Preparation of Carrageenan-Polyethyleneimine Nanocapsules Using Electrocapillary Emulsification Technique

Hideki SAKAI *,**, Hiroshi FUKUSHIMA *, Mayumi SATOH *, Tamotsu KONDO **, Kenichi SAKAI *, Koji TSUCHIIYA * and Masahiko ABE *,**,†

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Abstract

The present paper proposes a novel preparation method of carrageenan-polyethyleneimine complex capsules in the submicron size range using the interfacial gelling reaction at W/O type emulsion. A W/O emulsion was prepared by adding dropwise aqueous τ-carrageenan solution into cyclohexane containing sorbitan monooleate through a microsyringe. Carrageenan gel particles were then prepared by adding the emulsion into a cyclohexane solution containing Ca AOT (calcium bis(2-ethylhexyl) sulfosuccinate) to cause gelation of aqueous droplets. The gel particles thus prepared were coated with polyethyleneimine to improve their stability in water. Capsules with sizes ranging from 100 to 300 nm were obtained when an apparatus for electrocapillary emulsification was used in the preparation step of W/O emulsion. The use of calcium salt of dimyristoyl phosphatidyl glycerol, a constituent of biomembrane, as gelling agent gave capsules of sizes in the submicron range.

Key-words : Electrocapillary Emulsification, Nanocapsules, Biocompatibility, Carrageenan-Polyethyleneimine Complex

1. Introduction

Microcapsules (nanocapsules) can protect core substance from the external environment and control the rate of core substance release into the outside medium through changes in the wall material and wall thickness, thereby providing them with various functions1,2. Then, microcapsules are used in many fields including those of medicine, electronic paper, toner for printing, and agricultural chemicals3. In medicinal therapy, the ideal case is that a minimum amount of the medicine necessary is given to develop its effect at the morbid part of the body to be cured. Then, many investigations have been studied on the drug delivery system (DDS) in which a necessary amount of the medicine is delivered to the necessary part of the body during the necessary time period4,5. In addition, decrease in size of microcapsules to submicrometer size is required in order to increase the delivery efficiency of incorporated drugs to the organs.

While various biocompatible polymers have been studied as wall materials of microcapsules used for DDS, the present authors have chosen carrageenan, a natural polysaccharide polyelectrolyte, as the target material in this paper. Carrageenan is highly biocompatible and scarcely toxic to cells. Also, it has sulfate groups on its side chains and forms gels and becomes insoluble through ion complex formation with various cations including metal cations6. Then, many investigations using carrageenan have briskly been performed on the preparation of microcapsules and as a carrier for DDS7. Encapsulation is conventionally conducted by adding an aqueous carrageenan solution dropwise into an aqueous inorganic electrolyte solution through a syringe to form uniform sized droplets and gel the polyelectrolyte. Nevertheless, this method
hardly gives small gel particles because the reaction used occurs between aqueous solutions and the size of particles formed depends on the diameter of syringe needle. Much smaller gel particles are expected to be obtained if they are prepared at the water/oil interface via emulsion droplet. We have recently reported a new preparation method of calcium alginate gel particles in which an aqueous polyelectrolyte solution was dispersed in an oil phase containing oil-soluble gelling agent obtained by modifying a surfactant with calcium ion and gelation occurred on the surface of each aqueous droplets.

On the other hand, we have also reported a method for preparing W/O type emulsions containing very small aqueous droplets with the aid of electrocapillary emulsification. In this emulsification method, an electric potential is applied to the oil/water interface to obtain tiny liquid droplets with the great ability in lowering the interfacial tension. We have also succeeded in preparing polyurea capsules with sizes in the submicron range by the interfacial chemical reactions at the surface of the emulsion droplet prepared by the electrocapillary emulsification method. The present article proposes a method for preparing carrageenan- polyethyleneimine complex capsules in the submicron size range by the interfacial reaction at the surface of tiny emulsion droplet formed with the electrocapillary emulsification method.

2. Experimental

2.1 Materials

In the preparation of capsules, aqueous solution of τ-carrageenan (TCI Co., Ltd.) (Fig. 1 (a)), cyclohexane (TCI Co., Ltd.), sorbitan monooleate (Nikko Chemicals), an oil-soluble nonionic surfactant, and cyclohexane solution of Ca AOT (Fig. 1 (b)), an oil-soluble anionic surfactant, were used as the aqueous phase, oil phase, emulsifier, and gelling agent, respectively. Cyclohexane solution of Ca DMPG (Fig. 1 (c)), calcium salt of dimyristoyl phosphatidyl glycerol (NOF Corp.) and non-bioxic, was used as another gelling agent. The capsule wall material used was polyethyleneimine (Fig. 1 (d)) (Wako Pure Chemicals), a cationic polyelectrolyte.

2.2 Apparatus

An apparatus for electrocapillary emulsification was constructed (Fig. 2) and used in this work. A brief explanation of electrocapillary emulsification is given below. This emulsification method makes use of electrostatic pulverization phenomenon, especially electrocapillarity in which an electric potential applied across two liquids in contact with each other in a capillary changes the liquid/liquid interfacial tension, thereby causing increase in the interface area to produce a large number of minute droplets. Namely, this emulsification method allows to prepare fine liquid droplets electrostatically without mechanical agitation.
2.3 Preparation of oil-soluble gelling agent solution

Equal volumes of 1.0 M aqueous calcium chloride solution and 0.1 M Na AOT (sodium bis(2-ethylhexyl) sulfosuccinate) solution in heptane (Wako Pure Chemicals) were mixed to replace sodium ion with calcium ion as counterion. The aqueous and organic phases were then separated using a separating funnel and the organic phase (Ca AOT heptane solution) was taken out. The organic phase taken out was mixed with aqueous 1.0 M calcium chloride solution and the organic phase was taken. Finally, the organic solvent was removed and the residue was dried in a vacuum to give the oil-soluble gelling agent, Ca AOT\(^{12}\).

2.4 Preparation of carrageenan/polyethyleneimine complex capsules

A W/O emulsion was prepared by adding dropwise 1 ml of aqueous 1 wt% \(\tau\)-carrageenan solution into cyclohexane containing 2 wt% sorbitan monooleate through a syringe. Carrageenan gel particles were then prepared by adding the emulsion into 40 ml each of cyclohexane solutions containing various concentrations of Ca AOT to cause gelation of aqueous droplets. Finally, the solvent was replaced with distilled water and the aqueous dispersion obtained was added to aqueous polyethyleneimine solution to cover electrostatically each of negatively charged carrageenan gel particles with positively charged polyethyleneimine, thus giving carrageenan/polyethyleneimine complex capsules.

2.5 Colloid titration of polyethyleneimine

Optimization of polyethyleneimine concentration was conducted using colloid titration. This procedure was necessary to determine the optimum polyelectrolyte concentration for covering carrageenan gel particles effectively. Titration of 10 ml of aqueous 0.1 wt% polyethyleneimine solution with aqueous 2.5 \(\times\) 10\(^{-3}\) mol/l solution of potassium polyvinylsulfate (PVSK, Wako Pure Chemicals), a standard polyanion, using toluidine blue as indicator gave the equivalency of the former. Then, aqueous 5.0 \(\times\) 10\(^{-3}\) mol/l solution of methyleneglycol chitosan (Wako Pure Chemicals), a standard polycation, was added in an excess amount (10 ml) to 10 ml of aqueous 0.1 wt% \(\tau\)-carrageenan solution to precipitate the latter. The supernatant solution containing excess methyleneglycol chitosan was titrated with aqueous 2.5 \(\times\) 10\(^{-3}\) mol/l potassium polyvinylsulfate solution. Based on the results of the titrations, the equivalent point for carrageenan and polyethyleneimine was calculated and the optimum concentration of polyethyleneimine necessary for encapsulation was determined.

2.6 Capsule preparation using electrocapillary emulsification technique

A W/O emulsion containing tiny droplets of the aqueous phase was prepared using electrocapillary emulsification to decrease capsule size to a great extent. Thus, an emulsion was prepared by adding aqueous 1 wt% \(\tau\)-carrageenan solution into 40 ml of cyclohexane with 2 wt% sorbitan monooleate dissolved through a syringe under various electric potentials given by the electrocapillary emulsification apparatus. Carrageenan gel particles were then prepared by adding the emulsion into 40 ml of cyclohexane containing 50 mM Ca AOT to cause gelation of aqueous droplets in the emulsion, thus yielding a system with carrageenan gel particles dispersed in cyclohexane. Carrageenan/polyethyleneimine complex capsules were obtained after cyclohexane, dispersion medium, was replaced by distilled water and the resultant aqueous dispersion was added to 50 ml of aqueous 2 wt% polyethyleneimine solution.

2.7 Characterization of the microcapsules

The size of carrageenan/polyethyleneimine capsules was measured by the dynamic light scattering method using a NICOMP 380ZLS (Particle Sizing System). Light source is a diode pump solid state (DPSS) laser at a wavelength of 532 nm and scattering light was monitored at an angle of 90\(^\circ\).

The shape of carrageenan/polyethyleneimine complex capsules was observed with a differential interference optical microscope (IMT-2, Olympus). A sample of the capsules dried in a vacuum was observed using a transmission electron microscope (H-7650, Hitachi High Technologies). The qualification of capsule material was performed by the attenuated total reflection (ATR)-IR method using a spectrometer (FT-IR 6100, ATR PRO 410-S, JASCO). The surface charge of the capsules was evaluated with \(\zeta\) potential measurement using an apparatus for electrophoresis (Nicomp 380ZLS, Particle Sizing Systems).

3. Results and discussion

3.1 Novel preparation method of carrageenan/polyethyleneimine complex capsules

3.1.1 Optimum concentration of oil-soluble gelling agent

Ca AOT is known to be a surfactant that forms reverse micelle since it has four bulky hydrophobic groups as shown in Fig. 1 (b). The surfactant adsorbs to the oil/water interface with its hydrophilic group directing toward the aqueous phase (carrageenan solution droplet) of the W/O emulsion and Ca ion binds carrageenan molecules to form a gel. Then, the
optimum concentration of Ca AOT for gel formation was investigated.

The optimum Ca AOT concentration was determined by evaluating the shape of carrageenan gel particles after centrifugation. The particles were prepared fixing the volume of aqueous 1 wt% \(\tau\)-carrageenan solution at 1 ml while the concentration of Ca AOT was varied from 5 to 50 mM at every 5 mM step. Carrageenan gel particles prepared using the gelling agent concentrations higher than 20 mM were spherical in shape and stable without being destroyed after centrifugation because network formation is rigid at the high Ca AOT concentrations. Then, 50 mM was chosen as the optimum Ca AOT concentration in the present work because this concentration gave most stable gel particles.

3.1.2 Optimum polyethyleneimine concentration

Colloid titration was adopted to determine the optimum concentration of polyethyleneimine necessary to effectively cover the surface of carrageenan gel particles. The titration experiments revealed that the electric charges on polyethyleneimine and carrageenan are respectively equivalent to 5.03 and 12.82 m\(\text{mol}^{-1}\) of 2.5 \(\times\) \(10^{-3}\) mol/l PVSK solution. This means that the ratio of the electric charge on a carrageenan molecule to that on a polyethyleneimine molecule is 13 : 5. Then, 1 ml of 1 wt% \(\tau\)-carrageenan solution is equivalent to 50 ml of 0.52 wt% polyethyleneimine solution. Based on this finding, 50 ml of 2% (w/v) polyethyleneimine was used to cover all of gel particles prepared using 1 ml of 1 wt% \(\tau\)-carrageenan solution.

3.1.3 Confirmation of capsule formation

Particles with sizes ranging from 55 to 350 \(\mu\)m were observed under an optical microscope (Fig. 3). Peaks due to NH stretching vibration (3400 cm\(^{-1}\)) and deformation vibration (1650-1590 cm\(^{-1}\)) of polyethyleneimine were detected in the ATR-IR spectrum of particles dried in a vacuum (Fig. 4). Hence, negatively charged carrageenan gel particles were strongly suggested to be covered with positively charged polyethyleneimine to give carrageenan/polyethyleneimine complex capsules. Actually, the electrophoretic mobility of particles reversed its sign from minus to plus, indicating that the surface of the particles (capsules) is positively charged (Fig. 5).

3.2 Preparation of tiny capsules using electrocapillary emulsification

3.2.1 Effect of applied electric potential on droplet size

The optimum applied electric potential in electrocapillary emulsification was studied. In electrocapillary emulsification, the oil/water interfacial tension decreases abruptly at a certain applied electric
potential (critical electric potential) at which water (oil) disperses into oil (water) as a large number of very tiny droplets just like a shower. Then, the minimum electric potential (critical electric potential) necessary to emulsify the sample used in this work was determined. The critical electric potential was found to be 1000 V because the aqueous phase began to disperse as very tiny droplets into the oil phase like a shower at and above 1000 V.

A relation is known to hold between droplet size and applied electric potential in the electrostatic pulverization method and no monodisperse emulsion is obtained unless an optimum electric potential is applied. The effect of applied electric potential was then investigated on droplet size at applied electric potentials of 1000, 1500, and 2000 V. The mean droplet size was found to increase slightly with increasing applied electric potential and tended to increase with time elapsed after emulsion preparation. The increase in droplet size was the least and emulsion was stable when it was prepared at 1000 V. The droplet size distribution broadened with time in emulsions prepared at applied electric potentials of 1500 and 2000 V, probably due to a phenomenon characteristic of emulsion. When emulsion contains droplets of different sizes, small droplets are absorbed by large droplets through molecular diffusion (Ostwald ripening), thereby increasing the size of the latter. The reason why the droplet size distribution widened in emulsions prepared at applied electric potentials of 1500 and 2000 V would be due to increase in the droplet size with time after preparation caused by Ostwald ripening. The optimum applied electric potential was determined to be 1000 V in this work based on the above considerations.

3.2.2 Formation of tiny carrageenan/polyethyleneimine complex capsules

The same procedures of capsule formation as those described in the precedent section was applied to the emulsion prepared using electrocapillary emulsification. The formation of carrageenan/polyethyleneimine complex capsules was checked by transmission electron microscopy (TEM) and ATR-IR spectroscopy. Measurements of the ζ potential of particles were also conducted before and after coating them with polyethyleneimine to check the formation of the complex capsules.

A typical TEM image of the complex capsules is given in Fig. 6, which shows primary size of capsules ranging from 100 to 300 nm. Dynamic light scattering measurement also showed that capsule size distribution is from 100 to 300 µm. The ζ potential of the capsules had a positive value (+20 mV) indicating that the capsule surface is covered with positively charged polyethyleneimine. The ATR-IR spectrum of dried capsules was similar to that shown in Fig. 4 for the complex capsules prepared without using electrocapillary emulsification.

3.3 Preparation of biocompatible capsules

The same method as that used in the preparation of carrageenan/polyethyleneimine complex capsules was employed to prepare biocompatible nontoxic capsules using biocompatible Ca DMPG instead of Ca AOT as oil-soluble gelling agent and electrocapillary emulsification. The capsules prepared had sizes ranging from 200 to 700 nm as revealed from TEM observations.

4. Conclusions

Carrageenan/polyethyleneimine complex capsules were prepared by coating carrageenan gel particles dispersed in water with polyethyleneimine. The gel particles were obtained at oil/water interface by adding dropwise aqueous carrageenan solution into cyclohexane containing Ca bis(2-ethylhexyl) sulfosuccinate (Ca AOT), a gelling agent. Replacing cyclohexane with water gave carrageenan gel particles dispersed in water to be coated with polyethyleneimine.

Furthermore, the use of electrocapillary emulsification allowed to prepare carrageenan/polyethyleneimine complex capsules with sizes ranging from 100 — 300 nm. When Ca dimyristoyl phosphatidyl glycerol (DMPG), a phospholipid, was used as oil-soluble gelling agent instead of Ca AOT, biocompatible carrageenan/polyethyleneimine capsules of 200 — 700 nm in size were obtained.
電気毛管乳化法による生体適合型ナノカプセルの調製

酒井秀樹* • 福島 宏 • 佐藤真弓 • 近藤 保 • 酒井健一 • 土屋好司 • 阿部正彦**

*東京理科大学理工学部工業化学科 千葉県野田市山崎 2641（〒278-8510）
**東京理科大学総合研究機構界面科学研究部門 東京都新宿区神楽坂1-3（〒162-8601）
† Corresponding Author, E-mail: abemasa@rs.noda.tus.ac.jp

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要 旨

本稿では、サブミクロサイズを有するカプセル・ポリエチレンイミン複合カプセルの新製法を提案する。カプセルを調製した水相をヘキサン中に添加することによりW/O乳化法を調製した。さらに、この乳化法を油溶性グリセリソル（Ca AOT）を含むシクロヘキサン溶液に添加することにより、カプセルのゲル粒子ゲルが得られた。ゲル状のカプセルの表面をカプセルイミンで被覆することにより、複合カプセルを得ることに成功した。粒子の分散安定性は、カプセルの表面をポリエチレンイミンで被覆することで着しく向上した。また、乳化法の調製に電気毛管乳化法を適用することにより、乳化法イミンの粒子径が減少し、200－600 nmの複合カプセルを得ることが可能になった。さらに、Ca AOTの代わりにジミルクトライフォスファチジルグリセロールのカルシウム塩を用いることで、生体適合性のサブミクロサイズのカプセルを得ることができた。