Lipid-covered drug particles: combined action of dioctadecyldimethylammonium bromide and amphotericin B or miconazole

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Objectives: Coverage of antifungal drug particles (miconazole and amphotericin B) with cationic lipid and evaluation of a synergistic action between lipid and drug.

Methods: Miconazole and amphotericin B were mixed with cationic bilayer fragments (BF) of dioctadecyldimethylammonium bromide (DODAB) at extreme drug to lipid molar proportions (P). Light scattering for particle sizing and zeta-potential analysis evaluated effects of cationic lipid on drug particle size and charge. Colony counts evaluated viability of Candida spp. and Cryptococcus neoformans over a range of P.

Results: BF loading capacity for monomeric amphotericin B is 0.1 mM amphotericin B at 2 mM DODAB (P = 1:20). Above this low P, amphotericin B aggregates in the dispersion. At high P, addition of chaotropic K2HPO4 (0.2–2 mM) converts miconazole or amphotericin B aggregates into negatively charged particles with affinity for cationic lipid, which then surrounds each drug particle with a cationic layer. The combined in vitro action of lipid-covered drug particles against Candida and C. neoformans depends on P and interaction time. DODAB by itself kills C. neoformans and Candida at 2 and 2 to >250 mg/L minimal fungicidal concentration (MFC). In combination, over the first hour, fungicidal activity is due to DODAB with lipid capsules retarding drug action. At 48 h interaction time and 10^4 cfu/mL, MFC (mg/L) against Candida albicans is reduced from 4 to 1 amphotericin B (at 2 DODAB), and from 8 to 1 miconazole (at 1 DODAB).

Conclusions: DODAB may be a suitable candidate for use in combination with miconazole for antifungal therapy.

Keywords: antifungal activity, synergism, chaotropic effect, bilayer-covered drug particle

Introduction

The incidence of fungal infections is expected to increase continuously for the foreseeable future due to increased numbers of patients with immunodeficiency conditions caused by AIDS, ageing, organ transplantation or cancer therapy.1–3 There are two clinically important classes of antifungal hydrophobic drugs: the polyenes (e.g. amphotericin B) and the azoles (e.g. miconazole).4 On the other hand, some double-chained synthetic lipids such as dioctadecyldimethylammonium bromide (DODAB) or sodium dihexadecylphosphate (DHP) self-assemble in aqueous solution yielding closed bilayers (vesicles) or disrupted vesicles [bilayer fragments (BF), or discs] depending on the procedure used for dispersing the lipid.5 DODAB, in particular, bears a quaternary ammonium moiety as cationic polar head, which imparts to this cationic lipid outstanding anti-infective properties.6 Both amphotericin B and miconazole self-assemble and solubilize at hydrophobic sites of DODAB or DHP BF in water solution exhibiting in vivo therapeutic activity.7–10 Furthermore, sterically (PEGylated) stabilized BF have recently been characterized and employed in the rapid evaluation of amphipathic drug partition.11 In medicinal chemistry, aggregation of hydrophobic drug molecules in aqueous solutions has been shown to have an effect on drug bioavailability.12 The uptake rate into systemic circulation would depend on having hydrophobic drug aggregates of...
appropriate size available for absorption. At high drug to lipid molar proportion (P), hydrophobic drug aggregates (or particles) have been encapsulated by oppositely charged bilayers, whereas hydrophobic drug molecules could be easily solubilized by the rim of BF at low P. In this work, the interaction of hydrophobic antifungal drugs such as miconazole and amphotericin B with cationic BF of DODAB is systematically evaluated at extreme P by means of dynamic light scattering for particle size distribution and zeta-potential evaluation of changes on drug particle charge. Thereby, at low P, loading capacity of DODAB BF with drug was determined whereas, at high P, lipid coverage of drug granules produced some formulations acting synergistically against clinically important yeast.

Materials and methods

Drugs, lipids and microorganisms

DODAB, 99.9% pure, was obtained from Sigma Chemical Co. (St Louis, MO, USA). Amphotericin B (batch 0008000336) was purchased from Bristol-Myers Squibb (Brazil) and was initially prepared as a 1 g/L stock solution in DMSO/methanol 1:1. Miconazole nitrate (batch BEA53, Janssen Research Foundation, Beerse, Belgium) was initially dissolved in ethanol 43% v/v at 10 g/L. This solution was incubated for 1 h at 50°C to obtain total dissolution of drug crystals. Candida albicans ATCC 90028, Candida glabrata ATCC 90030, Candida krusei ATCC 6528 and Candida parapsilosis ATCC 22019 were purchased from ATCC. A clinical isolate of C. albicans, strain HU168, was obtained from the Mycology Laboratory Universidade de São Paulo Hospital, Brazil and Cryptococcus neoformans CBS 132 was obtained from Mycology Laboratory culture collection of the Universidade de São Paulo. For each experiment, yeasts were cultured freshly on Sabouraud dextrose agar (SDA, Merck) for 48 h at 35°C. For C. neoformans, which was grown for 72 h at 35°C. To prepare fungal cell suspension for antifungal activity assays, three to four colonies were picked from the plate, washed twice in isotonic glucose phosphate buffer (IGP; 1 mM potassium phosphate buffer, pH 7.0, supplemented with 287 mM glucose as an osmoprotectant), and a fungal cell suspension was prepared by adjusting the initial inoculum to 10⁶ cfu/mL. A working suspension was made by a 1:100 dilution in IGP, which results in 10⁸ cells per mL.

Preparation of lipid dispersions and analytical determinations of lipid concentration

Lipids were dispersed in water, using a titanium macrotip probe. This procedure dispersed the amphiphile powder in water using a high-energy input, which not only produced bilayer vesicles but also disrupted these vesicles, thereby generating open BF. Analytical concentration of DODAB was determined by microtitration and adjusted to 10 g/L.

Determination of zeta-average diameter and zeta-potential of dispersions

The size distributions for DODAB, miconazole, amphotericin B, DODAB/miconazole and DODAB/amphotericin B (zeta-average diameter, Dₚ) were determined by means of a ZetaPlus zeta-potential analyser (Brookhaven Instruments Corporation, Holtsville, NY, USA) equipped with a 570 nm laser and dynamic light scattering at 90° for particle sizing. The mean diameters referred to in this work from now on should be understood as the mean hydrodynamic diameters Dₚ. Zeta-potentials (ζ) were determined from the electrokinetic mobility μ and Smoluchowski’s equation, ζ = μ/εητ, where η and τ are medium viscosity and dielectric constant, respectively. For zeta-potential and size distribution measurements, different volumes of miconazole, (10 g/L) in 43% ethanol, or amphotericin B (1 g/L) in DMSO/methanol 1:1, were added to DODAB lipid dispersion, at low and high drug to lipid molar proportion. Mixtures were diluted in water or IGP at final volume of 2 mL. All Dₚ and ζ were obtained at 25°C, 1 h after mixing.

Determination of minimal fungicidal concentrations of miconazole, amphotericin B and DODAB for Candida strains and C. neoformans

Candida strains and C. neoformans were subcultured on SDA plates and grown at 35°C for 24 h prior to testing. The medium used for diluting cells was IGP supplemented with 287 mM glucose as an osmoprotectant. The interaction between DODAB and components of the culture medium would completely prevent targeting of the cationic DODAB-drug complex to the oppositely charged Candida cells. Usually, evaluation of combined action of antimicrobial drugs is presented as the standard fractional inhibitory concentration index FICI obtained from chequerboard experiments. Unfortunately, the chequerboard experiment is not feasible for the antifungal cationic lipid DODAB, which cannot remain in dispersion at usual ionic strengths of the culture medium. The DODAB bilayer dispersions flocculate at very low ionic strength (at and above 5 mM monovalent salt) and strongly interact with acidic components of the RPMI culture medium (e.g. negatively charged amino acids). Even small amounts of acidic amino acids cause DODAB dispersions to flocculate and lose antimicrobial activity. This is the reason why the microplate was used similarly to its use in a chequerboard experiment but just to promote the interaction of fungus and drugs over a broad range of drug concentrations but at very low ionic strengths and just for measuring cell viability of aliquots taken from the wells. Therefore, minimal fungicidal concentration (MFC) can be determined but MIC cannot. Briefly, twofold serial dilutions of miconazole, amphotericin B and DODAB BF were prepared at final volume of 100 μL per well in a 96-well flat-bottomed microtitre plates. The final concentration of the antifungal agents ranged from 32 to 0.25 mg/L for miconazole and amphotericin B, and from 250 to 0.5 mg/L for DODAB BF. Each well of the microtitre plate was inoculated with fungal cell suspension to a final concentration of 10⁷ cells per mL and the plates were incubated at 35°C for 48−72 h. An aliquot (50 μL) of cell suspension was taken from each well, diluted 1:10 and 1:100, and cell viability was determined by plating 0.5 mL on SDA plates and incubating for 48−72 h at 35°C. The MFC endpoint was the lowest drug concentration that killed ≥99.9% of the inoculum.

Determination of synergism from cell viability assay

In one dimension of a 96-well microtitre plate, twofold serial dilutions of amphotericin B, or miconazole, were added in a volume of 50 μL per well, yielding final concentrations from 32 to 0.25 mg/L after mixing with cells and the other antifungal agent (DODAB dispersion) to be tested for synergistic action, respectively. In the second dimension, a twofold dilution series of DODAB BF was added in a volume of 50 μL per well, giving a final concentration of DODAB BF from 100 to 0.5 mg/L. Drug−lipid mixtures were incubated together for 1 h before adding the cell suspension. Each well of the microtitre plate was inoculated with 100 μL of
C. albicans ATCC 90028 or C. neoformans CBS 132 cell suspension, to a final concentration of \(10^4\) cells per mL, and the microplates were incubated at 37°C for 48–72 h. Afterwards, an aliquot (50 \(\mu\)L) of cell suspension was taken from each well, diluted 1:10 and 1:100, and cell viability was determined by plating 0.5 mL on SDA plates and incubating for 48 h (C. albicans) or 72 h (C. neoformans) at 35°C. The lowest drug concentration that killed \(\geq 99.9\%\) of the inoculum of the fungal cells was used to determine the fractional fungicidal concentration (FFC), defined as the ratio of the MFC of a drug used in combination with the MFC of the drug tested alone. The FFC index (FFCI) was calculated as the sum of the FFCs for the most equally effective concentration of drugs. According to Odds, FFCI \(\leq 0.5\) corresponds to drug synergism, whereas an index \(>4.0\) represents antagonism. Each experiment was carried out in triplicate and no variation was obtained among them.

**Results and discussion**

**Effect of chaotrope dihydrogenphosphate anion and/or cationic lipid on drug particle size and zeta-potential at high P**

Figure 1 shows chemical structures of amphotericin B, miconazole and the cationic lipid DODAB. Two working hypotheses are illustrated: (i) at low P, DODAB BF or discs solubilize individual, non-aggregated drug molecules at their rim; and (ii) at high P, electrostatic attraction between drug granules or particles and oppositely charged lipidic BF drives formation of bilayer-covered drug particles (Figure 1).

In Table 1, mean diameters and zeta-potentials for drug particles, DODAB BF and mixtures of both at low or high P in different media are presented. Amphotericin B drug particles with 360 or 75 nm mean diameter, in water and IGP, respectively, are negatively charged with ca. –26 or –27 mV of zeta-potential, respectively. One should notice that IGP buffer (containing ca. 0.5 mM of the chaotropic dihydrogenphosphate large anion at pH 7) affected size distribution of amphotericin B particles at 50 mg/L reducing their mean particle size without changing zeta-potential. DODAB BF were slightly affected by the presence of 5% DMSO/methanol; they changed mean size from 69 to 85 nm and zeta-potential from 39 to 26 mV. Possibly, the solvent stabilized an interdigitated bilayer state with a reduced zeta-potential due to the increase in distance between cationic polar heads. For combinations of amphotericin B and DODAB BF, at low P (46 mg/L amphotericin B: 2500 mg/L DODAB), mean size for BF increased slightly from 85 to 110 nm and upon loading with amphotericin B, the BF zeta-potential of 42 mV decreased slightly to the 39 mV obtained in the absence of drug (Table 1). This corroborated previous work at low P and suggested once again that there is no effect of drug loading on bilayer structure as amphotericin B solubilization occurs at the rim of DODAB BF. At high P (50 mg/L amphotericin B: 50 mg/L DODAB), amphotericin B granules with 195 nm mean diameter exhibit a positive zeta-potential of 9 mV (Table 1). Thus, there is a certain extent of aggregation of the 75 nm negatively charged drug granules plus charge reversal and coverage caused by cationic DODAB BF resulting in the larger size and positive zeta-potential. This suggested bilayer adsorption onto the drug particles at least as BF but did not allow us to conclude that a continuous bilayer surrounded each amphotericin B drug granule because the zeta-potential was too low to correspond to the zeta-potential of a DODAB bilayer even in the interdigitated state. Another possibility would be an insufficient DODAB BF amount at 50 mg/L to cover all drug particles with one DODAB bilayer.

Figure 2 shows size distributions for DODAB BF in 1 mM IGP and DMSO/methanol 5% (Figure 2a), amphotericin B in water (Figure 2b) or in IGP 1 mM (Figure 2c). The effect of the chaotropic dihydrogenphosphate anion is a remarkable displacement of amphotericin B particle size distribution to a range of smaller sizes. This behaviour may be explained as a ‘salt-ling-in’ effect as observed previously for the strong association of chaotropes with hydrophobic solutes. In this case, the poorly hydrated anion (hydrophobe) might be adsorbing directly onto amphotericin B granule thereby stabilizing drug–water interface with a net increase in the exposure of total surface area of drug and decrease in drug particle size. Further addition of DODAB BF at high P to this amphotericin B dispersion changed the zeta-potential sign and value with a certain extent of aggregation (Figure 2d) as already described from Table 1. However,
aggregation was much more controllable than the one that occurs in pure water (Figure 2b).

The effect of potassium dihydrogenphosphate over a range of low concentrations (0.2–2.0 mM K₂HPO₄) on miconazole particle size distribution and zeta-potential was determined but not shown. As the drug particle in this case is positively charged, increasing salt concentration increases mean particle size and decreases zeta-potentials from 485 up to 638 nm and from –6 mV up to –16 mV, respectively. This experiment suggests that the anion directly adsorbs onto the cationic drug particle not only changing the sign of the zeta-potential which was positive in pure water at pH 6.3 but also turning this potential more negative as a function of anion concentration over a range of very low concentrations as is 0.2–2 mM (data not shown). One should consider that miconazole particles, in pure water, may be either positively charged at pH around the pKa of its imidazole moiety (see Figure 1) or neutral at pH values well above the pKa, which is 6.5. Therefore, the occurrence of negatively charged miconazole particles in the presence of IGP buffer at pH 7 (data not shown) reconfirms the previously raised possibility of chaotrope adsorption onto the drug particle.

Figure 3 shows coverage of the negatively charged miconazole particles obtained in IGP buffer with cationic DODAB BF. The usual miconazole dispersion in pure water at 500 mg/L and pH 6.3 is composed of positively charged particles with 49 mV mean zeta-potential and 206 nm mean particle diameter (Figure 3a). Miconazole particles in 1 mM IGP buffer at pH 7 and 80 mg/L have 604 nm mean diameter and –23 mV mean zeta-potential (Figure 3b) whereas DODAB BF in the same buffer has 69 nm mean diameter and 39 mV mean zeta-potential (Figure 3c).

### Action of cationic lipid-fungicides

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<td><img src="image" alt="At low drug to lipid molar proportion (P), solubilization of drug molecules at the rim of DODAB BF" /></td>
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<td><img src="image" alt="At high P, bilayer-covered drug particle" /></td>
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Figure 1. Chemical structures of amphotericin B, miconazole and the cationic lipid DODAB and illustration of the two working hypothesis for assemblies at low and high drug to lipid molar proportion (P).
At high P, miconazole particles are covered by DODAB BF so that the drug particles become positively charged with a zeta-potential practically equal to the one measured for DODAB BF alone, namely, 42 or 38 mV (Figure 3d and e, respectively) and a final size practically equal to the one measured in the absence of DODAB BF, namely, 663 or 532 nm (Figure 3d and e, respectively).

At low P, loading capacity of DODAB BF with miconazole was previously determined as 1 molecule of drug per 10 DODAB molecules in DODAB BF or 0.5 mM miconazole solubilized at the rim of 5.0 mM DODAB BF. In Figure 4, a similar experiment was performed to obtain DODAB BF loading capacity with amphotericin B over a range of low P. DODAB BF with a mean diameter of 99 nm and a zeta-potential of 52 mV in pure water (Figure 4a) were mixed with amphotericin B particles which presented a mean diameter of 364 nm and a zeta-potential of ~29 mV (Figure 4b) over a range of low P (0.05–0.15 mM amphotericin B: 2 mM DODAB). Figure 4(c) shows the disappearance of amphotericin B granules due to solubilization of amphotericin B at the rim of DODAB BF, i.e. the mixture is composed of particles with 110 nm mean diameter and 42 mV mean zeta-potential in agreement with the working hypothesis for drug solubilization by BF at low P (Figure 1). However, upon increasing amphotericin B final concentration in the mixtures with DODAB BF, mean particle size increases to 137 and 193 nm at 0.1 and 0.15 mM amphotericin B, respectively, whereas mean zeta-potential decreases to 39 and 36 mV, respectively (Figure 4d and e). This was due to the increased contribution of amphotericin B large particles, which started to appear in the dispersion after saturating the rim of DODAB BF with individual drug molecules (Figure 4d and e). Therefore, from Figure 4 saturation takes place slightly below P = 1:20 or at ca. 0.1 mM amphotericin B and 2 mM DODAB. Below saturation, amphotericin B was previously reported to have adsorbed at the rim of DODAB BF yielding optical spectra typical of individual, non-aggregated drug molecules that were similar to the ones obtained for the drug in its best organic solvent. Figure 4 quantitatively establishes the loading capacity of DODAB BF with monomeric amphotericin B.

DODAB lipid as fungicide acting synergistically with miconazole or amphotericin B at high P

From cell viability assays carried out for a variety of Candida species and Cryptococcus neoformans CBS 132 at 10^5 cells/mL over a range of DODAB, amphotericin B or miconazole concentrations, the fungicidal activity of DODAB can be compared with that exhibited by amphotericin B or miconazole from MFCs determined for each species (Table 2). DODAB itself is a potent fungicide against certain species such as C. neoformans and C. parapsilosis ATCC 22019, though less potent than amphotericin B or miconazole against some Candida strains such as C. glabrata ATCC 90030 or C. krusei ATCC 6258 (Table 2). The main point to be made here is that DODAB itself is acting as an anti-infective drug with fungicidal action. In fact, cationic lipids and surfactants bearing the quaternary ammonium moiety in their chemical structure, the QACs, have often exhibited antimicrobial properties (see Ref. 6 for a review).

The combined action of DODAB and amphotericin B or miconazole acting against C. albicans for 48 h produces values of MFC that are lower than those for drugs acting alone (Table 3). From the calculated synergism index for C. albicans, which is 0.65 and 0.325 for DODAB/amphotericin B and DODAB/miconazole, respectively (Table 3), the combined action of cationic lipid and drug can be considered synergistic over the long run for miconazole only. On the other hand, for C. neoformans, the combined action yields a synergism index of 1.0 both for DODAB/amphotericin B and DODAB/miconazole (Table 3) suggesting independent action for lipid and drug, according to Odds’ interpretation. Therefore, lipid–miconazole associations are synergistic to C. albicans and indifferent to C. neoformans.

After 1 h of interaction time between fungus and lipid-covered drugs, DODAB practically dominates the percentage killing of C. albicans (Figure 5a) or C. neoformans (Figure 5b), because either by itself or in combination with amphotericin B or miconazole very similar fungus killing percentiles are obtained over a range of DODAB concentrations. At first glance this result appears to contradict the synergistic action obtained over the long
run (48 or 72 h of interaction time) for DODAB/drug combinations (Table 3). Actually this time effect can be easily understood from the fact that both amphotericin B and miconazole need to be released from its lipidic layer before attaining its site of action in the fungus cell. DODAB is a very potent anti-infective drug which is already effective over the micromolar range of concentrations so that free DODAB BF that have remained in dispersion act first on the fungus cell surface possibly binding to anionic sites of the cell wall. One may speculate that this would cause channel blockades with inhibition of vital exchanges between fungus cells and their environment. In other words, DODAB action is immediate and takes place as soon as DODAB BF attaches to important anionic sites of the cell surface. In contrast, drug particles surrounded by a cationic DODAB layer, which also attach immediately to the cell surface, might not release the drug immediately to its site of action (the ergosterol enriched cell membrane of the fungus for amphotericin B or the cytochrome P450 system involved in ergosterol synthesis for miconazole). Our results indicate that a certain time longer than 1 h is required to obtain drug release (Table 3; Figure 5). One should notice that the most effective combinations which produced 100% killing are those given at high P with DODAB concentration in the range 1–5 mg/L for *C. albicans* (Figure 5a) and higher than 2.5 mg/L for *C. neoformans* (Figure 5b). DODAB itself was effective as a

Figure 2. Effect of IGP buffer and DODAB BF on size distribution and zeta-potential of amphotericin B (AMB) aggregates at high drug to lipid molar ratio. Effect of 1 mM IGP on amphotericin B size distribution is depicted from the comparison between (b) and (c) whereas the effect of DODAB BF on amphotericin B size distribution and zeta-potential in IGP 1 mM is depicted from the comparison between (c) and (d). The final DMSO:methanol concentration is 5% (a–d). Size distribution and zeta-potential were obtained 1 h after interaction at 25°C in IGP buffer 1 mM, pH 7.0. Values are expressed as mean ± SD.
fungicide by itself from 1 mg/L DODAB and above (Figure 5).

Figure 6 shows the effect of time on cell viability of *C. albicans* ATCC 90028. The potency of the combinations is revealed from the time effect. Whereas for DODAB or DODAB/miconazole at 1 or 1/10 mg/L, respectively almost complete loss of cell viability takes place over the first 20 min, at 1 mg/L DODAB/5 mg/L amphotericin B, loss of cell viability was much slower, though at 48 h loss of cell viability was complete also for this combination (Table 3).

The fact that the best combinations for drug and DODAB action require rather small DODAB amounts, just enough to produce a thin lipid layer surrounding hydrophobic granules (see previous section), is of utmost relevance for future pre-clinical and clinical tests as the usual toxicity of this cationic compound will be substantially minimized at the very low DODAB doses that will be required for optimal combinations. Furthermore, one should recall that the differential cytotoxicity of the DODAB lipid against a variety of pathogenic microorganisms was previously established by showing that DODAB is much less cytotoxic against mammalian cells than against bacteria or fungus.\(^5,6,10\) The therapeutic activity of a DODAB/amphotericin B formulation at low P, which reduced dose requirements of amphotericin B, presented the problem of very large DODAB concentrations required to solubilize the drug. At high DODAB...
concentrations, DODAB toxicity in vivo may become a problem due to hepatotoxicity (Lincopan et al., submitted for publication). Therefore, at high P, DODAB concentrations being much smaller and just the amount required to cover drug particles with a thin lipid layer, one may anticipate that DODAB general toxicity will not be a problem in vivo. Further evaluation of our DODAB/fungicide formulations should be performed in vivo in a mouse model of systemic candidiasis in order to prove efficacy and toxicity at high P. Finally, this is not the first instance of synergistic action exhibited by DODAB; recently, in vivo, the synergism between listeriolysin O, a recombinant protein and dimethyldioctadecylammonium bromide to activate CD8(+) T cells was reported. Thus, the prospects are good for in vivo testing of the combined formulations described in this work.

Table 2. Minimum fungicidal concentrations (MFCs) of DODAB bilayer fragments, amphotericin B (AMB) and miconazole (MCZ) for fungal isolates at a final cell concentration of 10^4 cfu/mL after a period of 48 h interaction for *Candida* species and 72 h for *C. neoformans*

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<th>Strains</th>
<th>MFC (mg/L)</th>
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The chaotropic dihydrogen phosphate anion from IGP buffer reduced amphotericin B particle size in dispersion and changed the zeta-potential sign of cationic miconazole granules to negative values thereby facilitating adsorption of the cationic

**Figure 4.** Loading 2 mM DODAB BF with amphotericin B over a range of low P. Particle size distribution and zeta-potential of 2 mM or 2500 mg/L DODAB BF (a); 0.01 mM or 17 mg/L amphotericin B (b) and combinations of both at 2 mM DODAB, P = 1:40, 92 mg/L amphotericin B (c), P = 1:20, 185 mg/L amphotericin B (d) and P = 1:13, 277 mg/L amphotericin B (e). Notice that final DMSO:methanol percentiles were 1.7 (b), 4.6% (c), 9.2% (d) and 13.9% (e). Data were obtained after 1 h of interaction at 25°C. Values are expressed as mean Dζ ± SD.
anti-infective lipid DODAB onto drug particles at high P. The lipid-covered drug particles exhibited synergistic action for DODAB/miconazole against C. albicans. At low P, the loading capacity of DODAB BF was determined as 0.1 mM amphotericin B at 2.0 mM DODAB. The general toxicity in vivo associated with DODAB/fungicide formulation at low P, which is mainly due to DODAB will possibly be circumvented by the use of novel formulations at high P for which DODAB will be required at very small amounts.

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Transparency declarations

None to declare.

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