Controlled drug release for oral condition by a novel device based on ethylene vinyl acetate (EVA) copolymer

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The application of drug delivery systems in oral environment is relatively a new area of research with the exception of release of fluoride ions from polyalkenoate cements and their predecessor silicate cements. The present study addresses development of a novel device based on ethylene vinyl acetate copolymer (EVA), a biocompatible material which enables constant drug release over several days to treat oral infections. Drugs incorporated in EVA included tetracycline, minocycline and nystatin together with combinations (C) of nytatin-tetracycline (1 : 1) and nystatin-minocycline (1 : 1). Polymer casting solutions were prepared by dissolving EVA and the drugs in the ratio of 10 : 1 in 70 ml of dichloromethane at 38 °C for 6 h. Thin square films of 3 × 3 cm and 1 mm thickness were cut from the dry sheet obtained by solvent evaporation. Drug loaded samples were extracted for a minimum of 15 days in 10 ml medium (water or water/ethanol (1 : 2) or 0.9% saline solution) which is replaced daily. Spectral measurements were made to follow changes in optical densities (OD) during release kinetics. Analysis of the data revealed that among all the drugs tested tetracycline exhibited the highest release rate (56.15 ug/cm^2/day) and % cumulative release (27.92).The observed enhanced values may be interpreted as due to the channels formed due to changes in free volume (microvoids). In case of nystatin-minocycline combination, the observed increased values of release rates and percent cumulative release, may be attributed to the swelling component or channels or relative hydrophobic interactions. Initial ''burst'' effects due to liberation of surface-bound drug molecules were observed with reference to all the three drugs and the combinations of drugs studied. Among all the drugs, minocycline exhibited the least ''burst'' effect suggesting that the drug is more homogeneously distributed in the copolymer. Drug loaded EVA thermoplastic copolymer may provide a favorable therapeutic material for the development of a novel, local treatment for oral, mucosal and periodontal infections.

1. Introduction

The administration of drugs to or via the oral cavity is a difficult problem that offers many challenges to scientists in dentistry and pharmacy. Although dental literature contains several publications on the general topic of oral drug delivery, very few have discussed with reference to the application of drug delivery systems. The use of these systems in dentistry is relatively a new area of research with an exception to the release of fluoride ions from polyalkenoate cements and their predecessor silicate cements [1, 2]. More recently, certain types of composite filling materials such as ''compomers'' [2], some orthodontic resins [3] and few methacrylate based systems [4] were reported to release fluoride ions in order to reduce dental caries [5]. The control of *candida albicans* is another area where drug loaded polymeric materials are being used to avoid repeated mouthwashes [6–10]. It was reported previously that tetrahydrofurfuryl methacrylate (THFMA)/poly(ethyl methacrylate) (PEM), and cold cure polymer systems (THFMA/PEMA) were proposed as drug delivery systems for chlorhexidine and other drugs [11].

Tetracyclines have bacteriostatic activity against a wide variety of pathogens that are responsible for many common and some exotic infections including periodontal condition [12]. The tetracyclines, among the first of antibiotics to become available about five decades ago, remain widely used. They are particularly valuable in the treatment of a typical pneumonia syndromes, chlamydial genital infections, lyme disease, etc. Based

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on pharmacokinetic considerations, doxycycline is the preferred agent among the tetracycline congeners. Minocycline may have a limited role in the treatment of methicillin-resistant staphylococcal disease in situations in which an oral antimicrobial may be appropriate. The tetracyclines possess association with dental staining and with food interactions. These drug and food interactions may limit gastrointestinal absorption [12]. Recently, the local delivery of antimicrobial agents such as tetracycline has been studied as a possible method for controlling and treating periodontitis. A number of agents have been investigated both as adjunctive therapies with scaling and root planning and as stand-alone chemotherapies. These agents have been administered in irrigation solutions and as single-dose formulations, but with little long-term efficacy in the treatment of periodontitis. Recent studies have focused on the delivery of antimicrobials in sustained-release formulations designed to maintain effective concentrations of drug within the periodontal pocket [13]. In a recent study of evaluation of antimicrobial efficacy in reducing subgingival microorganisms in periodontal pockets and release pattern of tetracycline and metronidazole using ethyl cellulose strips as local delivery system, results showed that tetracycline and metronidazole can both be applied locally to periodontal sites and markedly suppress the subgingival bacteria over a period of several days. It was observed that tetracycline showed a faster release than metronidazole [14].

Nystatin is a non-absorbable agent for oral administration and is considered effective, *in vitro* against a number of fungal micro-organisms and has a pronounced action against *candida albicans*. It may be useful clinically in the treatment of oral candidiasis and preventing the development of intestinal candidiasis and vaginal infections. During the course of protracted therapy with broad spectrum antibacterial agents, nystatin appears to be effective in suppressing the growth of yeast and other fungi which may overgrow [15].

A simple method, for incorporating various proteins and other macromolecules into non-inflammatory (biocompatible) polymers such as Hydron (a polymer of hydroxy ethyl methacrylate (HEMA)), ethylene vinyl acetate (EVA) copolymer (40% by weight vinyl acetate), polyvinyl alcohol (PVA), was developed in the past two decades and sustained release of biochemical active macro-molecules were demonstrated for periods extending to 100 days [16–19]. Sterile polymer casting solutions are generally made by dissolving polymers in appropriate solvents. With EVA copolymer (40 wt % vinyl acetate) in methylene chloride at 38 °C, slow release pellets were made by placing a mixture of casting solution and the macromolecule to be released in a conical mold, 2–4 mm in diameter and 1.5 mm deep. In order to avoid adhesion caused by the reaction of polymer casting solution with plastics, glass molds are commonly used for this preparation. The glass molds are dried under mild vacuum (overnight, causing the solvent to evaporate leaving the macromolecule trapped within the polymer matrix). To retard further release of macromolecules, ''sandwiching'' can be done by coating the matrix in pure polymer in casting solution. Coating was achieved by dipping the polymer pellet in a puddle of pure polymer in casting solution and drying [16]. Another application of EVA copolymer is in the use of intrauterine retaining device for controlling fertility over a long period of time [20]. The use of this material is further extended to include the device that contains an ophthalmic drug which is dispensed to the eye by diffusion through the copolymer. The device is adapted for insertion in the cul-de-sac of the conjunctiva [20].

The development of drug loaded, polymeric devices for the treatment of oral infections by controlled release of the drug for extended time periods is complex and multifactorial in the optimization of formulations. In general, three independent transport processes are involved in the treatment: (1) diffusion of the drug from the polymer into the surrounding fluid such as saliva or gingival crevicular fluid, (2) transport of the dissolved drug from the release site by the fluid, and (3) uptake of the drug from the fluid phase by the mucosa or targeted tissue (Fig. 1).

The main objective of the present study is to examine the delivery of antimicrobial agents in controlled-release from a biocompatible copolymer (EVA) and to maintain effective concentrations of drug over an extended period. Achieving the desired results in this study would have wide beneficial use (such as physiological or pharmacological effect) as a general drug delivery system.

2. Experimental procedures

2.1. Materials

Materials used in the study include non-inflammatory EVA copolymer obtained from Du Pont, Willmington DE (Elvax; Grade 140 Wg), water insoluble nystatin, water-soluble drugs such as minocycline and tetracycline (all drugs obtained from Sigma Chemical Company, St. Louis, MO), and low boiling dichloromethane (Cl_2CH_2) obtained from Mallinckrodt Baker Inc, Spctr AR, Paris, KY. In addition to individual drugs, combinations of nystatin/minocycline (C), nystatin/tetracycline (C) in equal proportion were also used in the study.

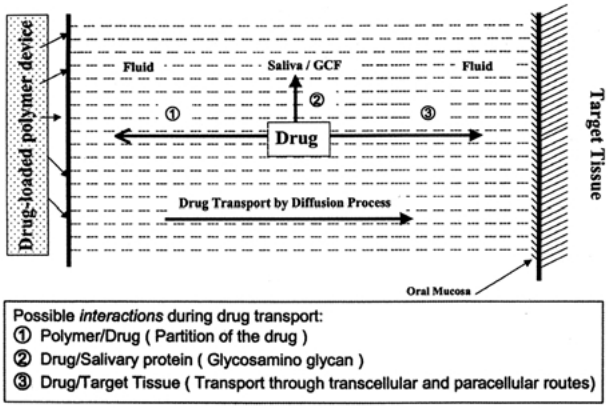


Figure 1 Mechanism of drug transport (diffusion) from the polymeric device through surrounding fluid to the infected site.

2.2. Method

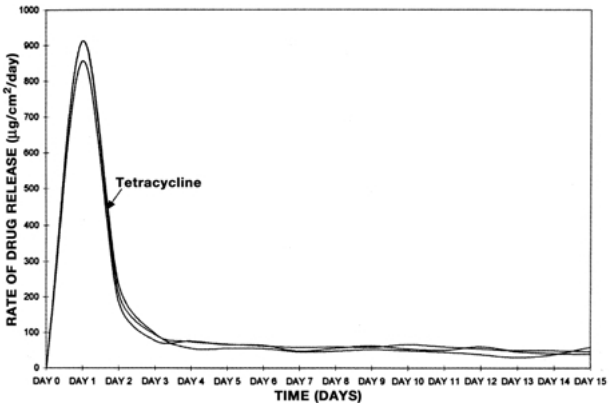
2.2.1. Drug solubility

Minocycline and tetracycline are water soluble and hence double distilled water was used in the study for these drugs. Nystatin is water insoluble and hence a mixture of water/ethyl alcohol system (1 : 2) was used to study the solubility characteristics and the kinetics of nystatin and for the combination involving nystatin. In order to mimic the oral condition, experiments were carried out at 23 °C performed in the aqueous medium (0.9% saline solution) [19].

2.2.2. Preparation of polymer thin films

The following method for incorporating the drugs into the copolymer such as EVA (40% by weight vinyl acetate) was adopted. EVA copolymer system is respectable from the point of biocompatibility, as this material has already been used as mouth guard material and contraceptive drug [20]. Polymer casting solutions were prepared by dissolving EVA copolymer (15 grams) together with the drug (1.5 grams) in 70 ml of dichloromethane at 38 °C for 6 h with stirring in a stoppered 500 ml conical flask. Glass petri-dishes (PYREX, 9.9 cm dia, 1.75 cm wall height) were used, because the polymer casting solutions reacted with plastics inducing adhesion. In order to prepare the film of thickness 1 mm or little less, a volume of about 3 to 5 ml of the drug containing polymer casting solution was poured into the glass petri-dishes (up to the mark at 3 mm high). These petri-dishes were dried under mild vacuum at 23 °C overnight to remove solvent by evaporation. Drug loaded polymer square films were cut from the dry films, and a minimum of four squares were used to follow the kinetics of drug release. A volume of 10 ml distilled water or water–ethanol system (pH 5.4) was used, depending on the solubility of the drug under examination, to collect the drug release daily. Fresh samples of 10 ml of the media were used daily for at least 15 days and decreases in drug concentrations were followed by measuring the optical density (OD) spectrophotometrically (Beckman Du® 70 Spectrophotometer) at wavelength (λ_{max}) where maximum absorption occurs. The values of λ_{max} for nystatin (306 nm), minocycline (350 nm) and tetracycline (276.7 nm) were determined separately by spectral measurements from 220 nm to 400 nm. Using standard plots between drug concentration and OD, decreases in drug concentration were determined each day [17–19].

Drug release profiles generated by following the decreases in the concentration OD of the drugs were determined spectrophotometrically for a period of at least 15 days. At least a minimum of four samples of each drug loaded polymer films was used in the kinetic study of drug release. Values of constant release rates and % total release of all the three individual drugs and combinations; as well were determined using the plots obtained from the drug release at various time intervals (days) (Fig. 2a–c). The observed drug release profiles conform to the usual trend representing initially first order followed by zero order kinetics.



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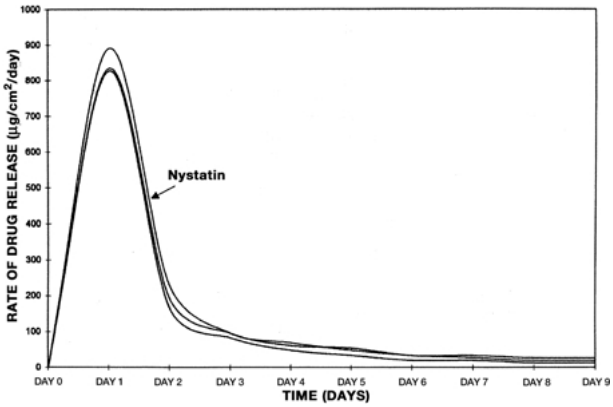

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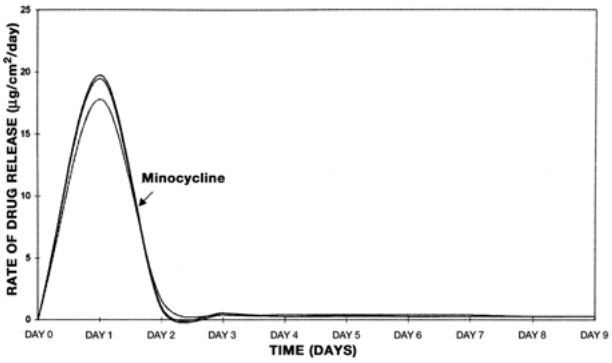


(c)

Figure 2 (a) Time release profiles of tetracycline from EVA copolymer system. (b) Time release profiles of nystatin from EVA system. (c) Time release profiles of minocycline from EVA system.

3. Results

Table I summarized the values of λ_{max} (maximum absorption wavelength) for all the drugs used in the study.

Rate data reported here is based on the part of the curve after the onset of initial burst. The initial burst is attributed to the surface-bound drug [20–23].

Table II summarizes the data with reference to the release rate and % of total drug release over a period of at least 15 days for individual nystatin in water/ethanol (1 : 2) and in 0.9% saline solution; minocycline in water and in water/ethanol (1 : 2); tetracycline in water and water/ethanol (1 : 2) and the combinations of nystatin/minocycline and nystatin/tetracycline in water/ethanol (1 : 2).

TABLE I λ_{max} (maximum absorption wave length in nm) for the drugs used in the study

Drug	λ_{max} (maximum absorption wavelength, nm)
Tetracycline (T)[a]	276.7
Minocycline (M)[a]	350
Nystatin (N)[b]	306
Nystatin (N)[c]	305
Nystatin-Tetracycline (COM)[b]	306–276.7
Nystatin-Minocycline (COM)[b]	306–350

[a]water medium; [b]water/ethanol (1 : 2); [c]0.9% saline solution; COM: combination of drugs (1 : 1).

Enhanced rate 0.4 mg/cm^2/day and ($\sim$ 1%) % release were observed in case of nystatin in water/ethanol (1 : 2) when compared to its data (0.24 mg/cm^2/day3), 0.5% obtained in 0.9% saline solution.

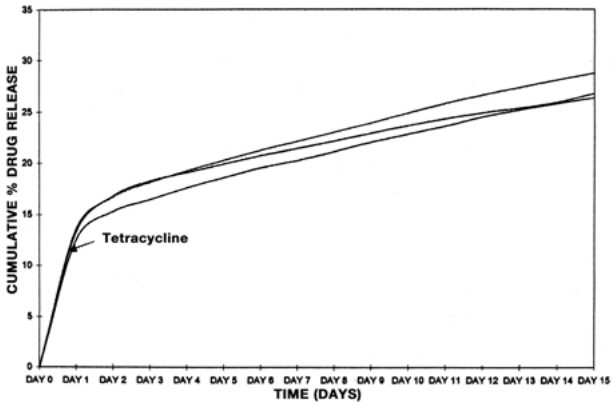
Figs 2a–c represent the release kinetic plots of tetracycline, nystatin and minocycline respectively. Three or four drug loaded samples were used for each drug in this study. The release profiles were found to be very reproducible. This is consistent with the expectation that in many therapeutic programs, such as a rate of release should be relatively constant or have a zero order time dependence, that is, the rate of release is independent of time [17].

Figs 3a–c represent the cumulative % drug release for all the individual drugs, namely tetracycline, nystatin and minocycline at 23 °C.

Fig 4a shows rate of release profiles at 23 °C for individual components of nystatin and minocycline when combined in equal proportions in water–ethanol system (1 : 2). The rate of release conformed to first order followed by zero order kinetics. Fig. 4b represents percentage cumulative release over an extended period of time for the same system under the same set of conditions.

Figs 5a and b show rate of release profiles and percentage of cumulative release (23 °C) respectively for individual drug component of nystatin and tetracycline in nystatin-tetracycline system (combined in equal proportions) in 1 : 2 water–ethanol medium. Release patterns followed the similar trend with reference to kinetic order.

Figs 6a and b show a comparison of release rates and cumulative % release respectively of nystatin, minocycline and tetracycline at 23 °C and under the same set of conditions.



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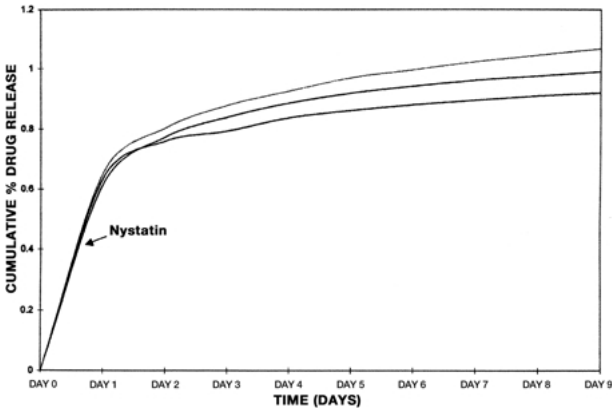

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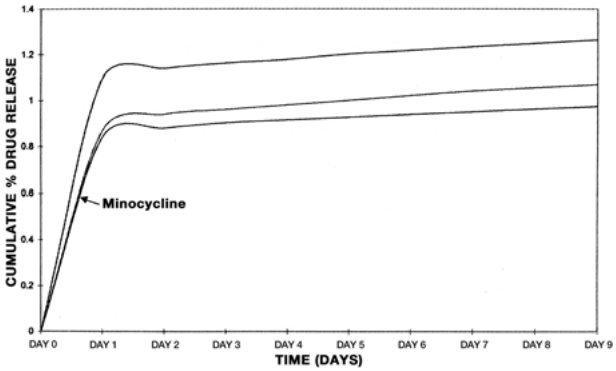


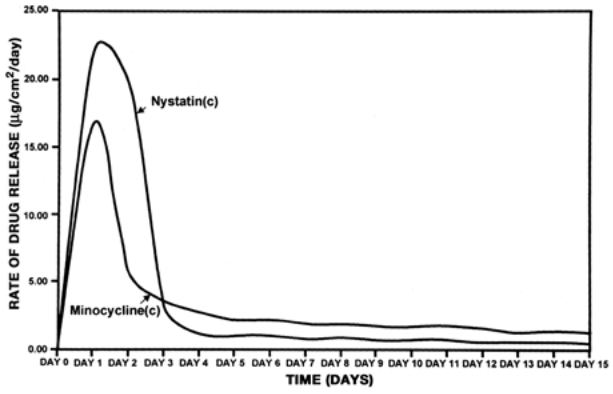
(c)

Figure 3 (a) Cumulative % tetracycline release from EVA copolymer system. (b) Cumulative % nystatin release from EVA copolymer system. (c) Cumulative % minocycline release from EVA copolymer system.

TABLE II Values of constant release rate and percent cumulative release for the drugs used in the study in water, water/ethanol (1 : 2) and 0.9% saline solution

Drug*	Release rate (μg/cm^2 per day)	% Cumulative drug release
Tetracycline (T)[a]	56.15	27.92
Tetracycline (T)[b]	50.44	56.20
Minocycline (M)[a]	0.33	0.97
Minocycline (M)[b]	0.85	1.00
Nystatin (N)[b]	0.40	0.96
Nystatin (N)[c]	0.24	0.05
Nystatin-Tetracycline (COM)[b]	2.28(N); 6.69(T)	3.03 (N); 8.39(T)
Nystatin-Minocycline (COM)[b]	1.23(N); 2.70(M)	7.84(N); 6.65(M)

*Drug in EVA (1 : 7); [a]water medium; [b]water/ethanol medium (1 : 2); [c]0.9% saline solution; COM: drug combination (1 : 1).



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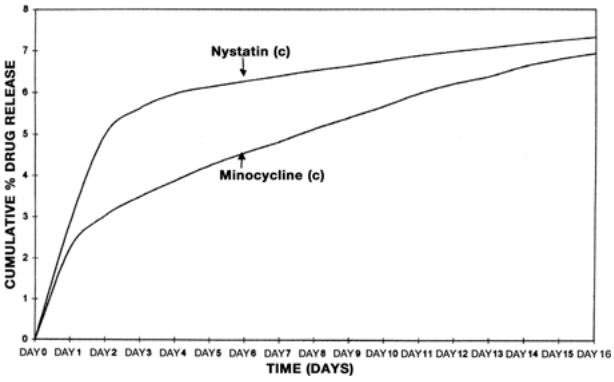
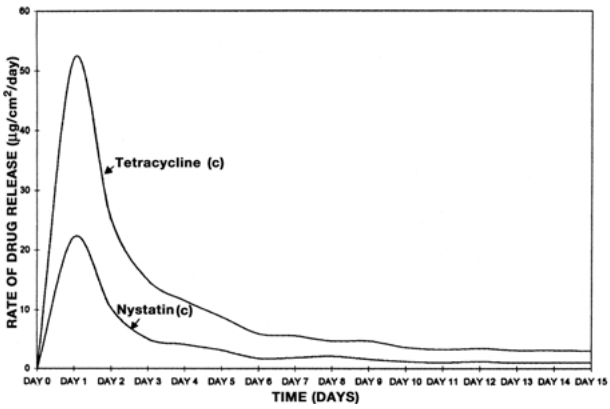


(b)

Figure 4 (a) Time release profiles at 23 °C for individual components of nystatin and minocycline when combined in equal proportions in water/ethanol system (1 : 2). (b) Cumulative % release profiles at 23 °C of nystatin and minocycline when combined in equal propotions in water–ethanol system (1 : 2)



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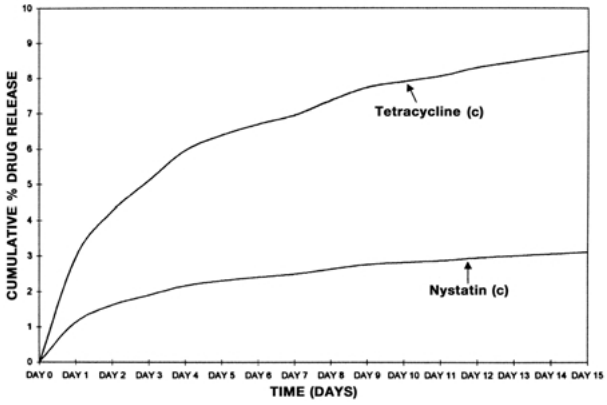


(b)

Figure 5 (a) Time release profiles at 23 °C for individual drug components of nystatin and tetracycline when combined in equal proportions in 1 : 2 water–ethanol system. (b) Cumulative % release profiles at 23 °C of nystatin and tetracycline when combined in equal proportions in 1 : 2 water-ethanol system.

4. Discussion

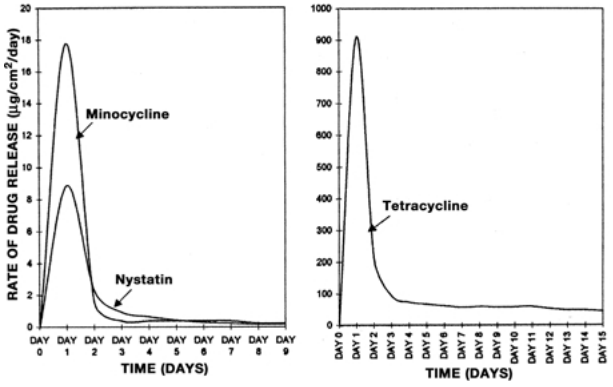
Frequently, release of loaded drug from films is characterized by an initial drug ''burst'', due to the presence of pores in the matrix or liberation of surface-bound drug [21–24]. Similar observation was made in all release profiles, with reference to tetracycline, nystatin and minocycline (Figs 2a–c, 3a–c).

The higher rate and % drug release observed with reference to nystatin in water/ethanol (1 : 2) relative to 0.9 % saline solution, may be interpreted as due to the formation medium which is less polar and swelling component induced by ethanol.

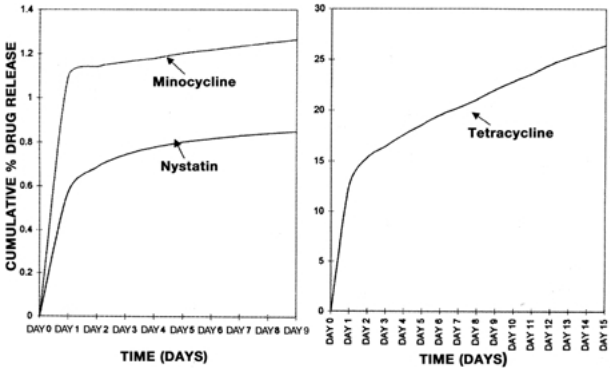
It is of interest to note that among the drug loaded samples, minocycline exhibited least ''burst'' effect which may be attributed to the drug being more homogeneously distributed in the polymer.

Most often, in therapeutic, medical and veterinary programs, it is desirable and important to provide for the slow release of a drug to the body at a controled rate over an extended period of time. In many therapeutic studies, rate of drug release showed to be nearly constant. Similar observation of near zero order kinetics was made with reference to release of tetracycline, nystatin and minocycline from drug loaded EVA films (Figs 2a–c and 3a–c). This is in agreement with the previous finding of the studies involving constant release of pilocarpine from thin film drug-delivery device inserted beneath the lower eyelid controlling glaucoma and release of progesterone at constant rate into the uterus from a drug-delivery device inserted in the uterine lumen in contraception [20]. This implies that the polymer develops a skin during solvent evaporation which presumably acts as a rate limiting factor in drug release.

Analysis of the data in Table II revealed that nystatin and minocycline when combined in equal proportion exhibited release rates about 3 and 8 times greater than those for individual drugs alone. Similar enhanced values of percent cumulative drug release in case of nystatin and minocycline ($\sim$ 8 times and 7 times) compared to individual drugs alone. However, in case of combined system of nystatin-tetracycline, reversal in trend was observed with reference to values of rate release and percent cumulative release of tetracycline from EVA films. About 8.4 times and 3.3 times higher rates and percent cumulative release respectively were observed for tetracycline than those when it is combined with nystatin. This may be interpreted as due to the formation of ''channels'' formed due to the ''free volume'' (voids) present in the polymer matrix facilitating faster diffusion compared to those when combined with nystatin [1]. Nystatin release rates and percent cumulative release in combination with tetracycline exhibited enhanced rates and percent cumulative release 6 and 3 times respectively higher than those when nystatin tested alone. Similar observation was made with nystatin when combined with



(a)



(b)

Figure 6 (a) A comparison of individual time release profiles of nystatin, minocycline and tetracycline. (b) A comparison of individual cumulative % release of nystatin, minocycline and tetracycline.

minocycline. This may be attributed to hydrophobic interactions, size of the drug molecule, drug–polymer interactions, partition effect and drug–drug interaction (Table II; Fig. 4a and b).

It seems clear that nystatin and minocycline in combination exhibited faster release compared to single drug in the EVA copolymer. Tetracycline in combination with nystatin gives increased rate of release of nystatin and decreased rate of release of tetracycline.

Among all the drugs tested, tetracycline exhibited the highest rate (56.15 μg/cm^2/day) (Table II; Fig. 6a), and the cumulative percentage drug release (27.92%) (Table II; Fig. 6b).

5. Conclusions

i. Among all the drugs tested, tetracycline exhibited the highest rate (56.15 μg/cm^2/day) and the % release (27.92) (Table II; Fig. 6b).

ii. The enhanced values of drug release rates and % total drug release in case of nystatin (c) and minocycline (c) may be attributed to their relative hydrophobic interactions or to swelling or to the channels formed due to changes in free volume in the polymer, facilitating faster diffusion.

iii. A nystatin/minocycline combination as well as tetracycline alone in a thermoplastic EVA copolymer may provide a favorable therapeutic material for the development of novel, local treatment for oral, mucosal and periodontal infections.

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