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COMMUNICATION

Preparation of Cross-Linked Sodium Alginate Microparticles Using Glutaraldehyde in Methanol

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ABSTRACT

Polymeric sodium alginate microparticles were prepared by precipitating sodium alginate in methanol, followed by cross-linking with glutaraldehyde. The extent of cross-linking was controlled by the time of exposure to glutaraldehyde. The topology of microparticles was characterized by scanning electron microscopy (SEM), which indicated smooth surfaces. The equilibrium swelling experiments were carried out in water to observe the effect of cross-linking and drug loading for better utility of microparticles. It was found that swelling decreased, but drug loading increased, with an increase in cross-linking of the matrix.
Key Words: *Cross-linked; Glutaraldehyde; Microparticles; Sodium alginate.*

INTRODUCTION

Alginate is a family of polysaccharides composed of α-L-glucoronic acid (G) and β-D-mannuric acid (M) residues arranged in homopolymeric blocks of each type (MM,GG) and in heteropolymeric blocks (MG). These are known to be hemocompatible and do not accumulate in any organs of the human body. In the literature, several formulations of calcium alginates in various combinations have been prepared and used as controlled release (CR) devices (1,2). Comparatively, not much attention has been focused on the preparation of sodium alginate (Na-Alg) microparticles (3,4). Earlier, it was shown (5) that the release rates of the drug-loaded Na-Alg microparticles are affected by the type of cross-linker and the method used for cross-linking. The Na-Alg microspheres

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prepared by emulsifying the Na-Alg aqueous solution into an oil followed by cross-linking with calcium will produce spherical particles initially, but after complete drying, the particles will collapse to yield irregular shapes with a wide particle size distribution.

Several methods are available in the literature for the preparation of drug-loaded Na-Alg microspheres, and all these methods are preferred for hydrophilic drugs. In our earlier studies on the CR of pesticides (6,7) and pharmaceuticals (8), we reported that Na-Alg can be cross-linked successfully with glutaraldehyde. In this paper, we report a new method adopted for preparing Na-Alg microparticles. A water-soluble drug, nimesulide is classified as a nonsteroidal anti-inflammatory drug (NSAID) (9) having a high solubility in most common solvents like water, methanol, and liquid paraffin; the drug was loaded into the Na-Alg microparticles. Because of the solubility of nimesulide in methanol and water, it leads to a low percentage of drug loading. However, the problem can be overcome by first preparing the microparticles and then loading them with the drug. The extent of cross-linking and the percentage of loading can be monitored to produce the stable microparticles for CR applications.

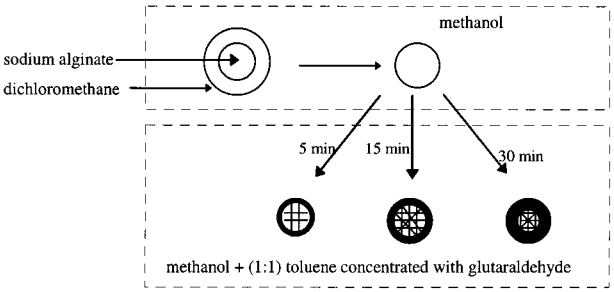
EXPERIMENTAL

Materials

A gift sample of nimesulide (purity 99.47%) was obtained from Bio-ethicals Pharmaceutical, Limited (Hubli, India). The sodium alginate, glutaraldehyde (GA) (25% w/v) solution, Tween-80, Span-20, dichloromethane, and methanol were all purchased from s.d. Fine Chemicals (Mumbai, India). Distilled water was used throughout the research.

Preparation of Microparticles

The microparticles were prepared as per Scheme 1. To 4% of Na-Alg solution in water, Tween 80 (2% of the Na-Alg solution) was mixed and stirred magnetically. Then, 25 ml of the above polymer solution (4% of Na-Alg + 2% Tween) was stirred with 100 ml of dichloromethane containing Span 20 (2% of dichloromethane) at 2000 rpm using an Ultra Turrax T-50 mixer (IKA Labortechnik, Germany). After 3 min, the emulsion was diluted with 50 ml methanol, and then stirring was stopped. The entire solution was transferred to 200 ml methanol containing 1 N HCl (1%) and toluene (10%) previously concentrated with an equal volume of 25% glutaraldehyde under constant stirring on a magnetic stirrer at 40°C. The



Scheme 1. Production of sodium alginate microparticles.

microparticles thus produced were removed from the medium at 5-, 15-, and 30-min intervals, filtered, and then washed thoroughly with water.

Drug Loading

To load the drug into microparticles, two procedures were adopted. In the first procedure (method 1), the fresh microparticles produced by the above method were soaked in the saturated solution of nimesulide in methanol for 6 hr, and these microparticles were filtered and then allowed to dry. In the second procedure (method 2), the fresh microparticles, after soaking in the saturated solution of nimesulide in methanol for 6 hr, were filtered and washed two times with methanol to remove the free drug as well as the drug adhering on the microparticles. The nimesulide-loaded microparticles thus produced were dried in air for 24 hr and used for further study.

Content Uniformity

Microparticles were evaluated for the nimesulide content by incubating the known mass of the microparticles with 50 ml of methanol for 24 hr. The microparticles were further broken down by sonicating the solution (Ikasonic U50, Labortechnik, Germany) for 2 min at a frequency of 60 MHz. The microparticles were then removed from methanol by centrifugation (Remi R24, India) for 5 min at 10,000 rpm. High-performance liquid chromatography equipment (Hewlett Packard, series 1100, USA) equipped with a UV detector was used to analyze nimesulide in the methanol using a C_{18} reverse-phase column; the mobile phase was 50% acetonitrile in water at 254 nm per the procedures published earlier (10,11).

Microscopic Studies

Completely dried microparticles were studied using optical microscopy to measure the particle size before

and after swelling in water. The particle size was measured by taking 5–10 particles on a glass slide under regular polarized light. The mean diameter was calculated by measuring the number of divisions of the ocular micrometer covering the microspheres. The ocular micrometer was standardized previously by a stage micrometer. The equilibrium swelling was carried out by adding a few drops of distilled water to 5–10 microspheres, and then the smear was covered by a glass cover. The diameter of the particles was noted after complete swelling, and the average value was calculated. Using these data, the percentage swelling was calculated.

Scanning Electron Microscope

The sample was deposited on a brass hold and sputtered with gold. The scanning electron microscopy (SEM) photographs were taken with a JSM 6400 scanning electron microscope (Japan) at the required magnification in room temperature. The working distance of 39 mm was maintained, and the acceleration voltage used was 5 kV, with the secondary electron image (SEI) as a detector. These measurements were done in RSIC at the Indian Institute of Technology, Mumbai, India.

RESULTS AND DISCUSSION

Because of the difficulty of cross-linking Na-Alg microspheres without precipitation in alcohol/methanol, the Na-Alg microdroplets produced in the oil/liquid paraffin phase may not be cross-linked by GA. But, the Na-Alg precipitated in methanol could be easily cross-linked with GA in an acidic media (12). Following these observations, we first precipitated the Na-Alg particles in methanol, and these were subsequently hardened by cross-linking with GA. The GA concentrated in toluene was used to cross-link Na-Alg to reduce the entry of GA molecules inside the microparticles. This favored cross-linking only on the outside periphery of the particles. However, theoretically it is not possible to cross-link Na-Alg microparticles only in the outer surface because GA will diffuse into the particles due to their smaller diameter even though GA is highly soluble in toluene.

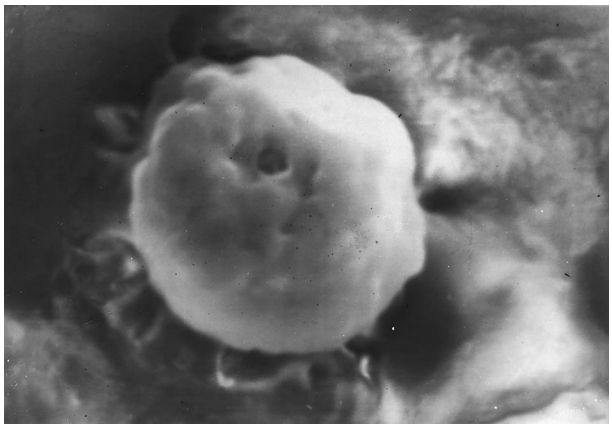


Figure 1. SEM of microparticles.

The encapsulation efficiency represents the percentage of encapsulated drug with respect to the total drug introduced into the polymer solution. The percentage content of nimesulide reflects the composition and rigidity of the microparticles produced as the percentage nimesulide content mainly depends on the space available within the microparticles. The extent of cross-linking of the Na-Alg microparticles produced in methanol was monitored by controlling the time of exposure to GA. The particles thus produced were characterized by swelling experiments in water and also by loading the particles with nimesulide. However, no previous studies are available for nimesulide encapsulation in Na-Alg microparticles, probably

Table 1

Percentage Loading and Swelling Results of Sodium Alginate Microparticles Produced at Different Extent of Cross-Linking with α-L-Glucoronic Acid (G)

Time of Exposure to GA (min)	Percentage Loading of Nimesulide (w/w) in Microparticles Produced by		Average Diameter of Particles (μm)		Percentage Increase in Diameter
	Washing	Without Washing	Dry	Swollen	
5	1.74	48.21	215	380	1.77
15	13.24	48.03	231	301	1.30
30	17.13	47.57	183	235	1.28

due to its leaching during formulation, thereby leading to problems of low percentage loading.

The morphology of microspheres as examined by SEM (Fig. 1) indicated the particles had smooth surfaces. However, the formation of dips was attributed to the rapid and massive solubilization of the drug at the beginning of preparation as well as during the drying stage.

The results of percentage loading of the drug and swelling of the Na-Alg matrix are presented in Table 1. The percentage entrapment efficiency varied significantly depending on the method adopted to load the drug. For instance, the highest efficiency was observed for microparticles loaded with nimesulide without washing (method 1) when compared to the microparticles produced by washing (method 2). The percentage entrapment efficiency decreased with an increase in cross-linking in the case of drug-loaded microparticles prepared by method 1. This may be due to the fact that the microparticles produced at higher cross-linking will swell less, thus resulting in low entrapment efficiency.

In case of drug-loaded microparticles prepared by method 2, the efficiency increased with an increasing amount of cross-linking. However, the nimesulide-loaded microparticles prepared by method 2 and produced at lower cross-linking time (5 min) showed the lowest nimesulide content (1.74% w/w of dry weight of particles). On the other hand, the microparticles produced at 30 min of exposure time to GA showed the maximum drug content (17.13% w/w of dry weight of particles). It may be noted that the particles produced at a shorter cross-linking time leached more drug during methanol washing, but the microparticles produced at higher cross-linking time were more rigid and exhibited reduced leaching of nimesulide from the Na-Alg matrix.

The encapsulation efficiency of the microparticles was further confirmed by studying swelling of the particles from a measurement of the diameter of the particles before and after swelling. These results (Table 1) indicate that the microparticles produced at 5 min of exposure to GA exhibited maximum swelling when compared to the particles produced at 30 min of exposure time. The percentage increase in diameter of the microparticles decreased to 1.77, 1.30, and 1.28 μm with an increase in cross-linking for particles exposed to GA at 5, 15, and 30 min, respectively.

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