Polysorbate 80 and Cremophor EL Micelles Deaggregate and Solubilize Nystatin at the Core–Corona Interface

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Received 2 November 2004; accepted 9 December 2004

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20301

ABSTRACT: The extent and the location of nystatin solubilization by nonionic surfactant micelles were determined. The critical aggregation concentrations (CAC) of nystatin in 4×10^{-3} M surfactant were determined by dynamic light scattering. The resulting CAC values for nystatin in Cremophor EL (CrEL), Tween 80 (T80), and Nofable ESO-9920 (NOF) were 150, 150, and 300 μM compared to 10 μM for the phosphate-buffered saline (PBS) control. The surfactants were able to solubilize and deaggregate nystatin from 50 to 75 times more than the PBS control. The core polarity of CrEL micelles, determined by pyrene fluorescence, was significantly lower than T80 and NOF micelles. The micelle–water partition coefficients (P) of nystatin and pyrene were determined by fluorescence spectroscopy. The partition coefficient values of 7.5 μM nystatin in CrEL and NOF micelles were 1100 ± 60 and 1000 ± 110, an insignificant difference ($p > 0.1$). However, there was a significant increase in pyrene partitioning in micelles with lower core polarity. Additionally, the P of nystatin decreased when the nystatin concentration was increased, whereas the pyrene P did not. The unusual partitioning behavior of nystatin revealed a good fit with the Langmuir adsorption isotherm, indicating solubilization at the micellar core–corona interface. © 2005 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 94:2345–2354, 2005

Keywords: polysorbate 80; Cremophor EL; surfactants; micelles; nystatin; aggregation; particle size; solubility; adsorption

INTRODUCTION

In the formulation of poorly soluble drugs for parenteral administration, surfactants are often relied upon for enhanced solubilization to achieve sufficiently high blood levels within a practical volume of administration. Although amphoteric and ionic surfactants have been used for formulation purposes, the nonionics generally offer the most advantages. Nonionic surfactants are typically less toxic, less hemolytic, less irritating to the skin, and tend to maintain near physiological pH values when in solution.[1] The two nonionic surfactants that are most widely used and studied in the pharmaceutical industry for their solubilization abilities are Cremophor EL (CrEL) and Tween 80 (T80). Cremophor EL is polyoxyethyleneglycerol 35 triricinoleate or polyoxyl 35 castor oil, the structure of which is shown in Figure 1a. The second surfactant, T80, is generically referred to as polysorbate 80, also known as polyoxyethylenesorbitan 20 monooleate (Fig. 1b). A highly purified version of polysorbate 80, called Nofable ESO-9920 (NOF) and produced by NOF Corporation (Tokyo, Japan), exists where low molecular weight contaminants such as aldehydes and peroxides have been minimized. It is argued that removal of these contaminants may decrease *in vivo* toxicities and drug degradation by oxidation. Both surfactants are generally

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Journal of Pharmaceutical Sciences, Vol. 94, 2345–2354 (2005)

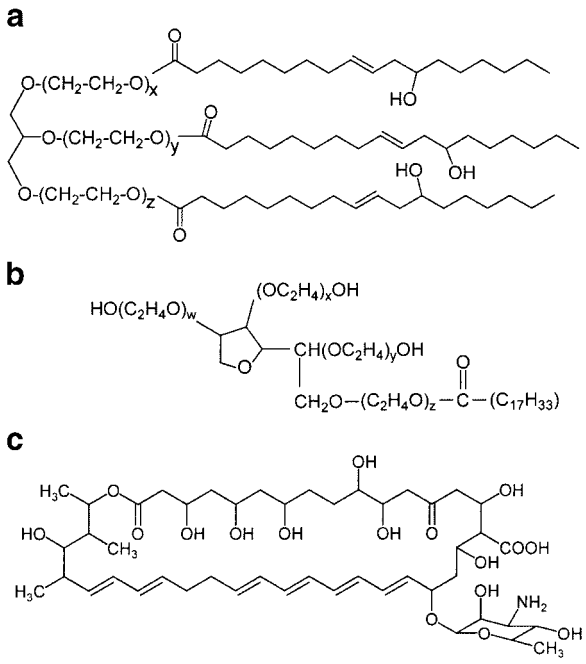

Figure 1. (a) Chemical structure of polyoxyethyleneglycerol 35 triricinoleate (Cremophor EL) where $x+y+z=35$. MW = 2560 g/mol as determined by time of flight mass spectrometry. (b) Chemical structure of polyoxyethylenesorbitan 20 monooleate (Tween 80 or Nofable ESO-9920) where $w+x+y+z=20$. MW = 1310 g/mol. (c) Chemical structure of nystatin A_1. MW = 926.11 g/mol.

recognized as safe, and are approved by the FDA for intravenous administration, with polysorbate 80 approved up to a concentration of 10% w/v and CrEL up to 65%.[2]

An increase in drug solubilization is achieved by the abilities of these surfactants to form micelles, which exhibit a typical core-shell architecture. Upon reaching a sufficient concentration known as the critical micellization concentration (CMC), the surfactants undergo a phase separation owing to the selective solubility of one of the segments. In aqueous media, the micellar cores are hydrophobic and relatively nonpolar, providing a more suitable environment for the solubilization of drugs over the surrounding media. Upon administration, the micelles will eventually fall apart through biodegradation or through simple dilution into unimers that are then below the renal molecular weight threshold (40–50 kDa), and that can be cleared through the kidneys or by other means of excretion. This opens the potential for micelles that are stable in plasma and that, by either preventing the drug from rapid renal clearance or protection from metabolic enzymes, are able to alter the blood disposition and pharmacokinetics of biologically active compounds.[3] CrEL has been shown to reduce the systemic clearance of multiple drugs, including doxorubicin and paclitaxel in humans. Furthermore, blood concentrations of CrEL that are above the CMC can be achieved in humans. It has therefore been argued specifically for paclitaxel that intact CrEL micelles in the blood are responsible for altering paclitaxel pharmacokinetics. Conversely, the majority of clinical investigations have shown little alteration in the pharmacokinetic profiles of agents when administered with polysorbate 80. It is argued that this is a result of the rapid degradation of polysorbate 80 by plasma esterases.[4]

The class of molecules known as polyene macrolide antifungals has been particularly difficult to formulate, owing to both the ease with which the molecules self-aggregate and the consequences of that aggregation. Nystatin is a member of this class of molecules, and delivers potent antifungal activity.[5] The chemical structure of nystatin reveals a molecule that is both amphoteric and amphiphilic, and these physical properties lead to poor solubility in aqueous media and incompatibility with a number of other drugs (Fig. 1c). Nystatin's mechanism of action exploits the differences between fungal cell membranes and mammalian cell membranes. It has been shown that monomers of nystatin bind, with great selectivity, to the ergosterol found only in fungal cells, causing significant perturbation of the membrane that leads to cell death. However, aggregates of nystatin that form due to poor aqueous solubility are nonselective and able to disrupt the integrity of both fungal and mammalian cell membranes, which leads to host cell death and toxicity.[6,7]

Nystatin is currently FDA approved only for the treatment of susceptible cutaneous and mucocutaneous fungal infections caused by the *Candida* species. However, nystatin exhibits a broad spectrum of activity against other fungi such as *Aspergillus* and *Cryptococcus*. Of particular interest, nystatin has shown action against some *Candida* species, where other antifungals, including its sister drug amphotericin B, have encountered resistance.[8–10] The incidence of disseminated fungal infections has risen over the past decade, leading to *Candida* becoming the fourth most commonly encountered nosocomial bloodstream pathogen.[10] For these reasons, it is desirable to obtain a safe and efficacious parenteral formulation of nystatin.

It is proposed that the delivery of unaggregated nystatin may improve its therapeutic index by preventing aggregation related toxicities. The aim of this article was to determine the extent to which CrEL and polysorbate 80 are able to deaggregate and solubilize nystatin, and to explore the means by which the solubilization occurs. The effects of surfactant composition, concentration, and micellar core polarity on nystatin aggregation were studied. Additionally, the nystatin micelle–water partition coefficient was determined and compared to that of a model hydrophobic compound so that the effect of the chemical properties of nystatin on partitioning could be studied. The data were subjected to the Langmuir adsorption model so that the identification of molecular solubilization sites could be facilitated.

EXPERIMENTAL

Critical Aggregation Concentration (CAC)/Particle Size Determination

Nystatin (Sigma, St. Louis, MO), Lot 033K0815, had a potency of 5957 units/mg and had a 99.4% tetraene content as determined by absorbance spectroscopy. Nystatin was first dissolved at a high concentration in *N,N*-dimethylacetamide spectrophotometric grade (DMAC, Sigma, St. Louis, MO). Cremophor EL (BASF, Mount Olive, NJ), Tween 80 (Aldrich, St. Louis, MO), and Nofable ESO-9920 (NOF, Tokyo, Japan) solutions were prepared by directly dissolving the surfactants in 10 mM phosphate-buffered normal saline (PBS, pH 7.4). These solutions were filtered through a 0.22-μm nylon filter (GeneMate, Kaysville, UT) to remove dust particles. The nystatin/DMAC solution was then added to the surfactant solutions and mixed at appropriate volumes with the pure surfactant solutions to give the desired nystatin concentrations. The final concentration of DMAC was always $<0.5\%$ v/v. Samples were then briefly vortexed and equilibrated with shaking at 55 strokes per minute in a water bath away from light at 25°C for 30–90 min. The CACs and particle size characterization of nystatin in surfactant solutions or controls were determined by dynamic light scattering (DLS) on a thermostatted NICOMP 380ZLS Particle Sizer (Particle Sizing Systems, Santa Barbara, CA) equipped in a right-angle geometry. A constant concentration of surfactant was used in the presence of increasing concentrations of nystatin for each experiment. Samples were maintained at 25°C throughout the experiment. Data was interpreted using either Gaussian analysis for single populations or NICOMP analysis (a proprietary algorithm based on nonlinear least-squares analysis) for multimodal populations. Data were fixed at an intermediate smoothing factor of 3, and are reported as volume weighted. The apparent CAC of nystatin is quantified by defining it as the concentration at which populations of particles are detected at sizes greater than can be attributed to surfactant micelles, namely 100 nm, while following an upward trend in size as the nystatin concentration is increased.

Micelle Core Polarity

Surfactant solutions were prepared in PBS, as described above, and diluted with PBS so that the desired surfactant concentrations could be obtained. A stock solution of pyrene (Aldrich, St. Louis, MO) in acetone was prepared at a concentration of 7.2 μM. A volume of 0.25 mL of the pyrene solution was placed in sample tubes with screw top caps. The tubes were then placed in a water bath at approximately 50°C to allow the acetone to completely evaporate. Solutions were placed in the pyrene-coated vials to give a final concentration of 6×10^{-7} M pyrene. Samples were incubated at 70°C for 2 h and then allowed to cool away from light for 16–24 h to 25°C. The fluorescence emission spectrum was obtained on a thermostatted Hitachi F3010 Fluorimeter with an excitation wavelength of 339 nm. The emission spectrum was collected at 10 nm/min from 350–530 nm with excitation and emission bandpasses of 3 nm. Polarity values were obtained by taking the ratio of pyrene fluorescence peak I ($\lambda \cong 373$ nm) over peak III ($\lambda \cong 384$ nm).

Micelle–Water Partition Coefficient

Nystatin at a concentration of 7.5–30 μM or pyrene at a concentration of 0.09–1.1 μM was dissolved in surfactant solutions in PBS. The amount of DMAC in the nystatin samples was fixed at 0.5%, while no DMAC was present in the pyrene samples. For each experiment, the solute concentrations were held constant in the presence of surfactants at concentrations ranging from well below their CMCs to well above. The samples were equilibrated at 25°C with shaking at 55 strokes per minute for 30–90 min. The fluorescence emission spectrums of the samples

were obtained on a thermostatted Hitachi F3010 Fluorimeter with an excitation wavelength of 320 nm for nystatin and 339 nm for pyrene. Spectra were then integrated using Spectra Calc software (Galactic Ind., Salem, NH) to obtain the area under the curve.

RESULTS AND DISCUSSION

As previously explained, the aggregation state of nystatin is believed to play a significant role in the toxicities observed with systemic administration. It is therefore necessary to carefully monitor the presence of aggregates in polyene antibiotics in formulations intended for systemic administration. When amphotericin B (AmB) aggregates form they induce exciton formation that results in significant changes in AmB's absorbance spectrum, allowing for easy detection of aggregate formation.[11] However, the chemical structure of nystatin is such that no changes in the absorbance spectrum occur with the formation of aggregates. The saturated bond on the polyene side of the macrolide ring breaks the chromophores into tetraene and diene species, thereby preventing coupling and exciton formation. Additionally, the presence of micelles in the tested formulations prevents the use of nystatin's fluorescence in the detection of aggregation, as the heterogeneity of polarity and viscosity in the formulations confounds the results. For these reasons, dynamic light scattering was used to determine the apparent CAC of nystatin through the detection of the aggregate particle sizes. This laboratory's previous work with nystatin and poloxamer formulations showed that the critical aggregation concentration of nystatin could be reproducibly determined with good sensitivity.[12]

An example of the typical size trends of particle size populations obtained by dynamic light scattering is shown in Figure 2a. Figure 2a shows the nystatin concentration versus the particle size population diameters detected in the presence of 1.59×10^{-3} M Cremophor EL. The concentration of nystatin was increased, in this case, from 0 to 270 μM in the presence of a constant concentration of CrEL. Up to a concentration of 120 μM, the only populations observed were those attributed to CrEL micelles in the range of 10–30 nm. Cremophor EL micelles in PBS without nystatin were 12.6 ± 1.2 nm in diameter, whereas T80 and NOF micelles were 9.1 ± 2.1 nm and 9.1 ± 2.2 nm, respectively. At a nystatin concentration of

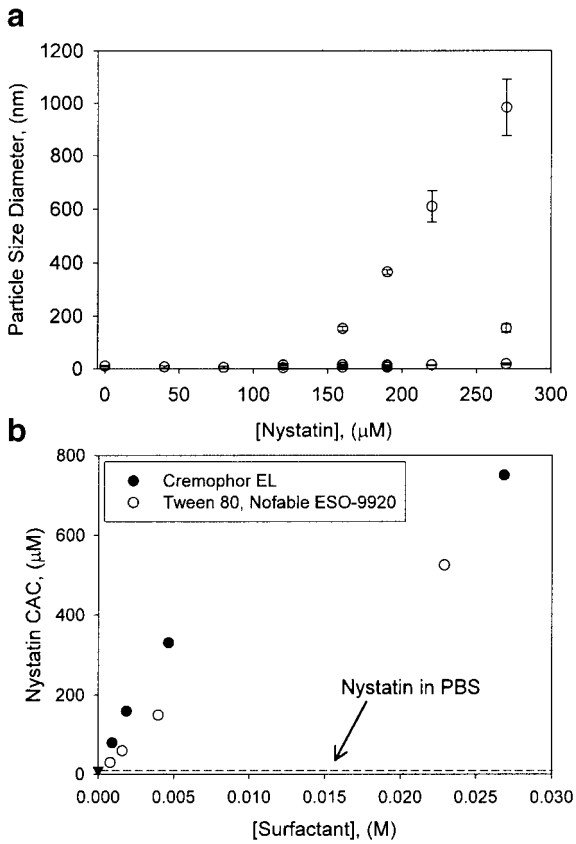

Figure 2. (a) Effect of nystatin concentration on the particle size populations observed in the presence of 1.59×10^{-3} M Cremophor EL as detected by dynamic light scattering. The points represent the mean particle size diameters with error bars representing the standard deviations of the populations. Multiple points at a single nystatin concentration indicate a multimodal particle size distribution. (b) Effect of surfactant concentration on the observed critical aggregation concentration of nystatin compared to the control of nystatin in phosphate-buffered normal saline, pH 7.4.

160 μM a particle size population appears at a mean size of approximately 200 nm. The size of the population increases as the nystatin concentration increases, eventually reaching into the order of microns. The highly reproducible appearances of large particle size populations that increase with the nystatin concentration were seen consistently across the DLS studies. For reference, the critical micellization concentrations of CrEL, T80, and NOF alone in PBS at 25°C were determined in this laboratory to be 2.9×10^{-6}, 3.6×10^{-6} and 2.1×10^{-6} M, respectively, by the pyrene solubilization method.[13]

The CAC of nystatin in PBS was determined, on the basis of the DLS method, to be 10 μM. This

value is in reasonable agreement with a concentration of 3 μM obtained, through fluorescence, in a Tris-HCl buffer, pH 7.4 at 25°C.[7] The nystatin CAC values obtained in the presence of CrEL, NOF, T80, or the control PBS are shown in Figure 2b. This plot shows the effect of surfactant type and concentration on the CAC of nystatin where the values were interpreted from the individual DLS plots. For all three surfactants the CAC of nystatin increased as surfactant concentration increased. Notable is that the CACs of nystatin in the presence of T80 and NOF are identical, a finding that was not unexpected. The surfactants were able to increase the CAC of nystatin up to 500–750 μM at the concentrations studied, a 50- to 75-fold increase in deaggregation over the control of nystatin in PBS. From these data, CrEL appears to have a greater solubilization capacity on a molar basis for nystatin than T80 and NOF as judged by the clearly greater CAC values seen in Figure 2b.

Because the CMCs of all three surfactants are so similar, one of the most probable explanations for the differences in the abilities of CrEL and the polysorbates to deaggregate nystatin concerns the polarity of the core. It is argued that the micellar core polarity of CrEL may be such that it provides a more suitable environment for nystatin solubilization and deaggregation. To test this theory, the probe molecule pyrene was used to identify the effect that surfactant composition and surfactant concentration have on micellar core polarity. Pyrene has been extensively studied for its ability to probe and report (in a predictable fashion) changes in its polar environment.[14] The relative intensity ratio of the first peak to the third peak within the vibrational bands of the pyrene emission spectrum has been shown to change greatly with local polarity. A reference scale generated from these ratios, known as the Py scale, correlates well with other scales of polarity such as the π^* scale of Kamlet and Taft.[15] High peak ratios reflect relatively polar environments, whereas lower ratios reflect lower polarity. As previously mentioned, the cores of micelles in aqueous systems are relatively nonpolar. These nonpolar cores preferentially solubilize pyrene to a high degree, allowing for detection of the polar environment of micellar cores. The peak ratio values are highly reproducible, but vary in relation to the wavelength of pyrene excitation. Solvent standards have been previously run and have shown good agreement with previous work at the same wavelength.[13] The effect of surfactant concentration on the pyrene peak I/III ratios is shown in Figure 3. For each surfactant, the polar environment detected by pyrene remains relatively high and then drops before again plateauing. The relatively stable values at low surfactant concentrations represent pyrene in the aqueous solution below the surfactant CMCs. The drop in polarity reflects the partitioning of pyrene into the forming micelles, and the final plateau value ratios at high surfactant concentrations are indicative of the micelle core polarities. The micelle core polarity values obtained with standard deviations for T80 and NOF were 1.127 ± 0.005 and 1.132 ± 0.009, respectively. Differences between these values are statistically insignificant ($p > 0.10$, two-tailed Student's t test). The polarity value obtained for CrEL was 1.077 ± 0.010, a significantly lower core polarity than the cores of T80 and NOF micelles ($p < 0.002$). These polarity values fall between previously obtained reference solvent polarity values of 1.18 ± 0.01 for methanol and 1.05 ± 0.01 for benzene, and are lower than the values of 1.20–1.22 for poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (poloxamers) micelles at 25°C.[12]

Previous studies of nystatin deaggregation by poloxamer micelles have shown no clear relationship between core polarity and solubilization capacity.[12] Therefore, studies were next undertaken to understand the effect of surfactant composition and core polarity on the nystatin micelle–water partition coefficient (P) as an alternative method by which to follow solubilization behavior. The coefficient P is defined as the ratio of the solute concentration in the micellar phase divided by the concentration of solute in the aqueous bulk phase.

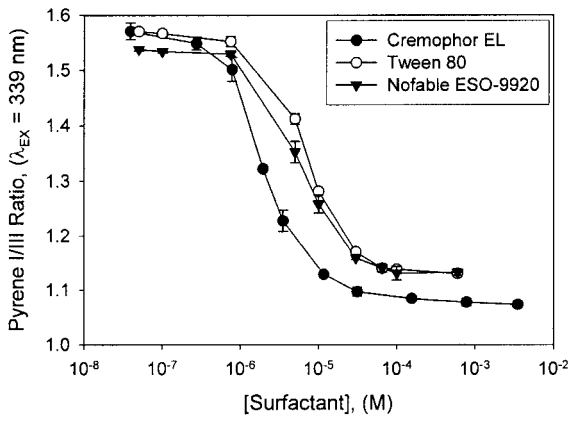

Figure 3. Effect of surfactant concentration on micellar core polarity as detected by pyrene fluorescence peak ratios.

This coefficient serves to estimate the amount of solute incorporated into the cores of micelles, and may give information on the location of the solute in the micelle structure. The P of pyrene was also analyzed in an effort to contrast the effect of nystatin's chemical properties on partitioning behavior. Whereas nystatin is a relatively large, zwitterionic, and amphiphilic molecule, pyrene is a smaller, nonionizable, and hydrophobic molecule. The P values of nystatin and pyrene were determined using the fluorescence properties of the molecules. Fluorescence methods have been used previously to determine the partitioning of solutes into micelles[12,13,16,17] and model membranes.[7] These models rely on a change in the fluorescence intensity of the solute upon transfer of that solute to the cores of micelles, a property that both pyrene and nystatin exhibit.[7,16] Complete descriptions of the calculations for the determination of P are presented in the appendix. Briefly, the total fluorescence emission of a constant concentration of solute is first plotted as a function of surfactant concentration from below the CMC to well above it. Figure 4a shows the effect of the NOF concentration on the fluorescence intensity of 22.5 μM nystatin, where the bars reflect standard deviations. At low concentrations of surfactant below the CMC, the fluorescence of nystatin is low and constant, reflecting nystatin mainly in the bulk aqueous phase. As micelles form, nystatin partitions into the core resulting in an increase in fluorescence. At sufficiently high surfactant concentrations, all available nystatin molecules have partitioned into their desired locations, and no additional surfactant will increase the fluorescence further. This same trend occurs when pyrene is solubilized by surfactant micelles. Baseline aqueous and micellar saturated solute fluorescence values are determined from the plot and labeled I_o and I_{max}, respectively. As shown in Figure 4b, the concentration of surfactant present as micelles is then plotted against nystatin fluorescence relative to I_o and I_{max} in double reciprocal coordinates. The result is a straight line with a slope equal to $1/(0.01Pv)$ where P is the micelle–water partition coefficient and v is the partial specific volume of the surfactant in solution. The partial specific volume is a thermodynamic parameter that can be calculated from density measurements using a pycnometer.[18] The partial specific volumes of 1% (w/v) CrEL and NOF surfactants in solution were calculated at 25°C, and were determined to be 0.920 and 0.923 mL/g, respectively.

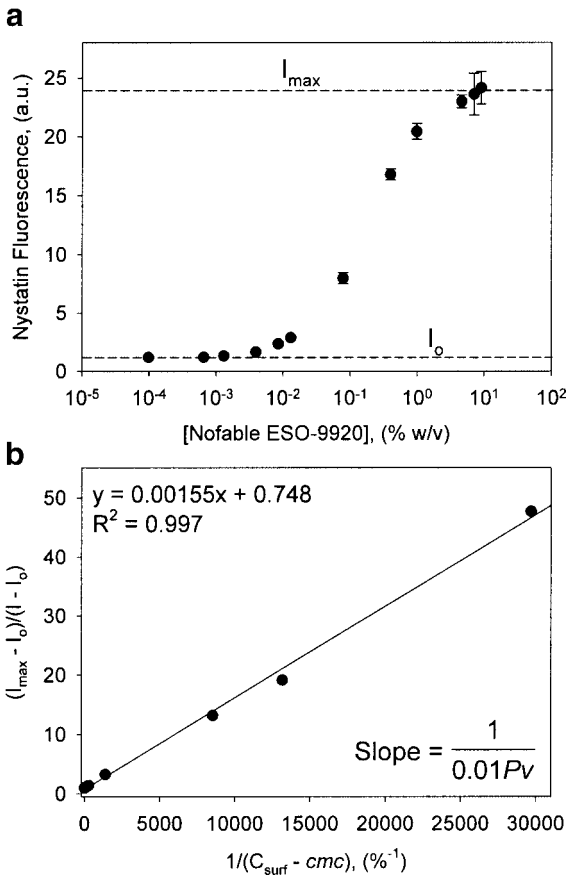

Figure 4. (a) Effect of Nofable ESO-9920 concentration on the fluorescence intensity of 22.5 μM nystatin. I_o and I_{max} are the determined baseline fluorescence intensity and the maximum saturable intensity, respectively. (b) Double reciprocal plot of 22.5 μM nystatin fluorescence intensity (relative to I_o and I_{max} from Fig. 4a) with respect to fraction of Nofable ESO-9920 present as micelles in solution. The resulting slope yields the micelle–water partition coefficient (P).

In Figure 5a, the P of nystatin in CrEL and NOF micelles is shown as a function of the nystatin concentration. With both of the surfactants the partition coefficient of nystatin decreases significantly as the nystatin concentration increases, a two- to threefold drop over the concentration range of nystatin studied. Additionally, there is no significant difference between the nystatin P in CrEL and in NOF at all concentrations tested (two-tailed Student's t test, $p > 0.1$). Nystatin concentrations above 30 μM were not tested, as aggregates are more likely to form in the bulk aqueous phase during partition coefficient determination at low surfactant concentrations. It is expected that aggregates of nystatin will partition differently and

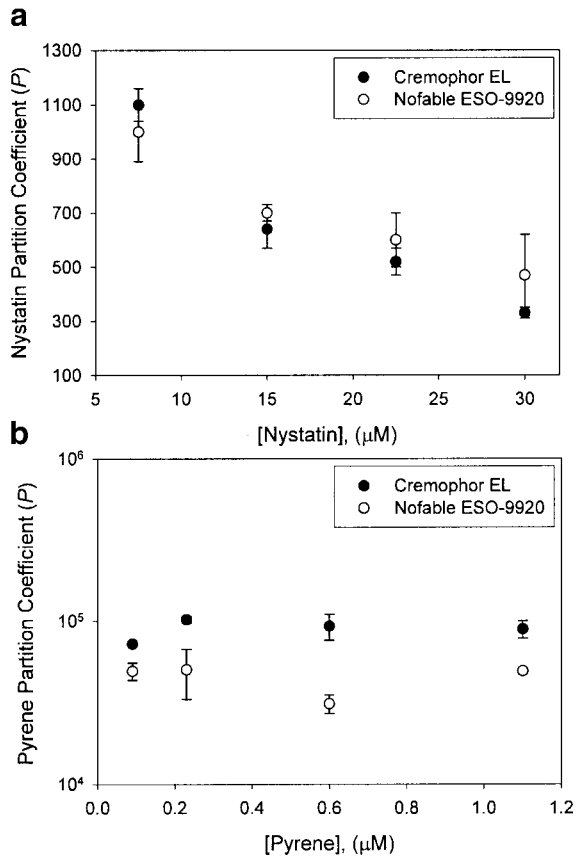

Figure 5. (a) Effect of nystatin concentration on the nystatin micelle–water partition coefficient (P) in surfactant micelles. (b) Effect of pyrene concentration on the pyrene micelle–water partition coefficient (P) in surfactant micelles.

can potentially confound results. The insignificant differences in nystatin partitioning between CrEL and NOF may be explained by the fact that many properties of these surfactants are quite similar, such as micellar sizes in solution, core-forming block components (ricinoleic acid versus oleic acid), and most importantly, the CMCs. However, the effects of architecture of the micelles, micellar association numbers, and corona-forming blocks have not been studied, and may be playing a role in other differences seen with the surfactants.

The results for the study of the effect that solute concentration has on the P of pyrene are shown in Figure 5b. First, the pyrene P is significantly higher than that of nystatin at all concentrations studied. Second, the partition coefficient into CrEL micelles is significantly higher than the partition coefficient into NOF micelles at all concentrations tested ($p < 0.002$). There also does not appear to be any upward or downward trend in the pyrene P as a function of the pyrene concentration in either of the surfactant formulations. These results reveal the great differences between the partitioning characteristics of nystatin and pyrene. Pyrene molecules partition to a significantly greater extent into the CrEL micelles because they have a lower core polarity and are thus more hydrophobic. This type of behavior for the highly hydrophobic pyrene was expected. The fact that nystatin is an amphiphilic molecule, combined with the significantly lower P values compared to pyrene strongly suggest that nystatin may not be solubilized in the core of the micelles. Additionally, the fact that core polarity has no impact on the nystatin P lends further credence to the conclusion that nystatin is being solubilized at the core–corona interface of the micelles. However, the strongest evidence may be the differences in the effect that solute concentration has on partitioning. For the P of nystatin to decrease in relation to an increase in its concentration may indicate a competitive solubilization process. As the quantity of nystatin solubilized in the micelles increases the finite amount of space available for solubilization decreases, making it progressively more difficult for further nystatin solubilization to occur. This phenomenon can be characterized as adsorption-like, where the nystatin molecules are localizing at the core–corona interface of the micelles. Studies have been performed with surfactant micelles where results indicate that the solubilization of solutes is driven by interfacial adsorption.[19,20] There are specific examples in which authors have described the interaction of small molecules with surfactant micelles as a binding phenomenon and have fit the data to a Langmuir isotherm.[21–24] Choucair and Eisenberg describe the binding of 2-nitrodiphenylamine at the core–corona interface of polystyrene-*b*-poly(acrylic acid) micelles, and find that the best fit is through the Langmuir binding isotherm.[25] The binding of the preservative parahydroxybenzoic acid esters to polysorbate 80 micelles has been described as Langmuir-like.[21]

Langmuir-like adsorption can be generally expressed in the following manner:

$$\frac{x}{x_m} = \frac{K_{ad}C}{1 + K_{ad}C}$$

where x and x_m are the solute mole fraction in the micellar phase and the maximum of the solute mole fraction adsorbed, and where K_{ad} and C are the Langmuir adsorption constant and the molar concentration of unbound solute molecules.[25,26]

Linearization of the isotherm is achieved by a rearrangement of the above equation:

$$\frac{C}{x} = \frac{1}{K_{\text{ad}} x_m} + \frac{C}{x_m}$$

such that a plot of C/x versus C will give a straight line where the constant of interest, K_{ad}, can be determined from the resulting slope and intercept. The value C can be calculated by working back from the partition coefficient equations from α, the fraction of solute solubilized by the micelles:

$$\alpha = \frac{\theta P}{[1 + \theta(P - 1)]}$$

where θ is the volume fraction of the surfactant micellar phase and can be described as

$$\theta = 0.01v([C_s] - \text{cmc})$$

where v is again the partial specific volume of the surfactant in solution, C_s is the surfactant concentration, and *cmc* is the critical micellization concentration of the surfactant. The value C is therefore calculated from $1 - \alpha$ multiplied by the molar concentration of nystatin in the formulation.

The Langmuir adsorption isotherms for nystatin in 4×10^{-3} M CrEL and NOF are shown in Figure 6. The two plots of nystatin in CrEL and NOF show good linearity and reveal K_{ad} values of 3.91×10^5 and 2.52×10^5 L/mol, respectively. The differences in these values appear to reflect the differences in the deaggregation of nystatin. The reported K_{ad} value for 2-nitrodiphenylamine adsorbed onto polystyrene-*b*-poly(acrylic acid) micelles was 5.7×10^5 L/mol, whereas values of 7 to 34 L/mol were obtained with salicylic acid and benzoic acid adsorbing onto polyoxyethylene glycol monoalkyl ether micelles.[24,25] As expected, great variability in adsorption constants can be found depending on the properties of the substrate and adsorbate. Pyrene in CrEL and NOF did not fit the Langmuir adsorption isotherm model with correlation coefficients of 0.30 and 0.07, respectively (data not shown). Both the partitioning behavior of pyrene and the correlation with core polarity suggest that interfacial solubilization was not occurring, or that it was not the principal mode of solubilization. Accordingly, pyrene's lack of fit with the Langmuir adsorption isotherm was expected, and supports the nystatin data. These results suggest that micellar solubilization and deaggregation of the amphiphilic nystatin is driven by interfacial adsorption between the surfactant core and corona. The micelle core surface area that is accessible, determined by surfactant CMC and concentration, would therefore primarily determine the extent of nystatin deaggregation.

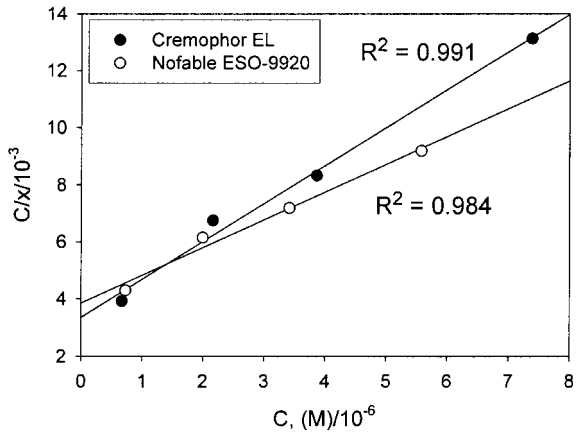

Figure 6. Langmuir adsorption isotherms for nystatin in 4×10^{-3} M surfactant, where C is the concentration of unbound solute in solution and x is the nystatin mol fraction in the micelle.

CONCLUSIONS

CrEL and polysorbate 80 micelles increased the CAC of nystatin by deaggregating nystatin at levels up to 75 times its CAC in phosphate-buffered saline. Research-grade Tween 80 and the highly purified Nofable ESO-9920, both of which are also known as polysorbate 80, showed no significant differences in all experimental results. Cremophor EL micelles had a significantly lower core polarity than the polysorbate 80 micelles. However, nystatin did not partition to any greater extent into CrEL micelles than into NOF micelles, whereas pyrene partitioned into CrEL micelles to a significantly greater extent. The P of nystatin decreased as the nystatin concentration increased, whereas the pyrene P did not. The nystatin micelle–water partition coefficient data for both CrEL and NOF fit well to the Langmuir adsorption isotherm, whereas the pyrene data did not. This information, combined with the fact that nystatin is an amphiphilic molecule, leads to the conclusion that nystatin is mainly being solubilized and deaggregated at the core–corona interface of CrEL and NOF micelles.

ACKNOWLEDGMENTS

This work was funded in part by the National Institutes of Health (NIH) Grant #AI043346-08.

Scott Croy is grateful to the American Foundation for Pharmaceutical Education (AFPE) for funding through a Pre-Doctoral Fellowship in the Pharmaceutical Sciences. Nofable ESO-9920 and Cremophor EL were generously donated by NOF Corp. and BASF Corp., respectively.

APPENDIX

Micelle–Water Partition Coefficient (*P*) Determination by Fluorescence Spectroscopy

The micelle–water partition coefficient (P) is a dimensionless number reflecting a ratio of values:

$$P = \frac{[S_m]}{[S_a]} \tag{1}$$

where S_m and S_a are the concentrations of solute contained in the micellar and aqueous phases, respectively. The bulk concentrations of micellar (S_m) and aqueous (S_a) phase solute can be expressed as:

$$S_m = \frac{\theta P C_o}{P\theta + 1 - \theta} \tag{2}$$

and as

$$S_a = \frac{S_o(1-\theta)}{P\theta + 1 - \theta} \tag{3}$$

where S_o is the total concentration of probe and θ is the volume fraction of surfactant present as micelles at a given surfactant concentration. The term θ is expressed as

$$\theta = 0.01v(C_s - cmc) \tag{4}$$

where C_s is the given concentration of surfactant and *cmc* is the critical micellization concentration expressed in % w/v. The term v is the partial specific volume of the surfactant in solution, and is a thermodynamic parameter that can be calculated from density measurements. When eqs. 2 and 3 are rearranged, the fraction of solute incorporated into the micelles (α) can be written

$$\alpha = \frac{S_m}{S_o} = \frac{P\theta}{P\theta + 1 - \theta} \tag{5}$$

The fraction of solute incorporated into the micelles (α) is also a function of fluorescence intensity, and is governed by the following expressions: $I_o = f_a S_o$ and $I_{\max} = f_m S_o$, where f_a and f_m are the molar coefficients of emission that correspond to the solute in the bulk aqueous phase and in the micellar phase, respectively. The emission in surfactant solutions consists of the emissions of the aqueous and micellar solute as shown:

$$I = S_o[(1-\alpha)f_a + \alpha f_m] \tag{6}$$

where I is the total fluorescence intensity of the solute at a given surfactant concentration. Therefore, α can also be expressed as:

$$\alpha = \frac{I - I_o}{I_{\max} - I_o} \tag{7}$$

Equations 4, 5, and 7 are combined, resulting in

$$\frac{I_{\max} - I_o}{I - I_o} = \frac{1}{0.01Pv(C_s - cmc)} + 1 - \frac{1}{P} \tag{8}$$

where a plot of $(I_{\max} - I_o)/(I - I_o)$ versus the inverse of $C_s - cmc$ yields a linear plot with the slope equal to $1/(0.01Pv)$, which can be solved to obtain the partition coefficient.

REFERENCES

1. Jonkman-de Vries JD, Flora KP, Bult A, Beijnen JH. 1996. Pharmaceutical development of (investigational) anticancer agents for parenteral use: A review. Drug Dev Ind Pharm 22:475–494.
2. Inactive ingredient search for approved drug products, "polysorbate 80" and "polyoxyl 35 castor oil." United States Food and Drug Administration, 2004 (8 December), http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm.
3. Adams ML, Lavasanifar A, Kwon GS. 2003. Amphiphilic block copolymers for drug delivery. J Pharm Sci 92:1343–1355.
4. Tije AJ, Verweij J, Loos WJ, Sparreboom A. 2003. Pharmacological effects of formulation vehicles: Implications for cancer chemotherapy. Clin Pharmacokinet 42:665–685.
5. Gunderson SM, Hoffman H, Ernst EJ, Pfaller MA, Klepser ME. 2000. In vitro pharmacodynamic characteristics of nystatin including time-kill and postantifungal effect. Antimicrob Agents Chemother 44:2887–2890.
6. Bolard J, Legrand P, Heitz F, Cybulska B. 1991. One-sided action of amphotericin B on cholesterol-containing membranes is determined by its self-association in the medium. Biochemistry 30:5707–5715.
7. Coutinho A, Prieto M. 1995. Self-association of the polyene antibiotic nystatin in dipalmitoylphosphatidylcholine vesicles: A time-resolved fluorescence study. Biophys J 69:2541–2557.
8. Johnson EM, Ojwang JO, Szekely A, Wallace TL, Warnock DW. 1998. Comparison of in vitro antifungal activities of free and liposome-encapsulated nystatin with those of four amphotericin B formulations. Antimicrob Agents Chemother 42:1412–1416.

9. Denning DW, Warn P. 1999. Dose range evaluation of liposomal nystatin and comparisons with amphotericin B and amphotericin B lipid complex in temporarily neutropenic mice infected with an isolate of *Aspergillus fumigatus* with reduced susceptibility to amphotericin B. Antimicrob Agents Chemother 43:2592–2599.
10. Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP. 1998. National surveillance of nosocomial blood stream infection due to *Candida albicans*: Frequency of occurrence and antifungal susceptibility in the SCOPE program. Diagn Microbiol Infect Dis 31:327–332.
11. Castanho MARB, Coutinho A, Prieto MJE. 1992. Absorption and fluorescence spectra of polyene antibiotics in the presence of cholesterol. J Biol Chem 267:204–209.
12. Croy SR, Kwon GS. 2004. The effects of pluronic block copolymers on the aggregation state of nystatin. J Controlled Release 95:161–171.
13. Wilhelm M, Zhao C, Wang Y, Xu R, Winnik MA, Mura J, Riess G, Croucher MD. 1991. Poly(styrene-ethylene oxide) block copolymer micelle formation in water: A fluorescence probe study. Macromolecules 24:1033–1040.
14. Kalyanasundaram K, Thomas JK. 1977. Environmental effects on vibronic band intensities in pyrene monomer fluorescence and their application in studies of micellar systems. J Am Chem Soc 99: 2039–2044.
15. Dong DC, Winnik MA. 1984. The Py scale of solvent polarities. Can J Chem 62:2560–2565.
16. Kozlov MY, Melik-Nubarov NS, Batrakova EV, Kabanov AV. 2000. Relationship between pluronic block copolymer structure, critical micellization concentration and partitioning coefficients of low molecular mass solutes. Macromolecules 33:3305–3313.
17. Kabanov AV, Nazarova IR, Astafieva IV, Batrakova EV, Alakhov VY, Yaroslavov AA, Kabanov VA. 1995. Micelle formation and solubilization of fluorescent probes in poly(oxyethylene-b-oxypropylene-b-oxyethylene) solutions. Macromolecules 28: 2303–2314.
18. Sun SF, Serpentino PM. 1974. The determination of partial specific volume. J Chem Educ 51:552–553.
19. Mukerjee P, Ko J. 1992. Solubilization of ethyl *o*-, *m*-, and *p*-aminobenzoates in micelles of different charge types: Interfacial adsorption and orientation effects. J Phys Chem 96:6090–6094.
20. Gonzalez M, Vera J, Abuin EB, Lissi EA. 1984. Solubilization of naphthalene derivatives in micellar assemblies. J Colloid Interface Sci 98:152–161.
21. Patel NK, Kostenbauder HB. 1958. Interaction of preservatives with macromolecules I. Binding of parahydroxybenzoic acid esters by polyoxyethylene 20 sorbitan monooleate (Tween 80). J Am Pharm Assoc 47:289–293.
22. Dougherty SJ, Berg JC. 1974. Distribution equilibria in micellar solutions. J Colloid Interface Sci 48: 110–121.
23. Donbrow M, Rhodes CT. 1964. Potentiometric studies on solubilization in non-ionic micellar solutions. Part I. Interpretation of pH changes and mechanism of solubilization of benzoic acid. J Chem Soc Suppl:6166–6171.
24. Donbrow M, Molyneux P, Rhodes CT. 1967. Potentiometric studies on solubilization in non-ionic micellar solutions. Part II. Solubilization of benzoic acid and the hydroxybenzoic acids and location of the solubilizates. J Chem Soc A 4:561–565.
25. Choucair A, Eisenberg A. 2003. Interfacial solubilization of model amphiphilic molecules in block copolymer micelles. J Am Chem Soc 125:11993–12000.
26. Crittenden B, Thomas WJ. 1998. Adsorption technology & design. Oxford: Butterworth-Heinemann, pp 31–65.