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Preparation and characterization of NaCS–CMC/PDMDAAC capsules

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Abstract

A novel capsule system composed of sodium cellulose sulfate (NaCS), carboxymethyl cellulose (CMC) and poly[dimethyl(diallyl) ammonium chloride] (PDMDAAC) was prepared for improving the properties of NaCS/PDMDAAC capsules. The process parameters, such as CMC concentration (0, 2, 4, 6 and 8 g/L), NaCS concentration (20, 25, 30, 35 and 40 g/L), PDMDAAC concentration (20, 30, 40, 50, 60, 70 and 80 g/L), reaction time and temperature were investigated to understand their effects on the diameter, membrane thickness and mechanical strength of capsules. The optimum operation conditions for preparing NaCS–CMC/PDMDAAC capsules were determined as 6–8 g/L CMC, 35–40 g/L NaCS, 60 g/L PDMDAAC and polymerization for 30–40 min. Diffusion of substances with low molecular weight into capsules was investigated, and diffusion coefficients were calculated according to the developed model. The yeast of *Candida krusei* was chosen as representative cell to evaluate the effects of different cell loading on capsule mechanical strength. Meanwhile the encapsulated osmophilic *C. krusei* cells were cultured in 250 mL shaking flasks for 72 h to determine the cell leaking properties in short and long term.
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Keywords: NaCS–CMC/PDMDAAC capsules; Membrane thickness; Capsule characterization; Cell cultivation

1. Introduction

Encapsulation is an important method for cell immobilization. Bioencapsulation aims to entrap viable cells and enzyme enclosed with a semi-permeable membrane. Each capsule can be regarded as a micro-bioreactor. The capsule membrane is expected to isolate viable cells and enzyme from harsh environmental conditions and form a favorable local condition for cells and enzyme. Simultaneously, substrates can freely permeate through the capsule membrane. As a result, encapsulation brings about some advantages in simplifying downstream processes, reducing the size of reactor and reutilizing the entrapped cells [1,2]. Over the past decades, encapsulation as one of immobilization technologies was widely studied in connection with the application of implantable bioartificial organs [3,4], successive processes [5] and immobilization of enzymes [6], plant cells, etc. Lim and Sun [7] reported that the Langerhans islets were encapsulated for producing insulin on physiological demands. Encapsulation of yeast cells in ethanol production [8], immobilization of hybridoma cells in the production of monoclonal antibody [9] and encapsulation of drugs in sustainable release systems represented some successful examples.

Calcium alginate beads were studied a lot in the past decades due to the simple preparation, low price and good biocompatibility. But their instability with chelating agents such as citric acid and phosphate which often presented in the culture medium, impeded its further application. The nutrients and oxygen diffusion into matrix, and cells leaking out of gel also brought some troubles [10,11]. To overcome these problems, the development of polyelectrolyte complex (PEC) microcapsules formed by oppositely charged polyions is a simple and effective method. The commonly employed polyelectrolyte capsule systems are sodium cellulose sulfate (NaCS)/poly[dimethyl(diallyl)ammonium chloride] (PDMDAAC), chitosan/alginate, chitosan/xanthan, etc. The characteristics of PEC capsules can be affected by the complex structure, concentration and charge density of

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Nomenclature

C_0	initial concentration of bulk solution (g/L)
C_t	concentration of bulk solution at time (t, g/L)
D_1	diffusion coefficient in the membrane of the capsule (m^2/s)
D_2	diffusion coefficient in the liquid core of the capsule (m^2/s)
D_m	overall diffusion coefficient (m^2/s)
D_w	diffusion coefficient in pure water (m^2/s)
n	the number of beads
q_n	nonzero positive roots of Eq. (2)
r	distance away from the core of a bead (m)
r_a	inner radius of the capsule (m)
r_b	outer radius of the capsule (m)
R	radius of a bead (m)
V	the volume of solution (m^3)

Greek letters

α	ratio of the liquid volume to the capsule volume

polyelectrolyte, and also PEC formation is strongly influenced by the presence of low molecular salts in polyelectrolyte solution.

Characterization of NaCS/PDMDAAC microcapsules such as preparation process, mechanical strength, biocompatibility and diffusion was systematically investigated by Dautzenberg and co-workers [12,13,2] and Yao and Cho [14]. In view that the formation of capsules was the reaction of one polyanion with the other polycation, it was difficult to regulate the characteristics of capsules membrane. Recently, the capsules composed of alginate, NaCS, poly(methylene-co-guanidine)hydrochloride (PMCG) and $CaCl_2$ have been put forward to improve the membrane thickness and diffusion properties [15–17]. Its preparation was a two-step procedure, which comprised the formation of calcium alginate beads followed by a membrane forming stage where the beads were suspended in the solution of PMCG. To obtain a liquid core capsule system, the beads had to be washed with phosphate or citrate buffer. In the whole process, all experimental conditions should be controlled strictly to obtain fine capsules.

In present paper, we will prepare a novel microcapsule system, i.e. carboxymethyl cellulose (CMC)–NaCS/PDMDAAC microcapsules, in which the mixture of CMC and NaCS is chosen as polyanions and PDMDAAC as a polycation. In the formation process of capsules, the characteristics of capsules can be regulated by adjusting CMC concentration. The mechanical stability, the uniformity of size, and cells encapsulation and optimal microenvironment are major challenges in the design and development of CMC–NaCS/PDMDAAC capsules for cell immobilization. In this paper, the characteristics of capsules made from polyelectrolyte NaCS, CMC and PDMDAAC are investigated in detail under different operation conditions. Diffusion coefficients of substances with low molecular weight into capsules are also calculated according to kinetic curves. The property of cell leaching and stability of capsules during the period of culture are studied in detail.

2. Materials and methods

2.1. Materials

PDMDAAC (MW 200,000–350,000) was obtained from Aldrich (Germany). NaCS was prepared by heterogeneous reaction as described before [19] in our lab. CMC was purchased from Sangon (Shanghai, China). All other chemicals and solvents were analytical grade and used without further purification. Osmophilic yeast *Candida krusei* (ICM-Y-05) was obtained from the Institue of Process Engineering, Chinese Academy of Sciences (Beijing, China).

2.2. Preparation of NaCS–CMC/PDMDAAC capsules

The microcapsules were prepared in hollow spheres in the experiments. A mixture of NaCS and CMC was dissolved in 100 mL water, degassed and added dropwise, through a syringe, into 100 mL PDMDAAC solution. Capsules formed while mild stirring for 40 min at room temperature. The microcapsules were washed with deionized water to eliminate excess unreacted PDMDAAC. The microcapsules were kept in 0.9 wt.% NaCl solution at room temperature.

2.3. Compression intensity and microscopic observation

The mechanical strength of capsules was tested through pressing 20 capsules on an electronic scale. The force exerted on the capsule was recorded as a function of the weight showing on the scale. The capsule was cut and its membrane thickness was visually examined under a standard light microscope. The capsule diameter was measured with a caliper after the capsule surface had been dried with filter paper.

2.4. Determination of diffusion coefficients

Diffusion of glucose, tyrosine, glycerol and Vitamin B_{12} from the well-stirred solution into capsules was measured. Firstly, equal volume of capsules was added into well-stirred substrate solution at a temperature of 25 °C, and samples of 50 μL were collected at a defined time interval. Diffusion coefficients were calculated from kinetic curves of substrate diffusion into capsules according to the developed diffusion model in our previous work [14].

In this paper, the capsule is postulated to be homogeneous. Under perfect stirring, the liquid film resistance around the capsules can be ignored. The diffusion of substrate into beads

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