PREPARATION OF CELLULOSE COATED HYDROGELS FOR CONTROLLED DRUG RELEASE

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ABSTRACT

In an attempt to improve material properties used for oral administration, which is restricted by fast dissolution in the stomach, chitosan hydrogels were coated with cellulose dissolved in ionic liquid. Using insulin as a model compound, the properties of these cellulose-coated microparticles for the controlled release of drug were investigated. The results showed that the coated microparticles were more stable than those which were uncoated at low pH and suitable for oral delivery without requiring any harmful and sophisticated cross-linkage treatment.

Keywords: Chitosan, hydrogels, cellulose, drug release.

1. INTRODUCTION

The use of natural polymers in dosage form has received extensive attention, especially from the viewpoint of safety. Among these polymers, chitosan, the *N*-deacetylated product of the polysaccharide chitin, is gaining increasing importance in the pharmaceutical field owing to its good biocompatibility, non-toxicity and biodegradability [1, 2]. In the early 1980s, chitosan was proposed as a useful excipient for either sustaining the release of water-soluble drugs [3] or enhancing the bioavailability of poorly water-soluble compounds [4]. More recently, it has been shown that chitosan is muco-adhesive [5, 6] and enhances the penetration of macromolecules across the intestinal [7] and nasal [6] barriers. These properties have opened promising prospects for the use of this polymer in the oral and nasal administration of proteins and peptides. Furthermore, chitosan has been presented as a useful polymer for colon-specific drug delivery because of its specific biodegradation by the colonic bacteria [8].

From a technological viewpoint, chitosan has unique properties which make it an excellent material for microencapsulation. Due to its hydrophilic and cationic character, chitosan has the ability to gel upon contact with counter-anions [9, 10]. Chitosan has also been demonstrated to possess very good film forming properties [11].

Four main approaches have been proposed for the preparation of chitosan microparticles (i) ionotropic gelation with an opposite charged polyelectrolyte, such as sodium tri-polyphosphate or alginate [9]; (ii) simple or complex coacervation [12, 13]; (iii) spray-drying [14] and (iv) solvent evaporation [15]. Independence of the particularities of microparticles produced by these techniques, a common limitation to all of them is their low capacity for controlling the release of the encapsulated compound and the necessity of a further covalent crosslinking process in order to avoid their rapid dissolution in the gastric cavity. This is due to the free amino groups in the chitosan molecule which become ionized

in acidic media leading to the almost immediate dissolution of the polymer. Chemical crosslinking with aldehydes has been used to overcome this problem [12, 13, 15]. However, this approach is not adequate for the encapsulation of proteins, peptides and other molecules with amino groups which can also undergo a covalent cross-linkage. Moreover, the toxicity of the aldehydes will significantly limit the exploitation of these cross-linked microspheres.

In the present study, we propose a simple yet useful method for coating cellulose on the surface of chitosan microspheres to improve the acidic stability of these spheres as well as to improve their controlled release properties. Cellulose has immense importance as a renewable raw material. The main restriction to the more extensive use of cellulose until now was a lack of suitable solvents for the chemical dissolution process. Cellulose is not soluble in water or conventional organic solvents because of the intermolecular hydrogen bonding. Therefore, technical processing of cellulose requires either chemical derivatization or physical dissolution in a suitable solvent. As a result of thorough investigation, ionic liquids have been considered a new type of cellulose direct solvent with superior dissolving power for cellulose.

By definition, ionic liquids are low melting salts with melting points of less than 100 ºC. Like common salt, they consist of 100% cations and anions. However, they are large volume organic ions whose low melting points are due to "softening" of the crystal lattice. On account of their interesting dissolving properties for organic and inorganic compounds and polymers, they can replace conventional solvents in many applications. Also, they have strong stability and are neither volatile nor readily flammable, which gives them advantages when used in a production process. The choice of cations and anions contained in an ionic liquid is vital in tailoring its physical and chemical properties in order to meet the requirements of a specific production process. A detailed screening with different types of ionic liquids selected and synthesized at BASF was performed searching for a suitable ionic liquid for dissolution of cellulose. Consequently, 1-ethyl-3-methylimidazolium acetate is one of a number of promising candidates. Herein, 1-ethyl-3-methylimidazolium acetate ([EMIM] [OAc]) was used to dissolve cellulose and the obtained cellulose solution was coated on the surface of chitosan microparticles. The coated hydrogels were loaded with model drugs and *in vitro* release studies were conducted to evaluate the performance of these microparticles.

2. MATERIALS AND METHODS

2.1. Materials

Chitin, sodium tri-polyphosphate (TPP), cellulose and insulin were purchased from Sigma (Korea). 1-Ethyl-3-methylimidazolium acetate ([EMIM] [OAc]) (Fig. 1.), with 95% purity, was obtained from IoLiTec (Germany) and was used without further pretreatment.

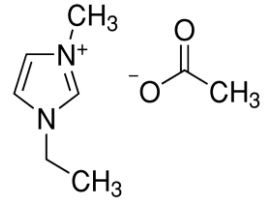


Figure 1. Structure of 1-ethyl-3-methylimidazolium acetate

2.2. Preparation of chitosan from chitin

Chitosan was prepared from chitin under deacetylation conditions of 60% NaOH, 120 ºC for 30 min to 180 min following the procedures described in [16]. After deacetylation, samples were filtered off, rinsed with distilled water to neutral pH and dried in oven at 60 ºC for 8 h and used for further experiment.

2.3. Preparation of cellulose-coated chitosan hollow hydrogels

The method was adapted from [17] and briefly summarized here. Chitosan was dissolved in 2% (v/v) aqueous acetic acid at room temperature and left overnight with continuous mechanical stirring to obtain a 2% (w/v) solution. Chitosan microspheres were fabricated by dropping 2% chitosan solution into 1% TPP solution at room temperature. The formed hydrogels were separated and washed with distilled water.

Cellulose was dissolved in [EMIM] [OAc] at 120 ºC and left overnight with continuous mechanical stirring to obtain 2% (w/v) solution. Coating was performed by mixing the prepared hydrogels in cellulose solution for 5 min, following by separation and washing with distilled water.

2.4. *In vitro* release studies

Microparticles (0.5 g) were suspended in 10 mL of phosphate-buffered saline (PBS) (pH 7.4) contained in a glass bottle, and maintained at 37 ºC, 120 rpm. Samples (2 mL) were periodically removed and the volume of each sample was replaced by the same volume of fresh medium. The amount of released was analyzed with a spectrophotometer at 276 nm [18].

3. RESULTS AND DISCUSSION

The polycationic polysaccharide, chitosan, forms gel when contacts with suitable counterions. The ionic interactions between the positively charged amino groups and the negatively charged counterion, tri-polyphosphate, were used to prepare chitosan hollow beads. The protonation of the amino groups facilitates the dissolution of chitosan by a large number of strong and weak acids [17]. Solutions of chitosan in acetic acid were dropped into TPP solutions and gelled spheres formed immediately by ionotropic gelation. The hollow beads were simply manufactured without any sophisticated equipment.

Chitosan is characterized by its degree of deacetylation and its viscosity in 2% (v/v) acetic acid solution. The shape and preparation of the beads were controlled by the viscosity of the chitosan solution. The anionic counterion, TPP, can form either intermolecular or intramolecular linkages with the positive charged amino groups [19]. The intermolecular linkages, which are responsible for the successful formation of the beads, increase in number with increasing molecular weight. Normal polyelectrolyte or intramolecular binding was probably prevalent with the low viscosity or low molecular weight chitosan samples. This may have prevented strong intermolecular crosslinking, and hence the formation of strong beads.

There is a big challenge to maintain the stability of the carriers as well as the target drug during encapsulation and release process. Generally, the chitosan particle was hardened by glutaraldehyde [17]. However, the use of glutaraldehyde might induce denaturation of drug, making it difficult to release drug from microspheres [17]. Due to this reason, the development of drug delivery shell/core microparticles, made of a combination of polymers, is receiving increasing attention in the field of microencapsulation. These models offer

critical advantages over the classical one-polymer based microcapsules: (i) by selecting appropriate core/coating polymer combination it is possible to achieve the encapsulation of hydrophilic and hydrophobic drugs simultaneously; (ii) the active component can be isolated and protected in the microcores; and (iii) the core material provides the coating polymer with an additional element for controlling the release. In the present study, we describe a new drug delivery microparticulate system which consists of microcore made of chitosan, a hydrophilic polymer, microencapsulated in a water insoluble cellulosic coat. The selection of chitosan was based on its interesting biopharmaceutical properties in addition to the drawbacks of chitosan microparticles developed until now as previously described. Therefore, it was the aim of this work to present a new approach for the preparation of chitosan microparticles suitable for oral administration and to evaluate their potential for the encapsulation and controlled release of hydrophobic drugs as well as hydrophilic macromolecules. As shown in Fig. 2, optical microscopy examination of the samples clearly indicated a thin film of coating layer on the surface of chitosan beads. The mean particle size ranged between 2.0 and 2.2 mm.

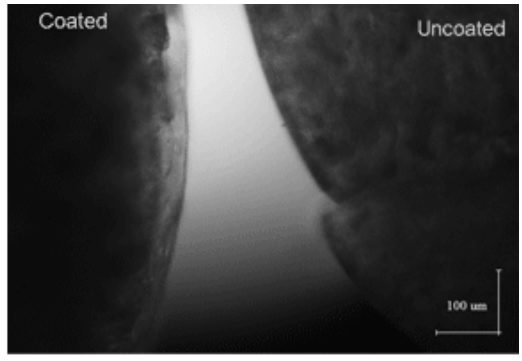


Figure 2. Optical microscopy examination of cellulose-coated and uncoated chitosan hydrogels

The hypothesized coating mechanisms were described in Fig.3. Briefly, the half-dried hydrogels were brought into contact with dissolved cellulose in ionic liquids. Then water was released from the inside to the surface of hydrogels, and cellulose surrounding the hydrogels began to precipitate on the surface, making the thin cellulose film. The generation of cellulose encapsulating coating layer was also reported in [20].

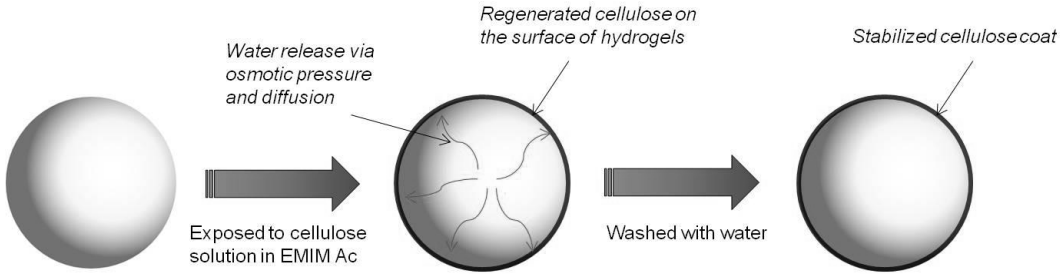


Figure 3. Schematic representation of hypothesized mechanisms of cellulose coating

To evaluate the potential of the proposed system in drug delivery system, insulin was selected as a model drug. Insulin was suspended in the chitosan solution and entrapped successfully. The TPP solution to chitosan solution ratio should be kept to a minimum for

maximum drug entrapment. More drugs were lost with increasing volume of the external phase. The drug content was independent of the total volume used, as long as the phase ratio was kept constant.

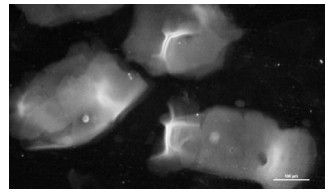
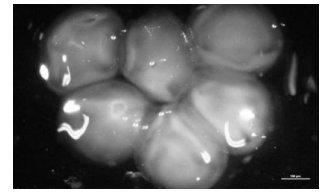
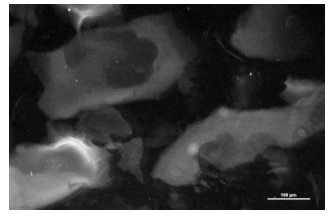
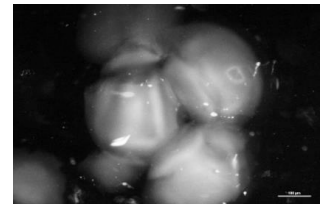
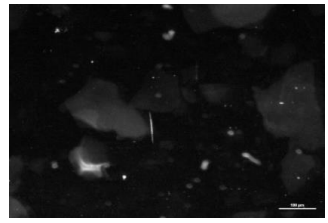
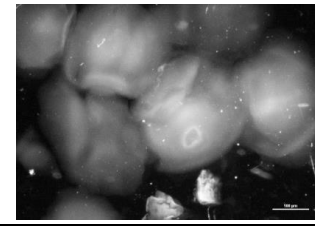
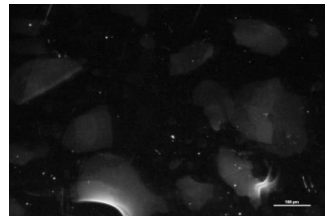
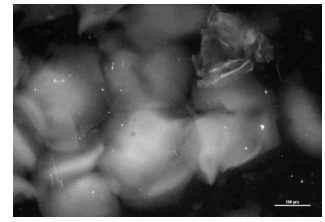
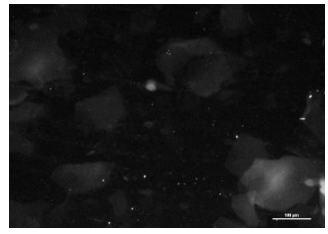
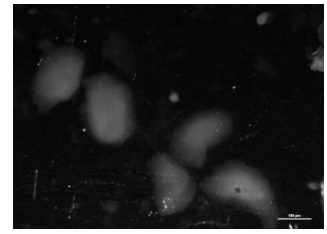
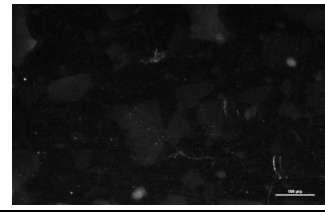
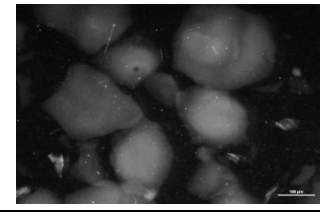
Time	Uncoated hydrogels	Coated hydrogels
30 min		
1 h		
2 h		
4 h		
6 h		
8 h		

Figure 5. Morphological observation of coated and uncoated hydrogels during incubation in simulated gastric fluid (pH 1.2) at 37 °C, 120 rpm

Other than the factors related to chitosan, it was expected that a key factor affecting the drug release would be the nature of the coating polymer. The release profiles corresponding to a formulation consisting of high molecular weight chitosan coated with cellulose and the corresponding control formulation (no coating) are displayed in Fig. 4. Coated hydrogels were hypothesized to swell inwards and force insulin to squeeze out while uncoated beads swelled out and restrained releasing of insulin, which was in agreement with the mechanism reported in [21]. In another attempt, the coated and uncoated samples were brought into contact with simulated gastric fluid (pH 1.2), it was observed that the hydrogels with cellulosic coating can be stable up to 8 hours while those without coating were dissolved within 1 hour of incubation at 37 °C (Fig. 5).

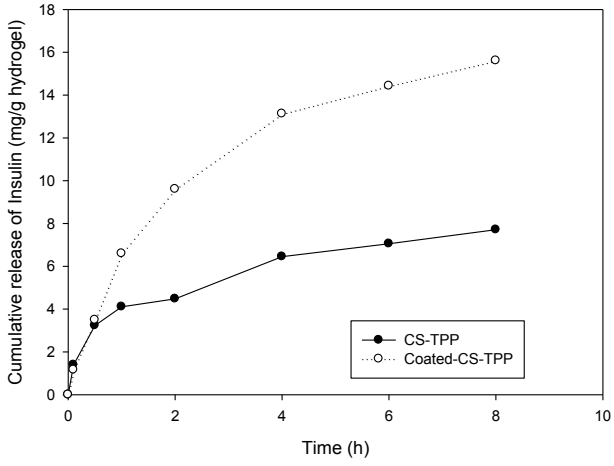


Figure 4. Release patterns of insulin from cellulose-coated and uncoated chitosan hydrogels in phosphate-buffered saline (pH 7.4)

4. CONCLUSIONS

The cellulose coating of hydrogels was first introduced with several merits compared to traditional method of crosslinking with glutaraldehyde. At first, the reaction could be completed in a very short time (less than 30 min). Secondly, the operation was very easy and no organic solvent was used in the reaction system. In a word, cellulose-coating as a post synthesis of a new scaffold is a fast and safe method to make drug carriers. The coated microparticles were more stable than those which were uncoated at low pH and thus, suitable for oral delivery without requiring any harmful and sophisticated cross-linkage treatment.

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TÓM TẮT

NGHIÊN CỨU TẠO HẠT VỚI MÀNG BAO CELLULOSE ỨNG DỤNG ĐỂ KÉO DÀI THỜI GIAN GIẢI PHÓNG DƯỢC CHẤT

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Nghiên cứu này nhằm mục tiêu tạo ra hạt chitosan với màng bao cellulose nhằm kiểm soát sự giải phóng hoạt chất và tăng độ bền của hạt khi tiếp xúc với dịch dạ dày nhân tạo có pH thấp. Mẫu dược chất sử dụng là insulin. Kết quả cho thấy các hạt với màng bao cellulose bền hơn các hạt không có màng bao trong môi trường có pH thấp và độ giải phóng dược chất insulin được kiểm soát tốt trong 8 giờ. Nghiên cứu cho thấy kỹ thuật tạo hạt với màng bao cellulose là kỹ thuật đơn giản, không sử dụng các hóa chất độc hại và có thể ứng dụng để kéo dài thời gian giải phóng dược chất.

Từ khóa: Chitosan, hạt, cellulose, giải phóng dược chất.