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Diffusion in and around alginate and chitosan films with embedded sub-millimeter voids

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ABSTRACT

Hydrogel scaffolds from biopolymers have potential use in the controlled release of drugs, and as 3-D structure for the formation of tissue matrix. This article describes the solute release behavior of alginate and chitosan films with embedded voids of sub-millimeter dimensions. Nitrogen gas was bubbled in a fluidic arrangement to generate bubbles, prior to the crosslinking. The crosslinked gel was dried in a vacuum oven, and subsequently, soaked in Vitamin B-12 solution. The dimensions of the voids immediately after the cross-linking of gel, and also after complete drying were obtained using a digital microscope and scanning electron microscope respectively. The porosity of the gel was measured gravimetrically. The release of Vitamin B-12 in PBS buffer on a shaker was studied. The release experiments were repeated at an elevated temperature of 37 °C in the presence of lysozyme. The diffusion coefficient within the gel layer and the mass transfer coefficient at the interface with the bulk-liquid were estimated using a mathematical model. For comparison, the experiment was repeated with a film that does not have any embedded void. The enhancement in diffusion coefficient due to the presence of voids is discussed in this article.

1. Introduction

A porous scaffold that provides a three dimensional support for the formation of a matrix, and also delivers the biological agents is a subject of extensive investigation. Use of natural and synthetic polymers, and hydrogels are known, where the biocompatibility and the biodegradability are important requirements. A gel layer has the potential to hold a significant volume of a biological agent that can diffuse into the host tissue over a period of time. Also the gel layer, loaded with a matrix forming cell can act as a scaffold, over which the tissue regeneration takes place. In these applications, it is important that a substantial porosity is induced in the gel layer. Additionally, the porosity has to be uniformly distributed so that the pore to pore distance remains uniform. This calls for a highly ordered pore structure.

Emulsion freeze drying, fiber bonding, solvent casting or particulate leaching, gas foaming, thermal phase separation, electrospinning, and use of supercritical CO_2 are some of the methods to induce the voids in a gel layer [4]. Direct introduction of bubbles using a fluidic arrangement is an alternative method that allows better control of the void size and the porosity [2,3]. Under most circumstances, the bubbles generated by this method are monodisperse. The bubbles rapidly self-assemble, and provide an ordered structure. Also, the gel is not exposed to any chemical or thermal treatment by this method. The method is inexpensive, in comparison with the solid free form fabrication techniques. This article describes a co-flow arrangement to generate voids in two types of gel systems. These are alginate and chitosan. Alginate is a naturally occurring polysaccharide, sourced from brown algae that grow in warm areas. Chitosan is the deacetylated form of Chitin, the second most abundantly available polysaccharide in the nature, other than the cellulose. Chitin is commonly sourced from the shells of crabs, prawns, lobsters, shrimps and exoskeleton of insects. Both of these gel systems are well known for their biocompatibility, and have potential use in drug delivery and tissue regeneration [19,28,32]. Alginate gel has been extensively characterized for controlled release applications [9,11,21,23,31].

Use of pulled microcapillaries to generate a gel scaffold of alginate has been reported [5,26,33]. In the co-flow arrangement for the present study, the pulled capillaries are arranged one inside the other. The inner gas thread is dragged by the co-flowing liquid until the gas stream snaps to form a bubble. An aqueous solution of biopolymer with dissolved surfactant made the liquid phase. After the collection of the ensemble of bubbles on a petridish, the crosslinker was sprinkled to form a free-standing porous gel film. The gel film was dried in a vacuum oven, and was dipped in Vitamin B-12 solution for uptake and release studies. Vitamin B-12 was considered here as a model drug. We anticipate that the voids in the gel structure will act as additional reservoirs, distributed uniformly over the entire film. Thus, more loading of Vitamin B-12 will be possible. Also, the diffusion resistance inside the film will be reduced leading to higher release rate. The enhancement in uptake and release

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can be controlled by the size and frequency of the bubbles, and the number of layers of bubbles in the film, apart from the physico-chemical tuning of the gel matrix.

Diffusion in gel has been studied using various methods, such as gravimetry, fluorescence, membrane permeation, radioactive labeling, holographic laser interferometry, and inverse sectioning method [6,16,27]. Different physico-chemical concepts of hindered diffusion e.g., obstruction effects, reduction in free volume of gel, additional hydrodynamic drag on the solute, steric factors, increased path length due to obstruction, and convective flow of liquid due to swelling or shrinkage were considered [1,18,27,30]. Diffusion of several solutes was studied in alginate [12,14,15,20,24,25,29] and chitosan [10,35]. In most of these studies, the diffusivity of solute in the gel matrix was derived from the release profile using a linearized analytical model with assumption of diffusion in the gel matrix as rate limiting, and negligible mass transfer resistance at the interface between gel and release sink. The diffusivity in gel was found to be less than that in water, and the reduction was influenced by the concentration of the polymer in the gel, and the molecular weight of the solute. On the contrary, for low Biot Number, the analysis of release profile was based on mass transfer around the scaffold as rate limiting with the assumption of instant equilibration of concentration within the gel matrix. For a composite scaffold as the one in this article, consideration of both the resistances in tandem may be important, instead of focusing on one regime or the other.

An effective design of biocompatible gel scaffold requires estimate of mass transfer rates in and around the scaffold. When the voids are present in the scaffold, the resulting matrix becomes a composite structure with mass transfer resistance varying in space. The effectiveness of the scaffold in controlled release, or tissue harvesting depends on the understanding and control of the mass transfer process within the scaffold. The object of this work is to study the uptake and release of Vitamin B-12. In particular, the diffusion coefficients that describe the release profile are estimated in this article for gel films, made from two different biopolymers. A mathematical model for unsteady state diffusion with error minimization algorithm was used in tandem to arrive at these estimates. In this paper, the mathematical model accounts for both in-scaffold diffusion and film mass transfer around the scaffold. The values of diffusion coefficients within the scaffolds were compared to demonstrate the effect of voids. The mass transfer coefficients at the interface were analyzed. The sensitivity of the release profiles to scaffold dimensions and the diffusion coefficients are discussed.

1.1. Materials

Alginate (low viscosity, CAS: 9005–38–3) and chitosan (low molecular weight, CAS: 9012–76–4) were purchased from Sigma Aldrich. Lutensol AT 25, a linear alcohol ethoxylate, was purchased from BASF, Germany. Pluronic F 127 is a non-ionic copolymer surfactant, purchased from Sigma-Aldrich(CAS: 9003-11-6). Calcium chloride dihydrate of extra pure grade ($CaCl_2 \cdot 2H_2O$, molecular weight = 147.02 g/mol) that was used as a crosslinking agent was purchased from Merck Specialities Private Limited, India (CAS: 10035-04-8). Lysozyme (3× crystallized, ex egg white, extra pure, CAS: 12650-88-3) was purchased from Sisco Research Laboratories Private Limited, India.

1.2. Methods

The preparation of the gel film involves the use of aqueous suspension of biopolymer. Added surfactant helps in formation and sustenance of bubbles. The crosslinker solution, when sprinkled on the bubbles develops a free-resting structure out of the aqueous film.

4% sodium alginate solution in distilled water was prepared by using a magnetic stirrer at 350 rpm for 12 h, and subsequently a mechanical mixer at 3000 rpm for 3 h. The pH of the solution was found to be 7.55. 4% pluronic F-127 solution in distilled water was used as a surfactant. The pH of the pluronic solution was found to be 6.58. The solution was stirred for 1 h using a magnetic stirrer at 350 rpm. The solution was then kept in a refrigerator at 4 °C for 24 h to ensure complete dissolution. The alginate and the pluronic solutions were mixed in even proportion using a magnetic stirrer at 100 rpm for 10 min.

The chitosan solution was prepared by dissolving chitosan beads in 0.2 M acetic acid solution with the use of a mechanical stirrer. The stirring at 2500 rpm was conducted for 16 h. The viscous chitosan solution was filtered through Whatman filter paper of Grade 4. Subsequently Lutensol AT 25 was added to the solution. The pH of 1.5 weight % of chitosan solution was found to be 4.50 prior to the addition of surfactant. The addition of Lutensol AT 25 (1 weight %) resulted in increase of pH to 5.5.

The viscosity of polymer solution with added surfactant was measured in the Brookfield viscometer, and also in Antonn Paar rheometer using the cone and plate arrangement. Surface tension and contact angle on glass and parafilm were measured in a Goniometer (Rantac, Germany). The solutions were prepared at least 1 h prior to the measurements and sonicated by Ultra-sonicator for 30 min.

In the co-flow device, made of pulled glass capillaries the gas is flowed through the inner capillary. The aqueous polymeric solution flowed through the outer capillary. A constant flow rate of this solution was maintained using a syringe pump from Harvard Apparatus, U.S.A. The flow of nitrogen was obtained from a gas cylinder through a mass flow controller from Alicat Scientific, U.S.A. The gas flow rate was maintained at 1 mL/min, whereas the liquid flow rate was set at 5.0 mL/min.

As the flow of the two phases proceeded through the co-flow device, thin gas thread from inner capillary broke up to form bubbles. The liquid with embedded bubbles were collected in a petridish. The digital images of the bubbles were acquired using a microscope with a camera, attached to the computer. The images were acquired under in line illumination, and were processed further using the Davis software from LaVision. An edge detection algorithm was utilized to digitally delineate the bubbles. The aqueous films with self-assembled bubbles were converted to the free-resting gel film by sprinkling crosslinker on the petridish. A solution of 4% $CaCl_2$ in deionized water was sprinkled on sodium alginate film, where the crosslinking involves exchange of sodium with calcium ions. A milky-white gel structure formed within minutes. In case of chitosan film, an aqueous solution of 40% formaldehyde was used as crosslinker. The amine bonds between amino groups in the chitosan and aldehyde group of formaldehyde formed an open chain ether linkage. It took less than an hour for the free-resting chitosan structure to develop fully. The chitosan gel has a tendency to stick to the petridish. This problem could be avoided by wrapping the petridish with parafilm. After five days, the gel structure was dipped in distilled water to remove the excess crosslinker solution. Similar gel-film, without any bubble was made in a separate petridish for comparison. Table 1 presents the list of samples used in this study. The gel films are circular. The diameter was varied from 60 to 100 mm. The thickness of the film was varied from 1 to 2 mm.

The vacuum drying of the gel-film to constant weight took about two to six hours at a temperature of 50 ± 2 °C, and an absolute pressure of 60 Torr. The internal structure of a sample of the dried gel was studied using JEOL JSM 5800 scanning electron microscope. The dried film was dipped in aqueous solution of Vitamin B-12. For alginate film, the Vitamin B-12 solution contained 0.02% Vitamin B-12 and 0.9% NaCl in deionized water. For chitosan film 0.02% Vitamin B-12 in Phosphate Buffer Saline (PBS) was used for uptake experiments. The objective here was to saturate the dry film with Vitamin B-12 solution, and quantify the uptake in the form of porosity. The porosity is defined as the ratio of the volume of Vitamin B-12 solution, held by the film to the total volume of the film after complete swelling. For gravimetric measurements a precision balance from Sartorius with the below balance weighing facility was used. It took 10 to 14 h for complete saturation that was ensured from constant value of suspended weight in the Vitamin B-12 solution.