Preparation and Characterization of Release Behavior

for Hydrophilic Dung Loaded Alginate Nanohydrogels

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ABSTRACT

Recently, there have been considerable interests in developing biodegradable nanoparticles as effective drug delivery devices. Alginate is one of the hydrophilic natural polymers having biodegradability and biocompatibility. It has possibility to apply in the controlled release of drugs, targeting particular organs/tissues, as carrier of DNA in gene therapy, and delivery proteins and peptides through a peroral route of administration. In this work, we proposed reverse emulsification-diffusion method to prepare alginate nanohydrogels containing hydrophilic drug and studied various parameters to influence in preparation of them. Physicochemical characteristics and in vitro release behavior of hydrophilic drug loaded alginate nanohydrogels were also investigated to establish them as a novel carrier for controlled release drug delivery system.

Keywords: Alginate nanohydrogels, Reverse emulsification-diffusion method, Hydrophilic drug, Controlled release

1 INTRODUCTION

Over the past few decades, various polymer have been used in drug delivery research as they can effectively deliver the drug to target site and thus increase the benefit, while minimizing side effects. Conventional methods like solvent evaporation, coacervation and *in situ* polymerization often require the use of toxic solvents and/or surfactants [1-3]. Therefore, research efforts have been directed to develop the environmentally safer encapsulation methods to produce the drug loaded nanoparticles. If impurities remain in the drug loaded nanoparticles, then these become toxic and may degrade the pharmaceuticals within the polymer matrix.

Biodegradable and hydrophobic nanoparticles have been extensively investigated as delivery systems for various drugs and biologically active genes. Sustained release characteristics of nanoparticles reduce the need for frequent administrations and enhance patient compliance by maintaining *in vivo* drug with the use of these systems for hydrophilic drug and gene delivery. However, insolubility of hydrophilic drug and gene instability has been observed during the preparation of nanoparticles when lipophilic polymers as carrier and conventional methods were used.

Therefore, sodium alginate as a hydrophilic polymer was used to deliver hydrophilic drugs and release by controlled velocity. Natural polymers like sodium alginate, chitosan and so on are used both as carriers and determinants of the release rate in controlled release systems. The main advantages of natural polymers are that they are biocompatible, biodegradable and produce no systemic toxicity on administration [4-6]. Sodium alginate is commonly used in pharmaceutical technology as natural polymers [7-9]. In this work, a reverse emulsification-diffusion method (REDM) was adapted to prepare alginate nanohydrogels (ANHs) containing a model drug. Then, we investigated characterization of release behavior for hydrophilic dung loaded ANHs.

2 EXPERIMENTAL

2.1 Materials

Sodium alginate was purchased from Junsei Chemical Co. (Osaka, Japan). Sodium salicylate (Fluka Chemical Co., Japan) was used as a model drug in this study. 1,2-Diacyl-sn-glycero-3-phosphocholine as a lipophilic surfactant was obtained from Sigma Chemical Co. (St. Louis, USA). Calcium chloride was used as crosslinking and lipophilizing agent for sodium alginate. Water was distilled by Milli-Q Quality (Millipore, USA). All organic solvents were either HPLC grade or American Chemical Society analytical grade reagents.
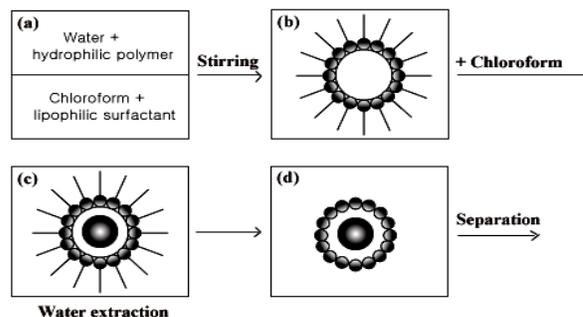

Figure 1: Schematic description of the proposed formation mechanism of ANHs by REDM.

	Alginate Conc. (wt%)	Surfactant Conc. (wt%)	Agitating speed (rpm)	Agitating time (min)
1	1.0	1.0	13,000	10
2	1.0	1.5	13,000	10
3	1.0	2.0	13,000	10
4	0.5	1.0	13,000	10
5	1.5	1.0	13,000	10
7	1.0	1.0	16,000	10
8	1.0	1.0	19,000	10
9	1.0	1.0	22,000	10
10	1.0	1.0	13,000	5
11	1.0	1.0	13,000	20
12	1.0	1.0	13,000	30

Table 1: Experimental conditions used in this study for preparing ANHs.

2.2 Preparation and Analysis of ANHs

The ANHs were prepared using the REDM. Figure 1 showed the mechanism for the formation of ANHs by this technique. After the mutual saturation of the two phases, the partially water-soluble solvent containing stabilizer and water containing alginate polymer, both liquids are in the state of thermodynamic equilibrium. (a) Stirring causes the dispersion of the aqueous solution as globules in equilibrium with the continuous phase: the stabilizing agent is then adsorbed on the large interfacial area created; (b) The addition of chloroform to the system destabilizes the equilibrium; (c) It causes the water to diffuse to the external phase. During this transport of water, new globules of nanometer size are produced which gradually become poorer in water; (d) As a result, the polymer of the globules aggregates because of the presence of a new, continuous non-water phase.

In detail, predetermined amounts of sodium alginate and sodium salicylate were dissolved in 20 ml of water. The aqueous phase was added into 20 ml of chloroform containing lipophilic surfactant. After mutual saturation of aqueous and continuous phase, the mixture was emulsified for several minutes with a high speed homogenizer.
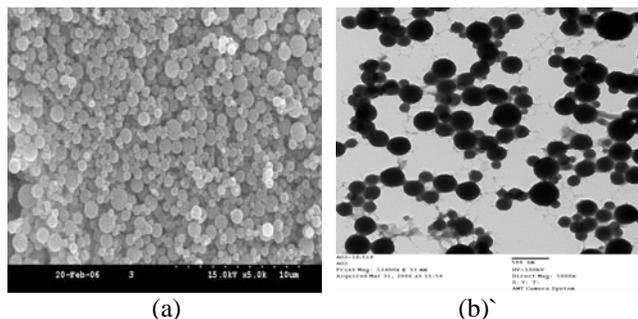

(a)   (b)`

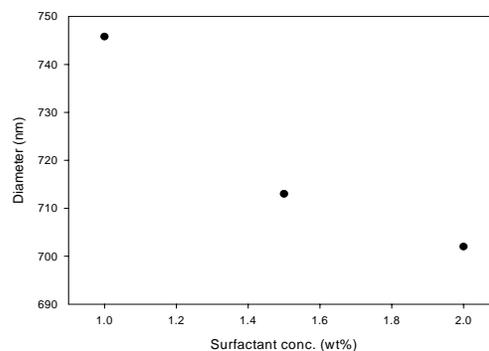Figure 2: (a) SEM and (b) TEM photographs of ANHs

Figure 3: Effect of surfactant concentration on average particle size of ANHs.

In order to allow for diffusion of water into chloroform, 200 ml of additional chloroform was subsequently poured into w/o emulsion, followed by moderate magnetic stirring for 3 days. Then, the results were crosslinked by adding 350 ml of 20% calcium chloride aqueous solution for 1 day. To control the size of ANHs, we conducted experiments of various conditions as shown in Table 1.

The prepared ANHs was then examined under scanning electron microscope (SEM, H-4300, Hitachi, Japan) and transmission electron microscope (TEM, H-7600, Hitachi, Japan) to investigate the shape of nanoparticles. The mean particle diameter and its distribution of the ANHs were also assessed by using electrophoretic light scattering spectrophotometer (ELS-8000, Otsuka Electronics, Japan).

2.3 *In vitro* Release Studies

100 mg of ANHs were placed into flasks containing 500 ml of release medium under the moderate magnetic stirring for 20 days. A total of 1 ml samples were taken at different time intervals, and analyzed by UV-spectrophotometer (UV-1700, Shimadzu, Japan) at 296 nm. High performance liquid chromatography (HPLC) is also adopted to characterize the drug release profiles of ANHs.
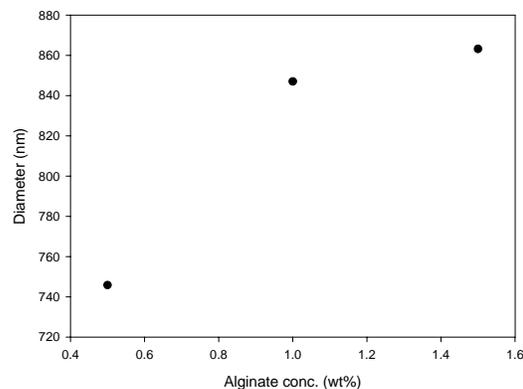

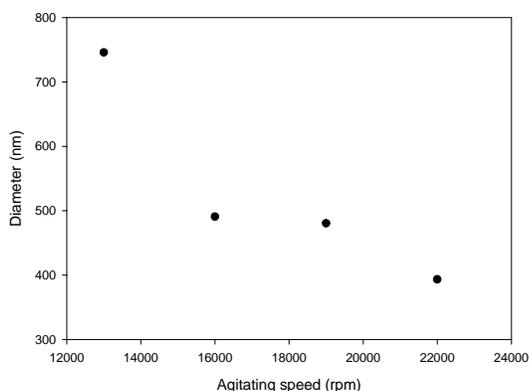Figure 4: Effect of polymer concentration on average particle size of ANHs.

Figure 5: Effect of agitating speed
on average particle size of ANHs.

3   RESULTS AND DISCUSSIONS

In this study, the REDM was adapted in order to get the ANHs containing sodium salicylate, and Figure 2 showed the formation of spherical and nano-sized partilces of ANHs. Particle size and its distribution can be affected by polymer and surfactant concentrations, agitating speed, agitating time, and so on.

Effect of surfactant concentration on average particle size of ANHs is shown in Figure 3. All specimens are prepared at fixed other conditions. This tendency of decreasing particle size with increasing surfactant concentration is ascribed to the micelle stabilization and destabilization by surfactant contents per micelle. Alginate is an adhesive hydrogel polymer. Therefore, in aqueous solution, these nanoparticles interact among themselves through their Brownian movement to form clusters. For interacting particles, the average particle size is always found higher than the actual size of the particles. Self-adhesive polymers tend to produce particle aggregation leading to the formation of large particles due to strong inter-particular interaction [10].
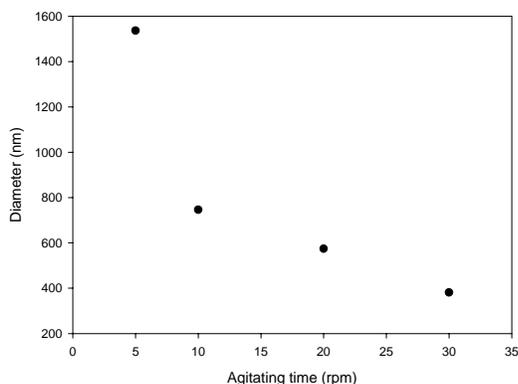

Figure 6: Effect of agitating time
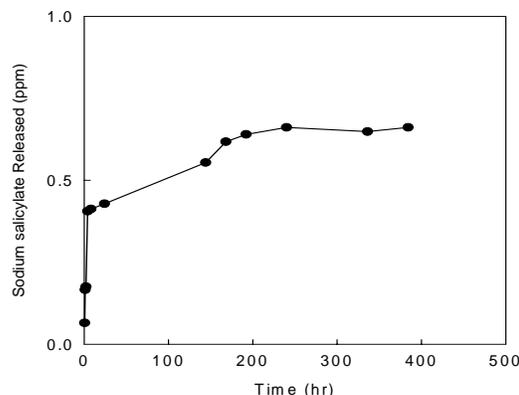on average particle size of ANHs.

Figure 7: Release profile of sodium salicylate
from ANHs.

Polymer concentration in the internal phase of a micelle is a crucial factor in increasing the size of nanohydrogels, as its concentration was increased. Figure 4 showed a good agreement with the findings of Schlicher *et al.* [11].

The influence of homogenizer speed on the average particle size of ANHs was also studied. The results are presented in Figure 5; this figure shows the expected tendency that a decrease of average particle size of ANHs can be correlated with an increase in homogenization speed.

Figure 6 showed that particle size of ANHs is decreased as emulsification duration is increased.

In vitro release property of sodium salicylate from ANHs is shown in Figure 7. It indicates an initial burst release for the first day followed by a sustained release for a period of 20 days. The initial burst release of hydrophilic drug is attributed to the predominant surface presence of the drug in the nanohydrogel formation.

4   CONCLUSION

The present work has shown that hydrophilic drug containing ANHs formation by the REDM. It demonstrates the potential process to control the size of ANHs. Preparative variables such as the concentrations of surfactant and polymer concentrations, homogenizer speed, and agitating time could be the crucial factors for the formation of ANHs:

1   Increasing surfactant concentration, stability of micelles was increased.
2   Particle size of ANHs was increased with increasing alginate concentration.
3   With increasing agitating speed as expected, particle size of ANHs was decreased.
4   Particle size of ANHs was decreased as emulsification duration is increased.

Sodium salicylate loaded ANHs showed sustained release property except for initial burst phenomena. From

this result, the necessity for lipophilic barriers for the release of hydrophilic drug from ANHs is immerged. As the future work, biodegradable lipophilic polymers including PLA and PLGA will be coated onto the hydrophilic drug loaded ANHs to eliminate the initial burst release and ensure zero order release profiles.

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