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Preparation of PHBV Foams and Investigation of Their Potential For Drug Release

Received: May 27, 1999

Abstract: Polymeric foams seem to have potential for use in many research areas such as cell seeding and macromolecule or drug loading. Three factors were studied to evaluate the efficiency of foams as drug carrier and releasing agents. Effect of polymeric properties was examined by using two types of polymers (PHBV 7 and PHBV 22) differing only in their valerate (copolymer) content. The other factor studied was the effect of loading extent on release kinetics and rate. The results showed an unexpected inverse proportionality between drug loading and release rate. They, however, were in accordance with foam physical properties such as porosity, which

dominate the control of release from the foam. When the concentration of the polymer solution increased the density of the resulting foam increased leading to a less porous structure and to a slower release rate. In all cases, complete drug release took place within a very short time period most probably due to the high water solubility and low molecular weight of the drug molecule. It is expected that it will be possible to achieve a longer term release with larger bioactive agents like enzymes, plasmids or antibodies.

Key Words: Controlled Release, Poly(hydroxybutyrate-co-hydroxyvalerate)-PHBV, Polymer foams, Tetracycline.

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Introduction

Controlled drug release systems are being used with increased frequency in the treatment of many diseases (1,2). Cases where long term delivery, and/or minimal fluctuation of plasma concentration of the drug are needed are when these systems mainly used. This is especially important in application of biotechnologically obtained oligomers and macromolecular bioactive agents (DNA, plasmids, enzymes, vaccines) to the tissues. Here, the protection of the bioactive agent from the environment and release controlling effect provided by the carrier system gains more importance. Since macromolecular structures have difficulty in diffusing through membranes, systems involving them release their contents either by the degradation of the carrier or by employing an open celled carrier foams or both. In addition foams carrying antibiotics could be very important in tissue engineering where foams are impregnated with the respective cell of the tissue aimed. Presence of an antibiotic would drastically decrease the risk of infection and thus failure. In a previous study poly (l-lactic acid) foams were prepared to study cell seeding and controlled release performance by using two model compounds, bromothymol blue and sulforhodamine B

(3). Poly (d,l-lactide-co-glycolide) foams were also prepared by Hsu et al (1996) and they investigated the release of isoniazid from these systems (4,5). In the present study preparation of a controlled release system using another biodegradable and biocompatible polymer, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV, for the release of an antibacterial agent, tetracycline (6,7,8) was aimed. The densities and drug release behaviors of the foams were studied and their morphologic properties were investigated by scanning electron microscopy.

Material and Methods

Materials

Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) with hydroxyvalerate contents of 7 and 22 percent (molar) and with molecular weights ranging between 400,000-750,000 D were purchased from Aldrich Chem. Co. (USA).

Methods

Preparation of PHBV Foams

Foams were prepared by lyophilization of polymer solutions after freezing in order to prevent the formation

of a compressed, dense foam or macroporous membrane. PHBV (ca. 1 g) was dissolved in chloroform (9 ml) and then acetic acid (18 ml) was added. This solution was then frozen at -28°C in an appropriate standard petri plate and freeze-dried at RT. After obtaining empty foams with this process, samples (ca. $2 \times 2 \text{ cm}^2$) were cut and submerged in tetracycline.HCl (TC.HCl) solutions the concentration of which was varied according to the loading ratio required (Table 1). By applying vacuum (ca. 1 mm Hg) and then rapidly releasing it by letting in the air, all the drug was forced into the foams which were then dried thus leading to 100% loading.

Release Method

Drug release was studied in a static release system. Samples (ca. $0.5 \times 0.5 \text{ cm}^2$) were introduced to phosphate buffer saline (250 ml, 0.1 M, pH 7.4) which was continuously agitated in a shaking water bath at 37°C . Samples (3 ml) were removed at different time intervals, tetracycline content was detected with UV spectrophotometer by measuring the absorbance at 360 nm and then returned to the release media. Release studies were carried out in triplicates.

Determination of Foam Density

Densities of the PHBV foams were determined with a pycnometer. Pycnometer was weighed when empty (w_0) and when it was completely filled with water (w_1). Then a previously weighed foam (w_f) was put into the pycnometer which was then topped with distilled water and weighed again (w_2). However, during this process some water was absorbed by the hydrophobic foam because of its porous structure and the capillary action, and this value was used in correcting the data. The foam was, therefore, weighed again after removal from the pycnometer (w_3). All these data were used to calculate the

volume of the foam (V_f), and eventually the density of the foam (D_f).

For the calculation of the foam density the following equations were used:

$$w_{w1} = w_1 - w_0 \quad [1]$$

$$w_{w2} = w_2 - w_f - w_0 \quad [2]$$

$$w_r = w_{w1} - w_{w2} \quad [3]$$

$$V_w = w_r + (w_3 - w_f) \quad [4]$$

$$V_w = V_f \quad [5]$$

$$D_f = w_f / V_f \quad [6]$$

where,

w_r : weight of water displaced by foam

V_w : volume of displaced water which is equal to its weight

Results and Discussion

Foam Density

By controlling the shape and the solvent volume (the size of the container), it is possible to produce foam structures with desired density and thickness (Table 1). The type of the polymer used also had its contribution (compare F2 and F4). It thus appears that foams with less void can be obtained by increasing the concentration of the polymer in the solution. The polymer type influences viscosity due to their crystallinities and solubilities in a particular solvent being different, the chain extend more or less leading to low or high densities.

Sample Code	Polymer Type	Solvent Ratio Chloroform: Acetic Acid (v/v)	Concentration of Polymer (g/ml)	Density (mg/ml)	Loading Ratios Drug: Polymer
F1	PHBV 7	1:2	0.02	33.8	-
F2	PHBV 7	1:2	0.04	52.7	1:5
F3	PHBV 7	1:2	0.06	67.1	1:5, 1:4, 1:3
F4	PHBV 22	1:2	0.04	41.4	1:5

Table 1. Effect of Polymer Type and Polymer Solution Composition on Foam Density.

Release Results

Release of TC.HCl from PHBV7 and PHBV22 foams are compared in Figure 1. When release from equal initial polymer concentration materials with different polymer types are compared, release of drug initially appears to be at similar high rates. This rate, however, decreases substantially for PHBV 7 at 1 h while it is maintained for another 3 h with PHBV 22. When the data is plotted to determine obedience either to diffusion controlled release (amount released vs square root of time) or the first order kinetics (natural logarithm of amount released vs time) the diffusion controlled release appear to be obeyed more. However, as the drug molecule is too small (MW: 481) in comparison to the size of the pores of the carrier, diffusion is not a very much release controlling factor here. Rather the drug release is thought to be mostly controlled by the solubility of the drug in the water present in pores of the foam structure and by convection through pores after being dissolved. So the surface to volume ratio of the drug crystals gains importance because it is directly proportional to the dissolution as well as the amount of drug that has been stored in the structure as they would affect the solubility of the drug.

In order to evaluate the effect of loading on release, three different drug loading ratios (drug: polymer weight ratios of 1:3, 1:4, 1:5) were used in the study. Figure 2

shows, the loading has an inverse effect on the rate of release. When the amount of drug loaded to foams increased, the release rate decreased. Although it appears to be contrary to the general expectation for release of a drug from a polymer matrix or capsule, the situation here is very different. The structure from which release is taking place is an open celled foam (Figure 3a). As can be seen from the figure, there is no diffusion restriction and the drug is loaded to a very high extent and is removed without changing the foam morphology to a detectable level (Figure 3b and 3c). When there is more drug loading, the interaction of drug crystals with the dissolution medium is decreased due to decreased surface to volume ratio of the drug, and due to occlusion of the pores by the drug crystals leading to a lower release rate when the drug content is high. This becomes apparent when the released amount of drug (not its ratio to the input) is plotted (not presented). There it is seen that during the first 5 hours the amounts released are the same even though the loadings are different, proving the point made here.

Release data presented in Figure 4 reveals that foam preparation from the more concentrated polymer solution leads to a foam with higher density (67.1 mg/ml vs 52.7 mg/ml, Table 1) and also to a foam from which the release rate is lower. Release kinetics, however, do not change from what was observed with F2 and F4.

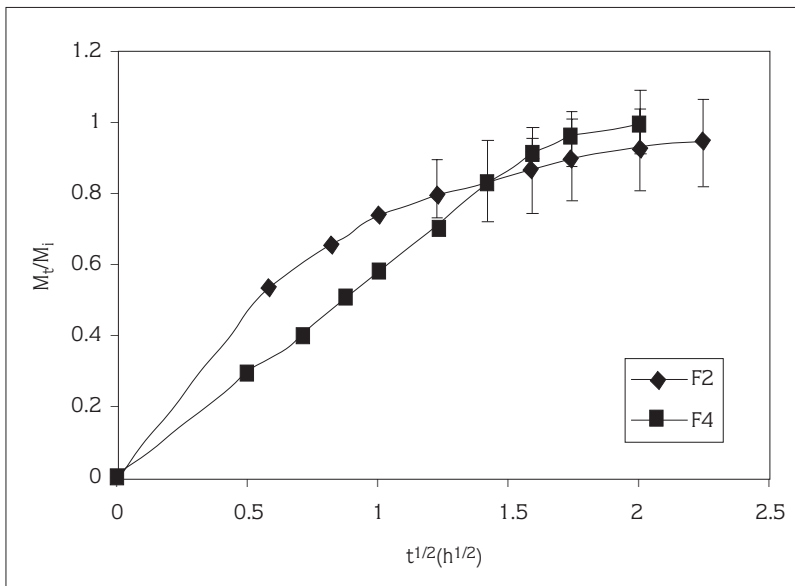


Figure 1. Effect of Polymer Type on Release From PHBV Foams

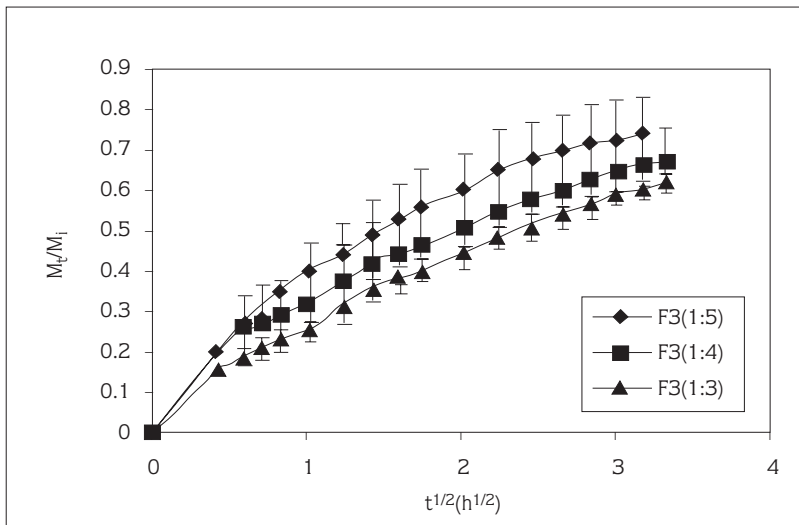


Figure 2. Effect of Amount of Drug Loading on Release From PHBV Foams

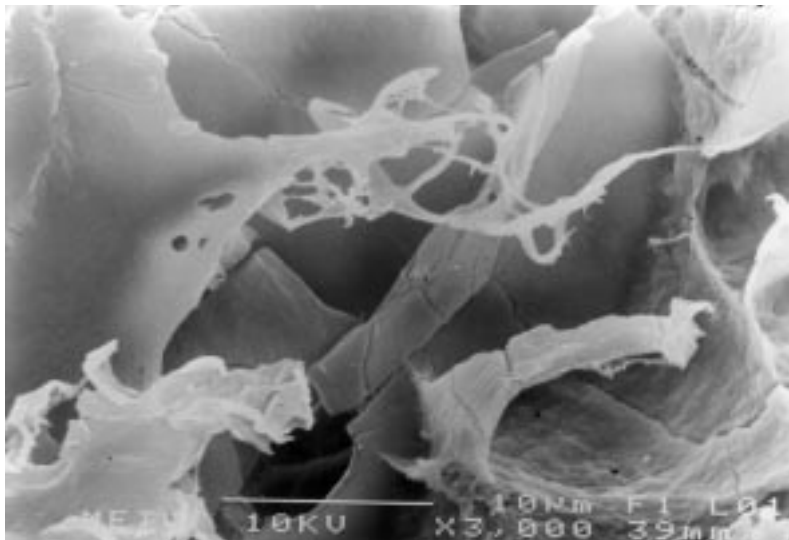


Figure 3a. SEM of PHBV 7 Foams Prepared From an Initial 6% Polymer Solution (F3). a. Unloaded.

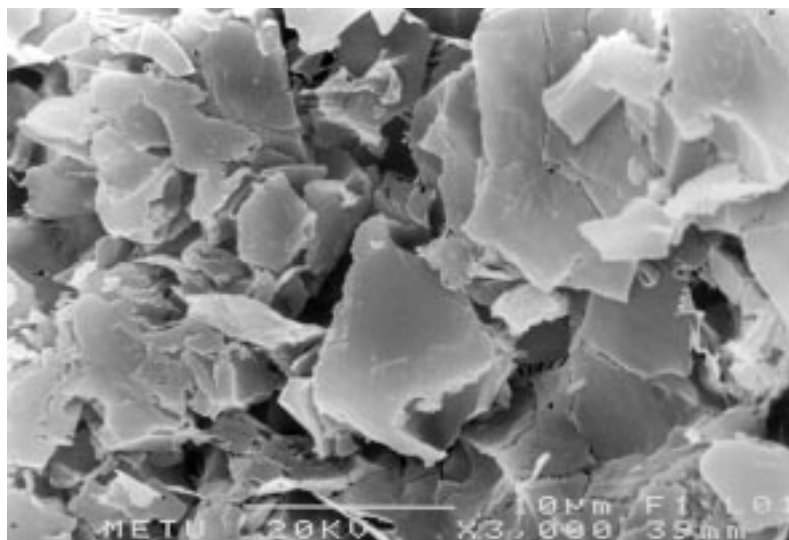


Figure 3b. SEM of PHBV 7 Foams Prepared From an Initial 6% Polymer Solution (F3) b. Tetracycline loaded.

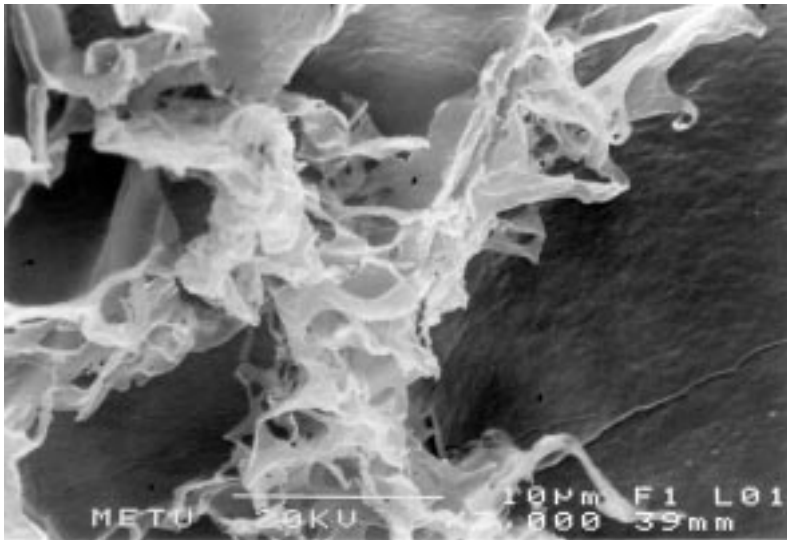


Figure 3c. SEM of PHBV 7 Foams Prepared From an Initial 6% Polymer Solution (F3) c. After release of tetracycline.

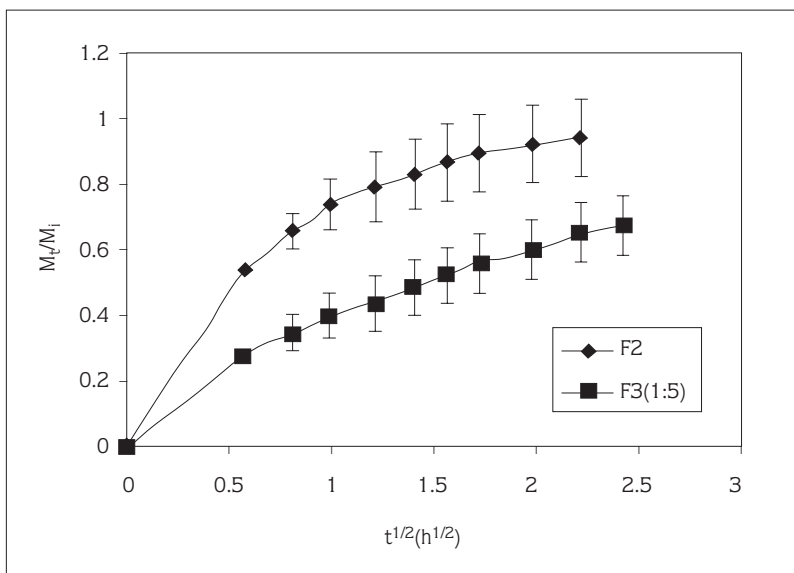


Figure 4. Effect of Polymer Concentration on Release From PHBV 7 Foams.

Conclusion

As the data presented above reveals, open celled foams have very limited control on release of low molecular weight substances. The parameters that have control on release rate are the foam density, drug loading and to very small extent, the polymer type. Such open-celled structures might, however, be very effective

carriers for controlled release of larger molecules such as proteins and polynucleotides. With the current enthusiasm about the biotechnological production of bioactive species prevailing we feel that these foams are going to be quite useful in nucleic acid and vaccine delivery.

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