Journal of Membrane Science 346 (2010) 296–301

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

# Quantitative characterization of membrane formation process of alginate–chitosan microcapsules by GPC

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ARTICLE INFO

*Article history:*
Received 23 June 2009
Received in revised form
23 September 2009
Accepted 23 September 2009
Available online 1 October 2009

*Keywords:*
Alginate–chitosan microcapsule
Membrane formation process
Quantitative characterization
Gel permeation chromatography

ABSTRACT

The semi-permeable membrane of alginate–chitosan (AC) microcapsules has been proven important to control the microcapsule stability and selective substance diffusion rate. Therefore, a novel and operable methodology based on gel permeation chromatography (GPC) was established for quantitative characterization of the membrane formation process, so as to provide guidance on performance improvement of AC microcapsules in biomedical applications. Not only the molecular weight ($M_w$) and its distribution of chitosan can be obtained by GPC, but also the area integral of molecular weight peaks can be linearly correlated to chitosan concentration in certain range. The dynamic membrane formation process was then obtained by quantitatively analyzing reaction amount of chitosan with time, which showed that for chitosan molecules with wide $M_w$ distribution, only parts of molecules bind with alginate to form microcapsule membrane. Moreover, the contribution of chitosan molecules participating in the membrane formation process was also different. These new findings, therefore, are helpful for adjusting and controlling the membrane formation process and properties of microcapsule membrane.



## 1. Introduction

Microencapsulation is the process of enclosing a substance inside a semi-permeable membrane to form a microcapsule [1]. Due to the protection and selective permeation properties of the semi-permeable membrane, microcapsules have been widely used not only in biochemical engineering such as cell immobilization fermentation, but also in biomedical fields such as drug delivery and cell transplantation [2]. Although many polymers have been attempted with various preparation technologies, alginate-based microcapsules are still the commonly used ones. Alginate–chitosan (AC) microcapsule, formed by two naturally occurred polysaccharides with opposite charges, has been attractive owing to the inherent properties such as biodegradability, nontoxicity, and biocompatibility [3]. Numerous papers have been published reporting studies on membrane permeability [4], mechanical strength [5] of AC microcapsules [6], as well as applications in drug delivery systems [7,8] enzyme immobilization [9], cell immobilization fermentation [10], and cell transplantation [11].

Whatever in short-term or long-term *in vivo* applications, AC microcapsules have to keep structural stability and exert function while facing, enduring, or interacting with complicated environments. Besides alginate gel core, the complex microcapsule membrane, formed by electrostatic interaction between alginate and chitosan, has been demonstrated to play an important role in controlling the microcapsule stability and substance diffusion rate [12]. The membrane formation process includes at least chitosan molecules diffusion into three-dimensional (3D) alginate gel network and simultaneous binding between protonated amino groups of chitosan and carboxyl groups of alginate, which is affected by complicated factors such as molecular weight ($M_w$) and wide $M_w$ distribution, chain flexibility, charge density of both polysaccharides [13]. Therefore, a better quantitative understanding on microcapsule membrane formation process, especially on chitosan amount and fraction binding with alginate will be helpful to improve the functional properties of membrane mechanical strength and selective substance permeability, and then the performance of AC microcapsules in biomedical applications. However, it is usually difficult to realize effective quantitative analysis of polysaccharides. Curotto and Aros introduced the use of ninhydrin reaction for quantitative determination of chitosan [14]. Prochazkova et al. demonstrated that the reaction of chitosans with ninhydrin was sensitive and reproducible only when a reliable calibration against a reference of similar composition was available [15]. Moreover, proteins hard to be removed from chitosan often affect the analysis accuracy. Gaserod et al. reported radioactive label method detecting the interaction between alginate and chitosan [16]. Although they gave quantitative reaction amount of radioactive labeled chitosan on alginate gel surface, the special

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doi:10.1016/j.memsci.2009.09.049

experimental condition and safety concern make it not acceptable for routine analysis means.

In this paper, a novel methodology for quantitative characterization of alginate–chitosan membrane formation process was established based on gel permeation chromatography (GPC), which has been generally used as a credible and rapid method to characterize the molecular weight and its distribution of polymers [17,18]. AC microcapsules were made by two-stage procedure, that is, calcium alginate gel (CAG) beads were produced firstly, and then the beads were immersed in chitosan solution to form the membrane on the surface of the beads. After establishing calibration curves with standard polymers, chitosan molecular weight and its distribution can be measured by GPC. And then the amount of chitosan molecules binding with alginate beads can be calculated by the integral of molecular weight peak area during AC microcapsule membrane formation process. Therefore, the membrane formation process can be characterized and further understood by quantitatively analyzing reaction amount of chitosan with time.

## 2. Materials and methods

### 2.1. Materials

Chitosan samples were chemically modified from raw material (Yuhuan Ocean Biomaterials Corporation, China) by our laboratory. The degree of deacetylation (DD) was 96–98%. The average degree of polymerization (DP) was 130, 400, and 560, respectively, which was calculated from intrinsic viscosity. Therefore, DP130, DP400 and DP560 were used to denote the different batch of chitosan samples in the study. Sodium alginate was purchased from the Chemical Reagent Corp (Shanghai, China), whose viscosity was over 0.02 Pa s when dissolved to form a 1.0% (w/v) aqueous solution at 20 °C. The powder size was less than 200 mesh. Nanoparticulate calcium carbonate was purchased from Taihua Corporation (Zhejiang, China). Pullulan standards (Shodex Standard P-82) were obtained from Shoko Co., Ltd., Japan. All other reagents were analytical grade and used as received.

### 2.2. Preparation of AC microcapsules

AC microcapsules were prepared according to the method developed in our lab [19]. Sodium alginate was dissolved in 0.9% (w/v) NaCl solution to form concentration of 1.5% (w/v). After being filtered through a 0.22 μm membrane filter, the solution was stored overnight before use to facilitate deaeration. Sodium alginate solution as above was extruded through a 0.4-mm needle into calcium gelling solution using electrostatic droplet generator (YD-06, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China) to form CAG beads. The beads were rinsed with distilled water and immersed in 0.5% (w/v) chitosan solution to form alginate–chitosan membrane around the surface.

### 2.3. Characterization of morphology and size distribution of AC microcapsules

The morphology of AC microcapsules was observed under convert optical microscope (Olympus CK-40, Olympus Corp. Japan). The size distribution of AC microcapsules was determined with laser diffraction particle analyzer (LS100 Q, Beckman-Coulter Corp., USA).

### 2.4. Gel permeation chromatography (GPC)

Pullulan standards (Shodex Standard P-82, Showa Denko K.K.) were firstly injected into a TSK G4000PWxl column (7.8 mm × 300 mm, 10 μm particle diameter, Tosoh Corporation, Tokyo, Japan) of GPC equipped with a refractive index detector (Waters, Model 2414, Milford, MA, USA) and an HPLC pump (Waters, Model 515), which would give a universal calibration curve showing the relationship between weight-average molecular weight ($M_w$) and the elution time. Then, chitosan samples (DP130, DP400 and DP560) dissolved in buffer of 0.2 M $CH_3COOH$/0.1 M $CH_3COONa$ were eluted through a TSK G4000PWxl column with a 20 μL injection volume at a flow rate of 0.6 mL/min with the same buffer used as eluent. The column and the detector were both set at the temperature of 30 °C. All data provided by the GPC system

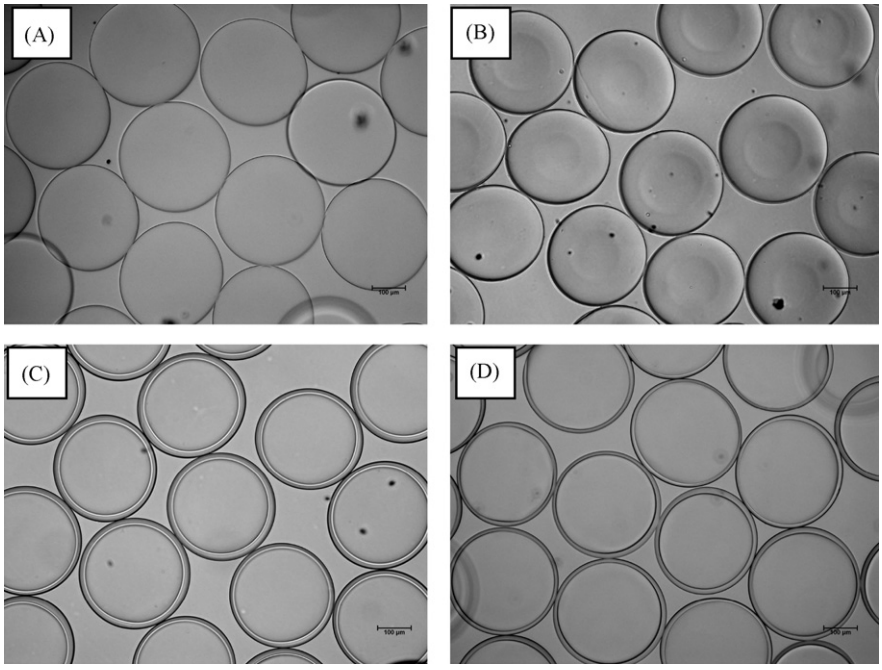


**Fig. 1.** Photographs of CAG beads (A) and AC microcapsules with chitosan of DP130 (B), DP400 (C), and DP560 (D).