Published in final edited form as:

Dent Mater. 2007 August ; 23(8): . doi:10.1016/j.dental.2006.08.005.

Effects of solubilizing surfactants and loading of antiviral, antimicrobial, and antifungal drugs on their release rates from ethylene vinyl acetate copolymer

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Abstract

Objectives—This study investigates the effects of surfactants and drug loading on the drug release rate from ethylene vinyl acetate (EVA) copolymer. The release rate of nystatin from EVA was studied with addition of non-ionic surfactants Tween 60 and Cremophor RH 40. In addition, the effect of increasing drug load on the release rates of nystatin, chlorhexidine diacetate and acyclovir is also presented.

Method—Polymer casting solutions were prepared by stirring EVA copolymer and nystatin (2.5 wt %) in dichloromethane. Nystatin and surfactants were added in ratios of (1:1), (1:2) and (1:3). Drug loading was studied with 2.5, 5.0, 7.5, and 10.0% wt. proportions of nystatin, chlorhexidine diacetate and acyclovir incorporated into a separate polymer. Three drug loaded polymer square films (3cm × 3cm × 0.08 cm) were cut from dry films to follow the kinetics of drug release at 37°C. 10 ml of either distilled water or PBS was used as the extracting medium that was replaced daily. PBS was used for nystatin release with addition of surfactants and water was used for the study on drug loading and surfactant release. The rate of drug release was measured by UV-spectrophotometer. The amount of surfactant released was determined by HPLC.

Results—The release of nystatin was low in PBS and its release rate increased with the addition of surfactants. Also, increasing surfactant concentrations resulted in increased drug release rates. The release rates of chlorhexidine diacetate ($p < 0.0001$), acyclovir ($p < 0.0003$) and nystatin ($p < 0.0017$) linearly increased with increasing drug loads. The amount of surfactants released was above the CMC.

Significance—This study demonstrates that the three therapeutic agents show a sustained rate of drug release from EVA copolymer over extended periods of time. Nystatin release in PBS is low owing to its poor solubility. Its release rate is enhanced by addition of surfactants and increasing the drug load as well.

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Keywords

Drug delivery; EVA matrix; nystatin; surfactants; chlorhexidine diacetate; acyclovir; drug loading

Introduction

Nystatin is an antifungal drug that is widely used for treating oral infections. It has low solubility in water and saliva [1]. It is known that the solubility of sparingly water soluble drugs can be increased through the addition of surfactants [2]. Solubilization of water insoluble drugs by micelles has long been investigated as a means of improving solubility for drug delivery [3] and the incorporation of a wide variety of drugs into micelles formed from a large variety of surfactants in particular non-ionic surfactants have been studied [4,5]. Nystatin release from a chewing gum formulation as drug delivery device with addition of non-ionic surfactants Tween 60 (polyoxyethylene sorbitan mono stearate), Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil) and Panodan AB 90 (diacetyl tartaric acid esters of mono and diglycerides of vegetable fats) was studied by Andersen et al [6]. The addition of surfactants promoted a far higher release of nystatin. There are other reports that have demonstrated an increase in drug release due to the addition of surfactants [7-10].

The main objective of this study is to investigate the effect of surfactants Tween 60 (Tween) and Cremophor RH 40 (Cremophor) on the release rate of nystatin from ethylene vinyl acetate (EVA) copolymer. Our second objective is to study the influence of drug loading on the rate of release of nystatin, chlorhexidine diacetate and acyclovir from EVA. Our previous studies have shown the ability of EVA, a biocompatible copolymer to deliver drugs at constant concentrations for an extended period of time [11-15]. This copolymer system is able to release the drug over several weeks and therefore may be useful as a drug carrier in the treatment of oral infections.

Materials and Methods

The materials used in this study are detailed in Table 1.

Preparation of thin polymer films

Drug loaded EVA polymer films were prepared by solvent evaporation technique according to our earlier studies [11-15]. Nystatin loaded samples with surfactants Tween and Cremophor were prepared similarly in drug to surfactant ratios of (1:1), (1:2) and (1:3). For the experiment on the effect of surfactants on nystatin release, nystatin incorporated was 2.5 wt % of EVA. The release rates from these samples were compared to those of nystatin alone in order to study the effect of surfactants. In addition, drug loading was studied with 2.5, 5.0, 7.5, and 10.0% wt. proportions of nystatin, chlorhexidine diacetate, and acyclovir incorporated into a separate polymer.

Determination of release rate

Three drug loaded polymer square films ($3\text{cm} \times 3\text{cm} \times 0.08\text{ cm}$) were cut from dry films to follow the kinetics of drug release at 37°C . 10 ml of either distilled water or PBS was used as the extracting medium. The release of nystatin with surfactants was studied in PBS while water was used for the release study of increasing loads of chlorhexidine diacetate, acyclovir and nystatin. Fresh 10 ml samples of the media were used daily for 12-14 days and the extracts were analyzed for a decrease in concentration by measuring the optical density (OD) spectrophotometrically (Hitachi U-2810 Spectrophotometer) at wavelengths (λ_{max}) where the maximum absorption occurred. The λ_{max} values were 306, 257.5 and 253 nm for

nystatin, chlorhexidine diacetate and acyclovir respectively. Using standard plots of OD vs. concentration, the drug concentration was determined each day.

UV spectral measurements were also made for the two surfactants Tween and Cremophor. The surfactants did not exhibit any absorbance in the region 200-400 nm and did not interfere with the determination of absorbance values for nystatin. Also, the standard plots of nystatin were similar with and without the addition of surfactants.

The amount of Tween and Cremophor released from the nystatin loaded EVA films in water at 37°C was determined by High Performance Liquid Chromatography (HPLC). The used HPLC set up was equipped with a Waters Atlantis dC18 3.9 × 150 mm, a Waters 2695 separation module, a Waters 2420 evaporative light scattering detector with settings 75% nebulizer heater level; 80 °C drift tube temperature; 20 gain; 30 psi nitrogen gas. The mobile phase was a mixture of A (85 % Fisher HPLC Grade Water) and B (15 % Fisher HPLC Grade acetonitrile) with a gradient elution of 70% A and 30 %B for the first 5 minutes and 85 % A and 15 % B for the next 11 minutes. The flow rate was 1.00 ml/min and sample injection was 20 µL. Using standard surfactant plots, the concentration of the surfactant released from the EVA films was measured.

Determination of solubility of drugs

Solubility of the three drugs chlorhexidine diacetate, acyclovir and nystatin was determined individually in 5 ml of water by stirring a saturated solution of the drug at 37 °C for 24 hours. The excess drug was filtered and the solution was analysed by UV spectrophotometer.

Statistical Analysis

For each study, one-way analysis of variance was applied to the drug release rates transformed to the log scale to achieve approximate normality and variance homogeneity. If the overall F-test for drug with/without surfactant was statistically significant at the .05 level, nine post-hoc contrasts were tested. Six of these comparisons involved the assessment of the impact of adding surfactant in various ratios: nystatin alone vs. nystatin + cremophor (1:1), nystatin alone vs. nystatin + Tween (1:1), mixtures of 1:1 ratio vs. those of 1:2 ratio, and of 1:2 ratio vs. those of 1:3 ratio for Cremophor vs. Tween, respectively. The final three contrasts pertained to comparing Cremophor vs. Tween with the drug to surfactant ratio fixed at one of the three levels. Bonferroni adjustments were made for multiple testing within these two sets of pairwise comparisons for ratios. For the assessment of ratios we defined $p\text{-value} < .05/6 = 0.008$ as statistically significant. For the comparison of surfactants we defined $p\text{-value} < .05/3 = 0.017$ as statistically significant. Finally, one-way ANOVAs were performed to assess the effect of loading of chlorhexidine diacetate, acyclovir and nystatin ranging from 2.5 % to 10.0 % wt. in EVA. When the percent weight was statistically significant (i.e. $p < .05$), pairwise comparisons of 2.5% vs. 5.0%, 5.0% vs. 7.5%, and 7.5% vs. 10.0 were conducted with Bonferroni adjusted $p\text{-value}$ criterion $< .05/3 = .017$.

Results

Table 2 shows the release rates of nystatin and nystatin with surfactants in PBS at 37°C respectively. Eighteen of 21 linear regression fits to the cumulative release data that had $R^2 > .95$ (with the lowest $R^2 = 0.89$) supporting use of linear regression to estimate rates for the range of time in days examined. The effect of surfactant content on nystatin release was studied in PBS medium (Figures 1 and 2). Analysis of the data shows that the cumulative release of nystatin is $0.87 \mu\text{g}/\text{cm}^2\cdot\text{day}$. Generally, addition of surfactants resulted in an increase in the release rate of nystatin (Overall F-test $p\text{-value} < .001$). The increase in surfactant content resulted in a 1.1 to 4.3 times higher nystatin release rate with the addition

of Tween and a 1.1 to 2.3 times higher release rate with the addition of Cremophor. A comparison of ratios resulted in no statistically significant differences between nystatin alone versus either Tween added ($p = .132$) or Cremophor added ($p = .031$) in a 1:1 ratio. However, in all four comparisons of 1:1 vs. 1:2 or 1:2 vs. 1:3 (for each surfactant) the higher ratio resulted in significantly higher release rate (all $p < .008$). At fixed ratio, addition of Tween did not result in a significantly different release rate than addition of Cremophor for 1:1 ratio, but Tween did result in a higher rate for ratios of 1:2 and 1:3 (both $p < .001$).

Table 3 shows the amount of Tween and Cremophor released from nystatin loaded EVA films in water at 37 °C. The surfactant released was between 1.2-2.6 mg/ml on day 1 and decreased by about 30-50 % by day 3.

Figure 3 shows the chlorhexidine diacetate release rate with increasing drug loads in water at 37°C. All linear regression fits to cumulative release data gave $R^2 > .95$. This graph is representative of the other drugs included in the study.

Table 4 summarizes the data on the effect of loading of chlorhexidine diacetate, acyclovir and nystatin ranging from 2.5 % to 10.0 % wt. in EVA on the release rate of the drugs. The release rate increased as the drug load increased for chlorhexidine diacetate, acyclovir and nystatin, (all p -values < 0.001). Regarding post-hoc comparisons, for Acyclovir, 2.5% was statistically different than 5.0 % ($p=.002$), but 5.0% was not significantly different from 7.5% ($p=.032$) nor was 7.5% statistically different from 10.0% ($p=.082$). For chlorhexidine diacetate, all drug loading pairs were statistically different (all $p < .002$). Finally, for Nystatin, 2.5% was statistically different than 5.0% ($p<.001$), 7.5% was statistically different from 10.0% ($p=.001$), but 5.0% was not significantly different from 7.5% ($p=.36$).

The solubility of chlorhexidine diacetate, acyclovir and nystatin in water at 37 °C were found to be 20, 2.67 and 0.715 mg/ml respectively.

Discussion

This study demonstrated that the three therapeutic agents show a sustained rate of drug release from EVA copolymer over extended periods of time. This study also showed that the rate of drug release increased with increasing addition of surfactant in the case of nystatin and with increasing drug loading in EVA up to 10.0 wt. %.

Effect of solubilizing agents on the rate of nystatin release

When surfactant molecules are dissolved in water at concentrations above the critical micelle concentrations (*cmc*), they form aggregates known as micelles. An important property of the micelles that has particular significance in pharmacy is their ability to increase the solubility of sparingly soluble substances in water. Release rate of nystatin increased with addition of surfactants owing to the micellar formation by surfactants that solubilizes the poorly soluble nystatin in aqueous environments. Also, it is possible that the surfactant lowers the interfacial tension between the polymer matrix and the dissolution medium; hence it will increase the dispersability of the polymer matrix containing the drug and will also increase the release rate. Perhaps the surfactant acts as a wicking agent, causing the fluid to enter the matrix, the surfactant may then dissolve and form channels from which the drug release may be affected [9].

At surfactant concentration above the *cmc*, the solubility increases linearly with the concentration of surfactant indicating that solubilization is related to micellization [2]. As the proportion of surfactant with respect to nystatin concentration is increased, the release rate of nystatin increased. This may be explained due to the increase of micelles, as a result

of the increased surfactant proportion. The increase in micelles enhances the solubilization of the drug. It was also speculated that increasing amounts of surfactants in the copolymer system together with the drug, increases the porosity facilitating the enhanced diffusion of drug molecules through the channels present in the matrix (EVA matrix), leading to an increase in the rate of drug release [9].

Analysis of the data in Table 3 revealed that the amount of Tween and Cremophor released from the nystatin loaded EVA films with respect to all nystatin to surfactant compositions [(1:1), (1:2) and (1:3)] was well above the *cmc*. The *cmc* for Tween 60 is 0.023 mM (30µg/ml) [16] and that for Cremophor RH 40 is ~0.039 % [17].

Addition of surfactants to nystatin in the EVA films has resulted in promoting release of nystatin that was not as high as reported in earlier studies of nystatin release from chewing gum formulation as drug delivery device [6]. This is perhaps due to the difference in the drug delivery device. The EVA material is a hydrophobic copolymer while the chewing gum formulation constitutes about 50-60% gum base and the rest are sweetening agents, flavoring agents etc that are hydrophilic [18] thereby rendering the chewing gum more hydrophilic than EVA. The extent to which solubilization of poorly soluble drug in aqueous medium depends on the formation of micelles which is facilitated in the drug carrier. It may be explained that the micelle formation is occurring more in the chewing gum than EVA and thereby the nystatin release was far higher in case of chewing gum than EVA. Additionally, chewing gum during service performance constantly undergoes conformational changes with an actively renewed surface over which mechanical forces are imposed. Therefore among several factors, perhaps the above two are responsible for the observed enhanced nystatin release from chewing gum.

Effect of drug loading

The release rate of the three drugs, nystatin, chlorhexidine diacetate and acyclovir, increased with increasing drug proportions in the polymer matrix as shown in Table 4 which shows a relationship between drug loading in the 2.5 to 10.0 wt % in EVA and the release rate of the drug. Increase in loading of the drug affecting the release kinetics has been reported earlier [19]. Water diffuses into the matrix through the dispersed phase to dissolve the drug upon contact. The drug particles, once dissolved, leave behind pores in the polymer matrix. The drug molecules can then diffuse out through the interconnecting pores.

Analysis of Table 4 shows that the release rate of acyclovir is more than chlorhexidine followed by nystatin. The release rate of nystatin is less owing to its low solubility in water. The solubility of chlorhexidine in water is more than acyclovir and based on the solubility of the drugs in water one would expect the release rate of chlorhexidine to be more than that of acyclovir and the observed results may be explained due to other factors that influence the release of a drug from a polymer matrix such as their relative solubility in the polymer matrix, drug-polymer interactions and their relative diffusion through the channels in the polymer matrix [8].

Increase in nystatin loading from 2.5 to 10 wt % resulted in rates that were 1.2-1.5 times higher than addition of surfactants except with nystatin and Tween (1:3) ratio.

Conclusions

1. The release rate of nystatin in PBS increased with the addition of surfactants. Furthermore, increasing surfactant concentrations resulted in increased drug release rates.

2. Increased loading of nystatin, chlorhexidine diacetate and acyclovir in the polymer matrix resulted in an increase in the release rate of the drug.
3. All three drugs exhibited a linear relationship between loading and release rate.

Acknowledgments

This work was supported by NIH-NIDCR grant R01 DE 15267. The authors are very grateful to Dr. Keith Beck, Professor and Department Head, Textiles Engineering Chemistry and Science, North Carolina State University, Raleigh, North Carolina for his great help with the determination of surfactant release by HPLC. The authors wish to thank Dr. Anton Schindler, Principal Scientist, Research Triangle Institute, Research Triangle Park, North Carolina, USA, for his valuable suggestions. The authors wish to thank Mr. Douglas Evans of DuPont packaging and industrial polymers, Chestnut Run Plaza, Wilmington, DE USA, for his constant help and generous supply of EVA (Elvax) beads.

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Figure 1.
Effect of increase in concentration of Tween on the release of nystatin in PBS

Figure 2.
Effect of increase in concentration of Cremophor on the release of nystatin in PBS

Figure 3.
Effect of drug load on the release of chlorhexidine diacetate

Table 1

Materials and suppliers

Material	Suppliers
Chlorhexidine diacetate	Sigma-Aldrich
Nystatin	Sigma-Aldrich
Acyclovir	Sigma-Aldrich
Ethylene vinyl acetate (Elvax 40Wg) (EVA)	DuPont
Dichloromethane	Mallinckrodt Baker Inc.
Phosphate Buffer Saline (PBS)	Sigma-Aldrich
Tween 60 (Tween)	Sigma-Aldrich
Cremophor RH 40 (Cremophor)	Bayer

Table 2

Release rate of nystatin and nystatin with addition of surfactants in PBS

Drug with /without surfactant	Release Rate in PBS ($\mu\text{g}/\text{cm}^2.\text{day}$) *
Nystatin alone	0.87(0.04)
Nystatin + Tween (1:1)	1.02(0.04)
Nystatin + Tween (1:2)	2.88(0.28)
Nystatin + Tween (1:3)	3.77(0.55)
Nystatin + Cremophor (1:1)	0.97(0.02)
Nystatin + Cremophor(1:2)	1.55(0.10)
Nystatin + Cremophor (1:3)	2.00 (0.10)

*
Rate expressed in terms of mean (SD)

Table 3

Concentration of Tween and Cremophor released in water at 37 °C

Composition of nystatin and surfactant	Duration of release * (Days)	Amount of surfactant released (mg/ml)
Nystatin + Tween (1:2)	1	1.121
Nystatin + Tween (1:2)	3	0.542
Nystatin + Tween (1:3)	1	1.933
Nystatin + Tween (1:3)	3	0.615
Nystatin + Tween (1:1)	6	0.320
Nystatin + Cremophor (1:2)	1	1.926
Nystatin + Cremophor (1:2)	3	0.678
Nystatin + Cremophor (1:3)	1	2.56
Nystatin + Cremophor (1:3)	3	1.30

* Surfactant release shown is measured for one day (24 hours) and the number denotes the particular day of release

Table 4

Release rate with increase in drug load in water

Drug loading in EVA (wt%)	Rate of drug release in water ($\mu\text{g}/\text{cm}^2\cdot\text{day}$) [*]		
	Acyclovir	Chlorhexidine diacetate	Nystatin
2.5	2.87(0.97)	2.58 (0.02)	0.97 (0.05)
5.0	6.2(0.24)	4.55 (0.50)	1.74(0.16)
7.5	9.2(0.80)	7.32 (0.53)	1.93(0.17)
10.0	12.0(0.90)	9.52 (0.17)	2.91(0.02)

^{*} Rate expressed in terms of mean (SD)