

Polyhydroxyethylmethacrylate/Polyhydroxybutyrate Composite Membranes for Fluoride Release

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ABSTRACT: Controlled release technology is a recent technology which has considerable potential in the fields of medicine, pharmacy, and agriculture. Fluoride ion is commonly used in the preventive treatment of decay, and when provided with extra fluoride, children living in regions that lack fluoride benefit from it. For the present study, clinical properties of an intraoral controlled release fluoride delivery system were considered. The preparation of a controlled release membrane system is described. Polyhydroxyethylmethacrylate/polyhydroxybutyrate composite systems were examined as fluoride carriers. Polymeric membranes were prepared by photopolymerization and then character-

ized. Contact angles and swelling ratios of fluoride-loaded membranes were determined. The surface morphology of the microporous membranes were examined by using scanning electron microscopy (SEM). *In vitro* fluoride release studies were carried out in an artificial saliva medium. The amount of released fluoride was determined and the effect of medium pH and temperature on fluoride release was investigated. © 2002 Wiley Periodicals, Inc. *J Appl Polym Sci* 87: 976–981, 2003

Key words: fluoride release; PHEMA; PHB; composite membranes

INTRODUCTION

Controlled release technology is a recent technology which has considerable potential in the fields of medicine, pharmacy, and agriculture.^{1–3} The fluoride ion is commonly used in the preventive treatment of dental decays. In addition, it is used for remineralization of opaque enamel lesions which have just developed. It has been found that fluoride plays an important role in the prevention and treatment of bacteria plaque.^{4–6} Fluorosis is generally reported in children who live in fluoridated areas and receive additional fluoride through a variety of sources.^{7–9} Traditional topical fluoride application has helped prevent caries by up to 30–40%. However, studies to increase enamel fluoride uptake to provide more protection have been continuing.¹⁰ Recent studies on fluoride cariostatic characteristics focus on the significance of fluoride in fluids around teeth.^{11–13} It has been noted that there is a strong correlation between tooth plaque fluid and fluoride level in saliva. This is an effective factor in fluoride's decay prevention mechanism.^{14,15} Various fluoride agents in toothpaste, gels, and mouthwash that are used at intervals temporarily increase the fluoride level in saliva.¹⁶ In these temporary applications, fluoride disappears at a rapid rate. This fact has

led to a search for systems to ensure a long-lasting fluoride source.

Fluoride-releasing polymers are matrices which surround fluoride and enable controlled release of fluoride. Fluoride release from a system formed by ethylcellulose and poly(ethylene glycol) has been studied *in vitro*.¹⁷ An increase in saliva fluoride concentration in the first 4 days was observed in an *in vivo* clinical research study of ethylcellulose containing sodium fluoride.

This study aims at forming polyhydroxyethylmethacrylate/polyhydroxybutyrate (PHEMA/PHB) composite membrane systems which release fluoride in a controlled way. For this purpose, fluoride-loaded PHEMA membranes were prepared by means of photopolymerization. The membranes were then coated with PHB by a spraying technique for controlling the diffusion rate of fluoride ions. Following characterization, fluoride-release studies were conducted *in vitro*. In these studies, the effects of fluoride loading ratio, pH, and temperature on the fluoride release rate were determined in artificial saliva to mimic the intraoral medium.

EXPERIMENTAL

Materials

The polymeric matrix used in this study consisted of two main components. 2-Hydroxyethylmethacrylate (HEMA) (Sigma, St. Louis, MO) was distilled at re-

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duced pressure under nitrogen atmosphere and the fraction of boiling point, 63°C/3 mmHg, was used. The second component, PHB, was supplied by Sigma. Polymerization initiator azobisisobutyronitrile (AIBN) was obtained from Riedel de Haen Seelze (Germany) and used as received. Chloroform and ethyl alcohol were obtained from Merck (Darmstadt, Germany). All other chemicals were of reagent grade and were purchased from Merck AG. All water used in the release experiments was purified by using a Barnstead (Dubuque, IA) ROpure LP[®] reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure[®] (Wisconsin) organic/colloid removal and ion exchange packed bed system. The resulting purified water (deionized water) had a specific conductivity of 18 mΩcm⁻¹.

Preparation of fluoride-loaded PHEMA membranes

The poly(2-hydroxyethyl methacrylate), PHEMA, membranes were prepared by UV-photopolymerization. The membrane preparation mixture (5 mL) contained 2 mL (HEMA), 5 mg AIBN as polymerization initiator and 3 mL 0.1M aqueous SnCl₄ as pore former. Selected amounts of sodium fluoride were added to this monomer. The monomer mixture was then poured into a cylindrical glass mold ($\phi = 4.5$ cm) and exposed to ultraviolet radiation (12 W UV lamp) for 10 min, while a nitrogen atmosphere was maintained in the mold. The resultant membrane was washed several times with deionized water.

PHB coating of fluoride-loaded PHEMA membranes

PHEMA membrane was placed in its glass mold and partially dried in a vacuum oven (600 mmHg, 50°C) for 30 min. The membrane was then cooled to room temperature. Each side of the fluoride-loaded PHEMA membrane was spray coated with a chloroform solution of PHB (7% w/w, 1 mL). A 0.5-mL portion of this solution was sprayed on, and after evaporation of chloroform at room temperature, a second 0.5-mL portion was applied on the membrane surface. The other side of the membrane was also coated in the same manner. After PHB coating, the composite membranes were agitated in 50 mL deionized water for about 1 min and were then placed in a vacuum desiccator (50 mmHg) at room temperature for 2 h. This procedure ensured the removal of the residual solvent and moisture. It should be noted that this washing step caused a sodium fluoride release (extraction from the composite matrix by water) of less than 5% in all cases. Thickness of polymeric membranes was measured by means of a micrometer (Miyoto, Japan). The PHEMA/PHB membranes were cut into small circular pieces ($\phi = 1.0$ cm) with a perforator.

Characterization of PHEMA/PHB membranes

Swelling test

Swelling ratios of the PHEMA/PHB membranes were determined in distilled water. Dry membrane samples (four pieces) with fixed surface area and thickness (ϕ : 1.0 cm; thickness: 350 μ m) were carefully weighed before being placed in 50-mL vials containing distilled water. The vials were put into an isothermal water bath (25 \pm 0.5°C) for 2 h. The membrane samples were removed from the water periodically every 15 min, blotted using filter paper, and weighed. The weight ratio of dry and wet samples was calculated by using

$$\text{Water uptake ratio \%} = [(W_s - W_0)/W_0] \times 100 \quad (1)$$

where W_0 and W_s are weights of membranes before and after uptake of water, respectively.

Contact-angle measurements

The PHEMA/PHB membranes were characterized by an air-under-water contact-angle measuring technique. The device consisted of a traveling goniometer with a $\times 15$ eyepiece, a variable intensity light source, and a micrometer-adjustable X-Y stage vertically mounted on an optical bench. The stage contained a Plexiglas container in which a Teflon plate was suspended. The polymer sample was held on the underside of the Teflon plate by means of small Teflon clips. The container was then filled with triple-distilled water at 25°C and the plate with the sample was lowered into the container until the sample was completely immersed. A bubble of air with a volume of about 0.5 μ L was then formed below the surface at the tip of the Hamilton microsyringe, detached, and allowed to rise to the polymer-water interface. The air bubbles were photographed at 25°C 5 min after reaching equilibrium in contact with the PHEMA/PHB samples. The equilibrium contact angle (θ_{air}) was calculated from the height (h) and the width (b) of the air bubble at the PHEMA/PHB sample surface by using

$$\theta_{\text{air}} = \cos^{-1}[(2h/b) - 1] \quad \text{for } \theta_{\text{air}} < 90^\circ \quad (2)$$

The mean value of five contact-angle measurements on bubbles at different positions was calculated. The reproducibility of contact angles was $\pm 2\%$.

Scanning electron microscopy (SEM)

Surface morphology and bulk structure of PHEMA/PHB membranes were observed in a scanning electron microscope (JEOL, JEM 1200 EX, Tokyo, Japan). PHEMA/PHB membranes were dried at room temperature and coated with a thin layer of gold (about

100 Å) in a vacuum and photographed in the electron microscope with $\times 800$ magnification.

Release studies

In vitro fluoride release studies were carried out in a continuous release system which is described in the United States Pharmacopeia XXII for achieving perfect sink conditions. The continuous release system consisted of a 20-cm length of a 1-cm diameter pipe with a total volume of 25 mL. The temperature-control jacket and the upper connector were made from polyethylene. The release cell temperature was controlled by circulating water through the jacket. The fluoride-loaded membranes (five pieces, ϕ : 1.0 cm) were placed in the release cell. Artificial saliva consisted of the following (in mg/mL of deionized water): NaCl, 0.4; KCl, 0.4; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.8; NaH_2PO_4 , 0.69; $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 0.05.¹⁸ Artificial saliva in the reservoir was introduced into the release cell at a flow rate of 0.75 mL/min by using a peristaltic pump (Cole Parmer, Model 7014-28) through the lower inlet. At the end of each 24 h, the collected sample was assayed with an ionmeter. During fluoride measurements, TISAB II solution (Orion Res., Inc., Beverly, MA) at a 1 : 1 ratio was added to the sample containing fluoride. A fluoride-specific electrode (Orion Res., Inc.) and a specific ionmeter (Orion Res., Inc.) were used.

Long-term stability of composite PHEMA/PHB membranes was performed in the release cell containing artificial saliva. The membrane samples were removed from the water periodically every 24 h up to 30 days, blotted using filter paper, and weighed. The degradation ratio was calculated gravimetrically.

RESULTS AND DISCUSSION

Characterization of PHEMA/PHB membranes

The PHEMA/PHB composite membranes prepared for this study were insoluble and swelled in water. The swelling behavior of the PHEMA/PHB and fluoride-loaded PHEMA/PHB composite membranes are shown in Figure 1. Because crystalline structures have lower swelling rates than amorphous structures, the decrease in the swelling rate of the polymeric structure as a result of the addition of sodium fluoride, which is of crystalline structure, is an expected outcome. As is known, crystalline polymeric structures are more regular than amorphous structures and so water diffusion is more difficult in crystalline structures. This view is supported by the results obtained from the dynamic swelling behaviors of the composite membranes. Although the maximum swelling ratio of the PHEMA/PHB composite membranes was 23.2%, that of the fluoride-loaded PHEMA/PHB composite membranes was 11.2%. It can also be observed from

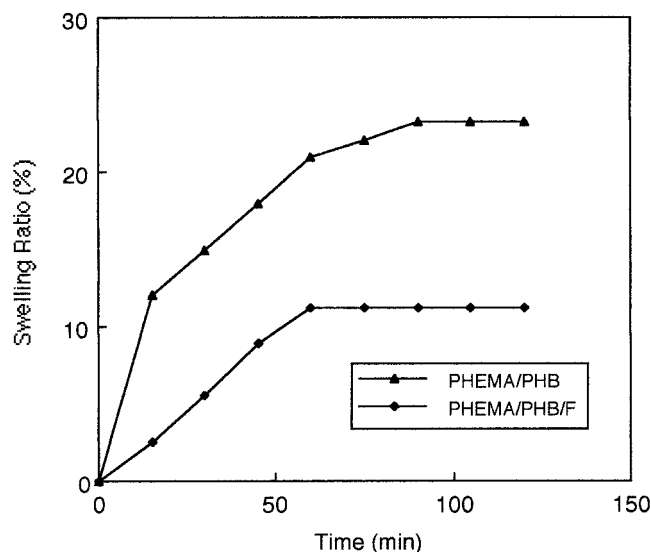


Figure 1 The swelling behavior of PHEMA/PHB and fluoride-loaded PHEMA/PHB membranes.

the graph of swelling ratios that the swelling of the polymers were quite rapid and approximately within 2 h an equilibrium swelling rate was reached.

Biocompatibility is an important factor to be taken into consideration whenever a polymeric biomaterial interacts with biological systems. Among the factors that determine the biocompatibility of the material, the extent to which the polymeric surface interacts with water is the foremost one. The surface wettability of a biomaterial has to be determined because the contact angles of the polymeric material and its biocompatibility are directly related.¹⁹ To demonstrate the relationship between the surface hydrophobicity/hydrophilicity, photographs of the bubbles were taken under water and the contact angles were measured. Considering that low contact angles represent hydrophilic surfaces and high contact angles represent hydrophobic surfaces, these PHEMA/PHB membranes can be defined as relatively hydrophilic. We had expected changes in surface properties and bulk structure of the membranes after the addition of fluoride to the polymeric structure. However, little change was observed in the contact angle as a result of the measurements. The contact angle was 54.3° in the PHEMA/PHB membranes and 54.7° in the fluoride-loaded PHEMA/PHB composite membranes.

The surface and cross-sectional scanning electron microscope photographs of the PHEMA/PHB composite membranes are shown in Figure 2. In the SEM photographs, it can be seen that both the surface and the cross section have pores which spread homogeneously. Pore size was measured as over $1 \mu\text{m}$. These large pores reduced the diffusion limitations for fluoride release and also enabled more fluoride loading as a consequence of their wider area. The SEM photo-

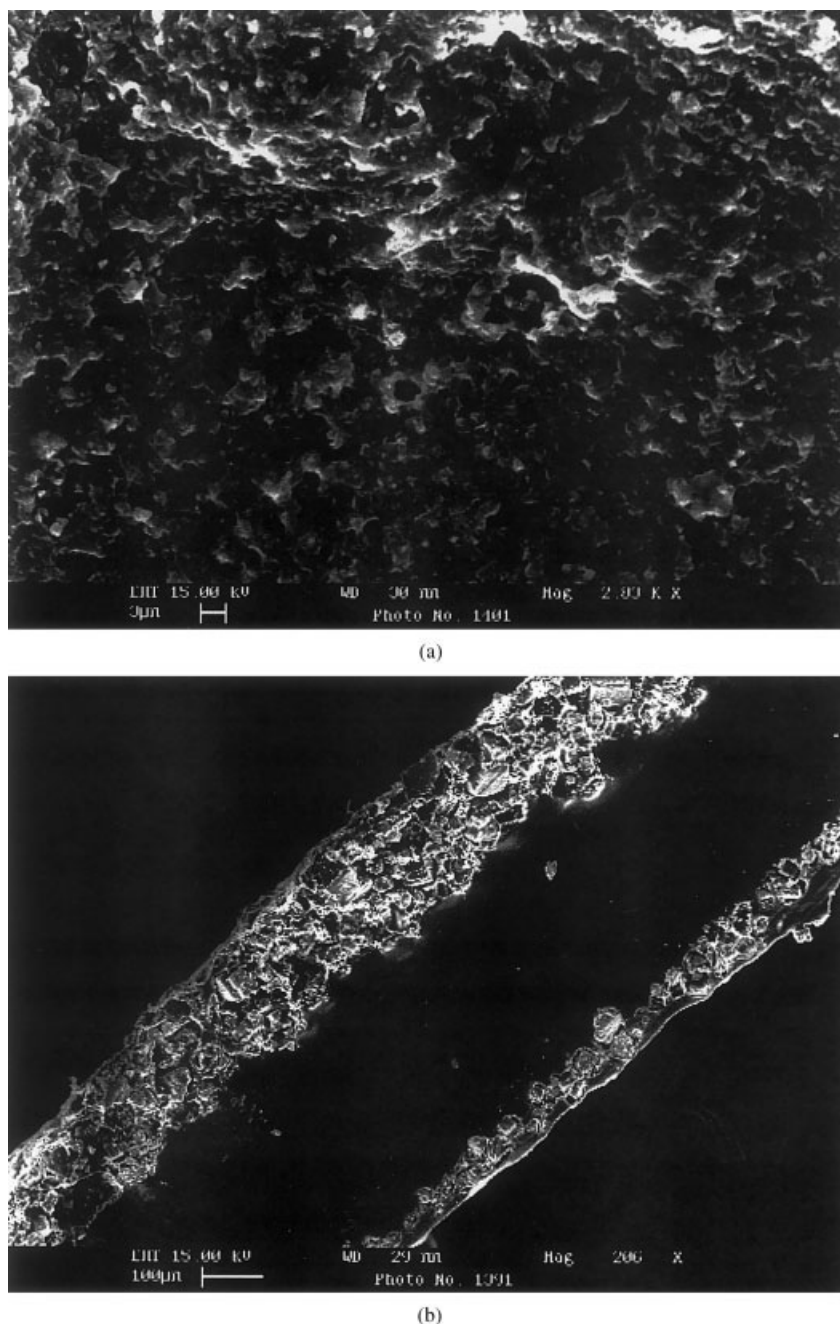


Figure 2 Scanning electron micrographs of fluoride-loaded PHEMA/PHB membranes showing (A) surface morphology (magnification, $\times 800$) and (B) cross section (magnification, $\times 800$).

graphs also allowed us to observe the PHB structure on the PHEMA structure. These structures were formed during polymerization under UV radiation after PHB was sprayed over the PHEMA membranes prepared previously. They have diffusion canals which allow fluoride to be released from the core. The width of these canals, the size of the pores in the membrane structures, and the pore distribution can be adjusted with changes in polymerization conditions. In the surface and cross-sectional SEM photographs of the fluoride-loaded PHEMA/PHB composite membranes, the PHB layer which controls fluoride release

can be seen as the outer layer of the polymeric membrane. This PHB layer enabled a homogenous fluoride release. Long-term stability of composite PHEMA/PHB membranes was also performed up to 30 days. It should be noted that no significant PHB degradation was observed.

In vitro fluoride release studies

The most important parameters that affect release rate from polymeric structures are the type of release mechanism and amount of substance loaded to poly-

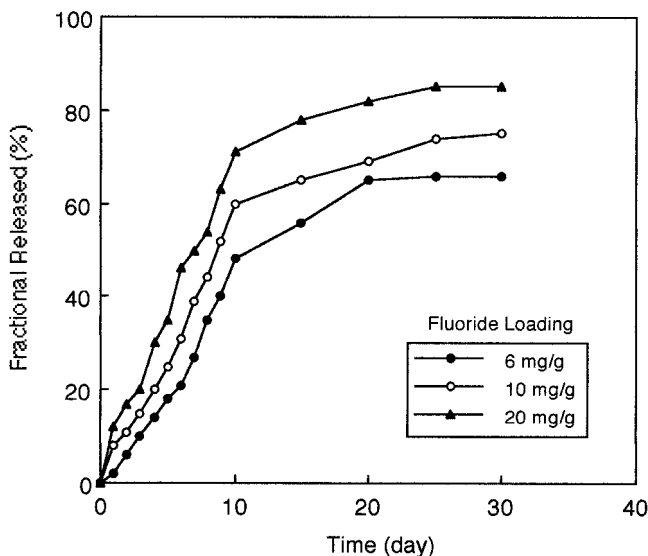


Figure 3 The effect of fluoride loading amount on fluoride release in PHEMA/PHB membranes. Release medium: artificial saliva. Release conditions: pH 6.0. Temperature: 25°C.

meric structure.³ When gradually decreasing release rates are observed, release rate decreases in relation to the square root of time. When release rates are independent of time, constant and linear release rates are observed.²⁰ In controlled drug-release systems, the mechanism that controls the release of the active agent and the application areas of the controlled release are taken into consideration. When the dynamic swelling graphs and the release rates of the PHEMA/PHB composite membranes used in the study are examined, it is observed that at the beginning (in the first hour) the fluoride release was a swelling-controlled diffusion mechanism. In the fluoride-release experiments, the polymeric membranes swelled in the first hour by absorbing water. Later on, the polymeric chains in the structure gained activity because of swelling and the pore size changed. As a result, fluoride started to be released. In other words, while the solvent diffused into the structure, fluoride ion diffused out of the swollen structure.

The effect of fluoride-loading rates on fluoride release

The maximum amount of active substance that can be loaded in the polymeric structure is very important. If the maximum loading amount is exceeded, the active substance prevents the polymer chains from growing and thus interface with the polymer structure formation. In this study, fluoride loading ranged between 6 and 20 mg per gram of monomer mixture. The effect of fluoride-loading amount on the fluoride release from the PHEMA/PHB composite membrane systems is shown in Figure 3.

The plots in Figure 3 show that increasing the fluoride-loading amount in the PHEMA/PHB composite polymer system accelerated the fluoride release. This can be explained in the following way: by increasing the loading amount, the concentration gradient triggering the diffusion process also increases. Another fact to be considered is that a considerable amount of the fluoride was leached from the structure during the 30-day release process. The release ratio of a PHEMA/PHB composite structure loaded with 20 mg/g fluoride was 80.4%. The fluoride release ratio for the PHEMA/PHB composite membranes loaded with the minimum of fluoride, that is, 6.6 mg/g, was 66%.

The effect of pH on fluoride release

Another parameter that affects the fluoride-release rate from the controlled release systems is pH. The microenvironment conditions are as significant as the structural characteristics of the polymer (pore size, pore distribution, etc.). In this study, pH in the release medium was varied between 4.5 and 7.4. Figure 4 shows the effect of the pH of the release medium on fluoride release. For the PHEMA/PHB composite membranes loaded with 20 mg/g fluoride when the pH was 4.5, 6.0, and 7.4, the release ratios were 71, 80, and 86%, respectively.

The effect of temperature on fluoride release

The effect of temperature on fluoride release from the PHEMA/PHB composite membranes of two different

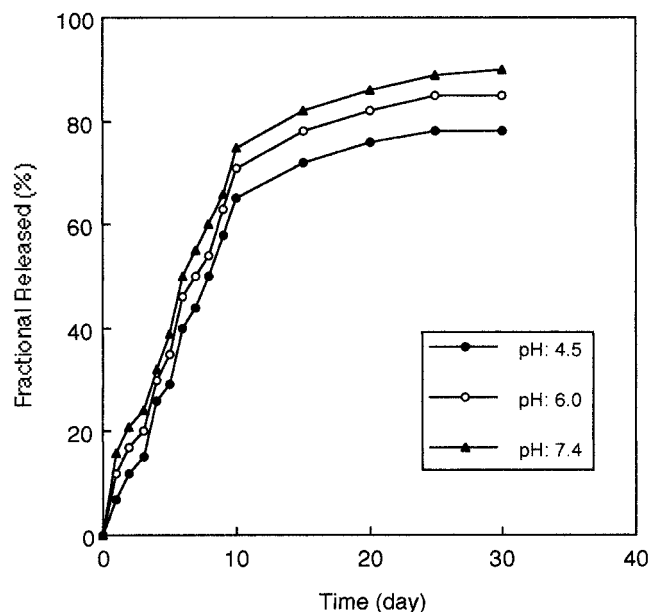


Figure 4 The effect of the pH of the release medium on fluoride release in PHEMA/PHB composite membranes. Fluoride loading ratio: 20 mg/g polymer. Temperature: 25°C.

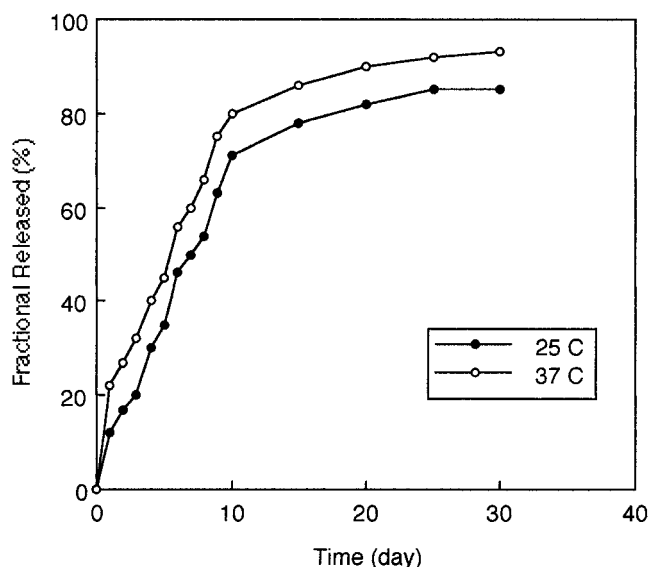


Figure 5 The effect of temperature on fluoride release from PHEMA/PHB composite membranes. Release medium: artificial saliva. Fluoride loading: 20 mg/g polymer. Release conditions: pH 6.0.

temperatures (25 and 37°C) were used in the study and results were shown in Figure 5. It is quite obvious that with an increase in the temperature the release amount also increases. The cumulative release amount observed at 25°C was 85% and at 37°C was 93%. One explanation for this behavior is that the chains in the polymeric structure gain more activity at the higher temperature and the pores in the polymer structure become wider, resulting in an increase in the fluoride release rate.

Comparison with related literature

Studies of different polymeric membranes, fluoride release rates, and applications have been reported. A structure containing a certain amount of NaF was formed by using a hydroxyethylmethacrylate/methylmethacrylate copolymer mixture which released fluoride in a controlled way. In this study, the daily release amount of the membranes was noted as 0.02–1 mg/day.²¹ In a study which was examined continuously and at intervals of membrane-controlled reservoir systems that released fluoride at different rates, Adderly et al. used membranes which released 100 and 350 mg fluoride.²² Drinkard et al. developed a membrane system which released 0.01, 0.02, and 0.04 mg/day fluoride.²³ Kula et al. studied controlled release systems pharmacologically; in their study, they loaded 42 ± 1 mg fluoride and developed copolymer membrane systems which released 0.1 mg fluoride per day.²⁴ Corpron et al. studied the effect of controlled release membrane systems on demineralized enamel

samples and noted that the fluoride release from the membranes containing 33 mg NaF was 0.5 mg/day.²⁵ Alaçam et al. used membrane systems which contained 8.4 mg ethylene-vinyl-acetate and 16.28 mg NaF released 0.32 mg fluoride a day.²⁶ In another *in situ* study, Corpron et al. examined the fluoride-releasing membrane system with respect to dose-remineralization relationship and for this purpose they used copolymer membrane systems which released 0.035 and 0.116 mg fluoride per day.²⁷ Adair et al. used HEMA/MMA copolymer membranes that released 1–2 mg fluoride a day.²⁸ Comparison of these results shows that the fluoride amount released in the PHEMA/PHB composite membrane system in our study is in agreement with the amounts noted in literature. In addition, when the structural characteristics and the microenvironmental conditions are varied, fluoride release at a desired level can be attained.

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