

\[
\text{Encapsulation efficiency (weight %)} = \frac{\text{Weight of drug in the micelles}}{\text{Weight of drug used initially}} \times 100 \quad (1)
\]

\[
\text{Loading capacity (weight %)} = \frac{\text{Weight of drug in micelles}}{\text{Weight of micelles tested}} \times 100 \quad (2)
\]

2.2.3. Determination of particle size and polydispersity index.

The micelle hydrodynamic diameter ($D_h$) and polydispersity index (PDI) were determined using a Malvern ZetaSizer (Nano-ZS, Malvern Instruments, Worcestershire, UK). The instrument had a He-Ne laser operating at 633 nm and an avalanche photodiode detector. The samples were measured directly after preparation and filtration through a 0.45 μm PVDF filter without further treatment. Measurements were done in triplicate at room temperature.

2.2.4. Transmission electron microscopy (TEM) measurements.

The size and morphology of voriconazole-loaded nano-micelles were characterized using a high-resolution transmission electron microscope (Hitachi-H7650, Santa Clara, CA). An aliquot of the sample (100 μL) was added onto a 200-mesh TEM copper grid. The sample was left to dry at room temperature for 5 min, and then the excess sample was carefully removed by a piece of filter paper. Subsequently, the sample was negatively stained using an aliquot of aqueous uranyl acetate solution (2%, w/v). The instrument operated at an accelerating voltage of 80 kV. The data acquisition was made using an AMT-700 capture image camera (Advanced Microscopy Techniques, Woburn, MA).

2.2.5. In vitro drug release studies.

\textit{In vitro} drug release studies were carried out using a standard semi-permeable dialysis membrane. The dialysis membrane was firmly stretched over one end of a glass tube opened from both sides and had an internal diameter of 2.4 cm by means of a rubber band. The tested formulation (3 mL equivalent to around 3.5 mg voriconazole) was introduced over the membrane in the dialysis tube. Similar drug concentration in dimethyl sulfoxide (DMSO) was tested and used as a control. The tube was suspended in 25 mL of pH 7.4 phosphate buffer. The experiment was carried out in a shaking water bath adjusted to 37 ± 0.2 °C and 50 RPM. Aliquots (3 mL) were withdrawn from the release medium at different time intervals (1, 2, 3, 4, 5, 6, 12, 24, 48, 72 h) and replaced with an equal volume of release medium kept at the same temperature. The concentration of the drug in the release samples was determined spectrophotometrically by taking the absorbance at 256 nm after proper dilution against a blank (UV-spectrophotometer, Schmidzu-50–02, Schmidzu Corp, Kyoto, Japan) and using a constructed calibration curve ($R^2 > 0.998$). The cumulative amount of drug released was calculated and plotted as a function of time. All experiments were carried out in triplicate, and the mean ± SD was calculated.
2.2.6. *In vitro* antifungal activity studies.

2.2.6.1. Fungal strains.

*Candida albicans*, Assiut University Mycological Center (AUMC) strain No. 1299, and *Aspergillus flavus*, AUMC strain No. 1276 were obtained from AUMC. Fungal strains were individually grown on Sabouraud's dextrose agar species and incubated at 28 °C for 5 days [38]. Fungal cells were harvested and transferred to sterile vials containing autoclaved distilled water. The colony-forming units (CFU) were counted in each cell suspension by a hemocytometer and adjusted to $10^4$ CFU/mL.

2.2.6.2. Assay medium.

Sabouraud's dextrose agar medium (10 g/L peptones from meat, 40 g/L glucose, 15 g/L agar, and chloramphenicol 0.5 g/L) was prepared. One mL of the fungal cell suspension was transferred to sterile Petri plates (9 cm diameter), followed by the addition of 20 mL of the autoclaved medium just before solidification. Plates were rotated clockwise and anticlockwise to allow thorough mixing of fungal cells with the medium then left for solidification. A sterile metallic cork borer was used to make holes accepting 50 µL of the tested solution.

2.2.6.3. Assay procedure.

Various concentrations of voriconazole-loaded nano-micelles were prepared (equivalent to voriconazole concentrations of 1155, 577, 288, 144, 72 µg/mL) and transferred to holes made in cultures of *C. albicans* and *A. flavus*. Identical drug concentrations in DMSO were also prepared and used as controls. The assay cultures were kept at 28 °C, and the inhibition zones around the holes were measured (in mm) after 2 days. Three replicates were used for each drug concentration. Clotrimazole was used as a positive reference control. Blank nano-micelles were prepared above but without drugs and used as a negative control.

2.2.6.4. Statistical analysis.

All the experiments were run in triplicate, and the mean ± SD was calculated. Statistical studies were performed using GraphPad Prism software version 8.0.1 (GraphPad Software Inc., La Jolla, CA). One-way analysis of variance (ANOVA) with Newman–Keuls posthoc test was used to test the differences. A $p$-value less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Preparation of voriconazole-loaded polymeric nano-micelles.

The PEG2-b-PCL5 copolymer is known for its ability to form polymeric micelles in water due to the presence of a hydrophilic block (PEG) and a hydrophobic one (PCL) with sufficient balance between them and to minimize the system interfacial free energy [39]. Other studies showed that these micelles had a size in the nanometer range and successfully served to enhance the aqueous solubility and biological activities of several hydrophobic drugs such as sertaconazole and triamcinolone acetonide [35,37]. This study aimed to extend the applicability of PEG2-b-PCL5 polymeric micelles to improve voriconazole aqueous solubility and antifungal activity and eventually provides an aqueous-based delivery system that avoids the side effects of the currently available systems. We first confirmed nano-micelle formation...
by dynamic light scattering studies. Results showed that the nano-micelles, both free and drug-loaded, had narrow size distribution. This is reflected in a small polydispersity index (PDI) of 0.12±0.003 for blank nano-micelles and 0.15±0.003 for the drug-loaded ones (formulation F4 prepared at 100% drug/polymer weight ratio) (Table 1). It was shown that PDI smaller than 0.05 indicates highly monodispersed nanoparticles, whereas values greater than 0.7 indicate a very broad particle size distribution [40]. This confirms the unimodal size distribution of voriconazole nano-micelles. Uniform size distribution is an important asset for nanoparticles to ensure drug delivery to the same target within the body and hence maximize drug efficacy. The nano-micelles were also characterized by TEM measurements to study their size and morphology. Figure 1 shows that voriconazole-loaded nano-micelles (formula F4) had a uniform spherical shape with no clusters or agglomerates. The particle size was 53.4±12.1 nm. The size obtained by DLS measurement for the same formulation showed a hydrodynamic diameter of 60.38±1.24 nm. This size is larger than that observed by TEM measurements which are commonly observed in the literature due to the dry nature of TEM samples. In contrast, the size measured by DLS reflects the hydrated status of the nanoparticles in solution [41].

3.2. Effect of voriconazole/polymer weight ratio on the drug encapsulation efficiency and loading capacity.

Nano-micelles prepared for drug delivery applications should have the highest possible drug content to take full advantage of the drug/polymer ratio and thus ensure the use of minimum polymer amounts. To reach the highest voriconazole content of the nano-micelles, they were prepared at various drug/polymer weight ratios (25-100%), and their drug encapsulation efficiency and loading capacity were determined. Table 1 shows that the solubilized drug concentration increased with the drug/polymer ratio increase. Thus, the drug solubility increased from around 0.7 mg/mL to around 1.2 mg/mL when the drug/polymer ratio was increased from 25 to 100%. Despite this increase in solubility, the percent encapsulation efficiency decreased from 95.22±0.15% (F1) to 38.54±0.02% (F4). This different trend is attributed to how the percent encapsulation efficiency was calculated. The solubilized drug amount was divided over increasing drug amounts used initially in nano-micelle preparation. Since the increase in the solubilized drug is not proportional to that of the drug used initially, this resulted in decreased encapsulation efficiency. Nonetheless, the encapsulation efficiency was high in the range of around 65-95%, especially for the nano-micelles prepared at lower drug/polymer ratios. It is noteworthy that the same copolymer micelles showed lower drug encapsulation efficiency for other drugs such as sertaconazole (around 40%) and paclitaxel (around 70%) [35,42]. The different encapsulation efficiencies for different drugs might be related to several factors, such as the drug hydrophobicity, molecular weight, and drug-copolymer compatibility [43,44]. Table 1 also shows that the percent loading capacity increased with the drug/polymer ratio increase.

Table 1. Voriconazole encapsulation efficiency and loading capacity for various nano-micellar preparations at a 3 mg/mL polymer concentration.

| Formula code | Drug /polymer weight ratio | Solubilized drug (mg/mL) | Encapsulation efficiency (%) | Loading capacity (%) |
|--------------|---------------------------|--------------------------|----------------------------|---------------------|
| F1           | 25%                       | 0.71±0.001               | 95.22±0.15                 | 19.23±0.02          |
| F2           | 50%                       | 1.01±0.001               | 67.36±0.58                 | 25.19±0.16          |
| F3           | 75%                       | 1.01±0.004               | 45.07±0.16                 | 25.26±0.07          |
| F4           | 100%                      | 1.16±0.001               | 38.54±0.02                 | 27.82±0.01          |
Thus, the increase in the ratio from 25 to 100% resulted in an increase from around 19 to around 28% for the percent loading capacity. This is significantly higher than the percent loading capacity seen in the literature for most drug/copolymer micellar combinations, usually in the range of 5-10% [44].

While the encapsulation efficiency measures the efficiency of the preparation method to get the drug encapsulated into the micelles, the loading capacity measures the percent drug content in the micelles (equations 1 & 2). Accordingly, the percent loading capacity is more important for clinical applications since the higher its value, the lower the polymer/excipients needed to achieve a given therapeutic effect. Formulation F4 showed the highest loading capacity among the studied formulations (27.82±0.01%, Table 1) and the highest solubilized drug concentration (around 1.2 mg/mL), making it the most appropriate one for further studies.

Figure 1. A TEM photomicrograph of voriconazole-loaded nano-micelles (Formula F4).

3.3. Effect of voriconazole/polymer weight ratio on the particle size and polydispersity index.

Particle size affects the colloidal stability of the micelles and their in vivo behavior, bioavailability, and therapeutic efficacy [45,46]. Table 2 shows that the nano-micelle hydrodynamic diameter was in the range of 50 to 60 nm. The size generally increased with the increase in the drug/polymer ratio and the drug content of the nano-micelles.

| Formula code | Drug/polymer weight ratio | Hydrodynamic diameter (nm) | Polydispersity index |
|--------------|---------------------------|----------------------------|---------------------|
| F1           | 25%                       | 56.19±0.05                 | 0.18±0.02           |
| F2           | 50%                       | 49.57±0.41                 | 0.14±0.005          |
| F3           | 75%                       | 58.59±0.33                 | 0.14±0.01           |
| F4           | 100%                      | 60.38±1.24                 | 0.15±0.003          |

The size difference between any two drug/polymer ratios was statistically significant ($p<0.05$). Thus, increasing the ratio from 25% (drug content 19.23±0.02%) to 100% (drug...
content 27.82±0.01%) resulted in increasing the particle size from 56.19±0.05 to 60.38±1.24 nm (Table 2). All the micellar formulations (F1-F4) had a size below 100 nm, a property that increases their potential to improve the drug permeability through biological membranes and thus, augment therapeutic drug efficacy.

Previous studies showed that nanoparticles having a size of 50 and 100 nm had an oral absorption in rats of 34% and 26%, respectively. In contrast, the 300-nm particles had negligible oral absorption [47]. The polydispersity index for all the formulations was in the range of 0.14 to 0.18. This confirms the monodispersity of voriconazole-loaded nano-micelles [48]. Based on the above results, formulation F4 was selected for further studies since it had the highest percent drug loading capacity (27.82±0.01%), highest solubilized drug concentration in water (1.16 mg/mL at a polymer concentration of 3 mg/mL), reasonably small particle size (60.38±1.24 nm) and unimodal size distribution (PDI: 0.15).

3.4. In vitro drug release studies.

Figure 2 shows the in vitro drug release profiles of voriconazole nano-micelles (formulation F4) compared to the drug solution in DMSO, which was used as a control. The dialysis method is a simple and convenient method that is widely used to study drug release from nanoparticles. Previous studies showed its applicability in distinguishing the release profiles of various formulations from each other and from the control [37,49,50]. Drug solution in DMSO showed rapid release through the dialysis membrane, and complete release was achieved within 24 h. This rapid diffusion confirms that the dialysis membrane did not hinder drug release.

On the contrary, voriconazole was released much slower from the nano-micelles, especially after 8 h. Drug release from the nano-micelles followed a biphasic fashion; the first phase had fast drug release and lasted for about 8 h. This was followed by another phase where the drug was released much slower. Biphasic drug release was previously observed for several other nanoparticle formulations and was attributed to the presence of some drug near the surface of the nanoparticles, which rapidly dissolved, resulting in the faster release. The other drug portion was incorporated deep inside the nanoparticles, and thus it needed more time to be released, resulting in the second slower phase [51,52].

![Figure 2](https://biointerfaceresearch.com/)

**Figure 2. In vitro release profiles of voriconazole from its micellar solution (formulation F4) compared to the drug solution in DMSO. The experiment was done at 37 °C in phosphate buffer pH 7.4.**

3.5. In vitro antifungal efficacy studies.
The *in vitro* antifungal efficacy of voriconazole incorporated into PEG2-\-b\-PCL5 nano-micelles (formulation F4) was tested against *Aspergillus flavus* and *Candida albicans*. These fungal strains were selected based on their known ability to cause human diseases [53,54]. The two fungal strains were subjected to serial dilutions of voriconazole micellar stock aqueous solution having a drug concentration of 1.155 mg/mL. Same drug concentrations in a mixture of water/DMSO (1:1, v/v) were used as controls.

**Figure 3.** Photomicrographs of *Aspergillus flavus* treated with different concentrations of voriconazole. (a) voriconazole in DMSO water/DMSO (1:1, v/v), (b) voriconazole in nano-micelles. A: drug concentration of 1.55 mg/mL, B: 0.577 mg/mL, C: 0.288 mg/mL, D: 0.144 mg/mL, E: 0.072 mg/mL.

Photomicrographs of *A. flavus* and *C. albicans* plates treated with various voriconazole concentrations in either DMSO or nano-micelles are shown in Figures 3 and 4, respectively.
The diameters of the inhibition zones for various samples were measured and plotted against drug concentration (Figure 5).
Figure 4. Photomicrographs of Candida albicans treated with different concentrations of voriconazole. (a) voriconazole in water/DMSO (1:1, v/v). (b) voriconazole in nano-micelles. A: drug concentration of 1.55 mg/mL, B: 0.577 mg/mL, C: 0.288 mg/mL, D: 0.144 mg/mL, E: 0.072 mg/mL.

Figure 5A shows the diameters of voriconazole nano-micellar solution inhibition zones and control against A. flavus. The inhibition zone diameter depended on the drug concentration for both the nano-micelles and control. There was a nearly linear increase in the diameter with the increase in drug concentration to 0.288 mg/mL. Subsequently, the increase in the diameter was not as sharp for the nano-micelles and control.

The diameters were higher for voriconazole nano-micelles for most of the studied concentrations compared with the control. However, the difference was statistically significant ($p<$0.05) only for the samples having a drug concentration of 1.155 and 0.288 mg/mL. Despite this similar antifungal efficacy for voriconazole nano-micelles and control solution against A. flavus, the nano-micelles offer significant advantages compared to the water/DMSO solution. First, the control solution contains 50% DMSO which is not suitable for clinical use at this high concentration. Second, the nano-micelles had better control over the drug release rate than the drug solution (Figure 2). In addition, the drug solution had easier diffusion through the agar medium, which might lead to better and faster interaction with the fungi. Taken together, all these observations support the conclusion that voriconazole nano-micelles are better than their DMSO/water solution despite having similar antifungal efficacy. Several other studies reported similar antifungal activities for drug-loaded nanoparticles and control solutions [55,56].

Figure 5B shows that the inhibition zones against C. albicans were larger than those of the control at all the studied concentrations. Statistical analysis showed that the differences were statistically significant ($p<$0.05) for all the tested concentrations. The differences were more pronounced at lower drug concentrations. Thus, the diameters of the zones were 31.33±1.15 and 20.33±0.57 mm for voriconazole nano-micelles and control solution, respectively, at a drug concentration of 0.072 mg/mL. Enhanced antifungal efficacy for the micellar drug solution at lower drug concentration might result in smaller drug doses, reduced drug adverse effects, and better patient compliance. The enhanced antifungal activity for the nano-micelles might be attributed to their ability to solubilize the drug and improve its interaction with the fungi walls. In addition, the nano-micelles had a small size (around 60 nm) which probably facilitated drug uptake through the fungal walls. These results agree with previous studies, which showed better antifungal efficacy of drugs incorporated into polymeric micelles [7,35,57].
Figure 5. Diameter of inhibition zones (mm) as a function of drug concentration for voriconazole nano-micelles and voriconazole solution in water/DMSO (1:1, v/v) against (a) A. flavus and (b) C. albicans.

4. Conclusions

Voriconazole-loaded nano-micelles were successfully prepared using PEG2-b-PCL5 copolymer at various drug/polymer ratios. The nano-micelles had a particle size smaller than 100 nm and monomodal size distribution. High drug encapsulation efficiency (up to ~95%) and loading capacity (up to ~27%) was obtained. These two important micelle attributes could be modulated by changing the drug/polymer ratio used in micelle preparation. The optimum formulation of drug encapsapsulation efficiency, loading capacity, and particle size was tested further for drug release patterns and in vitro antifungal activity. Drug release from the nano-micelles showed a biphasic pattern with a relatively fast phase for 8 h followed by a slower phase. In vitro antifungal activity of the nano-micelles was tested against A. flavus and C. albicans and compared with the drug solution in DMSO/water. The nano-micelles and drug solution had similar activity against A. flavus. However, the nano-micelles were much more effective against C. albicans. Collectively, these results confirm that PEG2-b-PCL5 could serve as an efficient delivery system of voriconazole against fungal infections, especially those caused by C. albicans.

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None.

Conflict of interest

The authors declare no conflict of interest.

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