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Effect of ultrasound on the diffusion properties of casein entrapped in alginate–chitosan gel

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ABSTRACT

The effects of ultrasound-assisted and pre-ultrasound treatment on the diffusion properties of casein imbedded by alginate–chitosan gel were investigated. The fluorescence spectrophotometry for determining the fluorescence intensity of casein was established to calculate the diffusion coefficient (D_e). Scanning electron microscope (SEM) was used to observe the microstructure of gel beads. The results showed that two different kinds of ultrasonic treatments had obvious distinctions on the casein diffusion. As the frequency increased, the value of D_e decreased from 28.56×10^{-4} m^2 s^{-1} (28 kHz) to 2.57×10^{-4} m^2 s^{-1} (135 kHz) during the ultrasound-assisted process. While, the minimum D_e of 8.6×10^{-4} m^2 s^{-1} was achieved at the frequency of 50 kHz for the pre-ultrasound treatment. The impact of power on the diffusion showed that D_e increased with the increase of ultrasound power until it reached the highest value 28.56×10^{-4} m^2 s^{-1} (0.45 W/cm^2) in the ultrasound-assisted process. It would reach the maximum value (16×10^{-4} m^2 s^{-1}) when the power was 0.25 W/cm^2 in the pretreatment ultrasound process. SEM analysis exhibited that the gel structural changes (area ratio) were in accordance with D_e through different ultrasonic treatment. This was mainly due to the mechanical action and cavitation of the ultrasonic treatment. This study is important to explain the diffusion properties of large molecules and explore the mechanism of enzyme immobilization treated by ultrasound.

1. Introduction

Enzyme immobilization is a novel technology in recent years and has attracted a wide range of interest from fundamental academic research to many different industrial applications [1]. Because of the simple preparation, mild experimental conditions and high enzyme activity, embedding immobilization becomes the most widely used in the food, pharmaceutical and biomedical application [2,3]. Many researchers have investigated the effect of embedded immobilization on enzyme properties. Di found that the gold nanoparticles and horseradish peroxidase embedded in silica sol–gel network on gold electrode surface in the presence of cysteine exhibited direct electrochemical behavior toward the reduction of hydrogen peroxide, and the biosensor exhibited high sensitivity, rapid response, long-term stability and an excellent electrocatalytic response to the reduction of H_2O_2 without any mediator [4]. Wu showed that compared with free α-amylase, the immobilized α-amylase entrapped by agar retained a higher enzyme activity, good reusability and reaction stability, longer storage time and wide pH range [5]. However, the grid structure of polymer carriers may hinder the macromolecules' diffusion and restrain the release of substance [6]. Wojcieszyńska reported that compared with the free state, the K_m and v_{max} of epigallocatechin gallate dioxygenase immobilized by sodium alginate were 0.42% and 37.7%, the immobilized enzyme and substrate's affinity increased 230 times, but the enzymatic reaction rate decreased - more than 60%, which proved that the structural rigidity of sodium alginate gel blocked the substrate and the product of the reaction [7]. Therefore, to overcome the block is the essential problem of the embedding immobilization.

The diffusion of macromolecules, such as casein, embedded with alginate–chitosan gel beads in liquid phase may be explained by a shrinking core model. Hsu showed that the resistance for the diffusion of solute molecules was mainly depended on the surface layer [8]. The mass transfer rate could be improved by increasing mass transfer coefficient, driving force and the contacting area [9].

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At present, the research on the diffusion of protein in gels has received some progress. Gutenwik used diffusion cell to measure the effective diffusion coefficients of lysozyme and BSA [10]. Johnson studied the effects of electrostatic interactions on the diffusion and equilibrium partitioning of fluorescein-labeled proteins in charged gels by fluorescence recovery after photobleaching and gel chromatography. They concluded that for diffusion of globular proteins through gel membranes of like charge, electrostatic effects on the effective diffusivity are likely to result primarily from variations in equilibrium partitioning coefficients with only small contributions from the intramembrane diffusivity [11].

Ultrasound is able to produce cavitation, mechanical and heated effects, which may affect the mass transfer and diffusion of molecules in ultrasonic field [12]. Qin found that binary diffusion coefficient in the capillary was enhanced with the increase of ultrasonic power [13]. Luque–Garcia proved that ultrasonic cavitation turbulent effect caused the particles' high speed oscillations and collision, therefore the cleaning effect of microjet and shockwave on the interfacial layer accelerated the mass transfer [14]. At present, the impact of ultrasound on macromolecular diffusion and the relationship between the microstructure of carrier and molecular diffusion were unclear.

The objective of this paper was to study the effects of ultrasound-assisted and pre-ultrasound on the diffusion of casein in the alginate–chitosan gel beads by evaluating the diffusion coefficients and observing the changes of alginate–chitosan gel beads by the fluorescence spectrophotometry and scanning electron microscope techniques, to establish the relationship between the diffusion coefficients and the gel surface area ratios.

2. Materials and methods

2.1. Materials

Sodium alginate (1.05–1.15 Pa s viscosity) was purchased from Tianjin City Guangfu Technology Development Co., Ltd. (Tianjin, China). Chitosan (80.0–95.0 deacetylated degree) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Anhydrous calcium chloride (>96% purity) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Casein ($\geqslant$13.5% total nitrogen) was purchased from Beijing Ao Bo Xing Bio-Tech. Co., Ltd. (Beijing, China). Three hydroxyme thyl aminomethane (Tris, $\geqslant$99.0% purity) was purchased from Sigma Chemical Co., Ltd. (Beijing, China).

2.2. Ultrasonic equipment

An assemble ultrasonic bath system equipment with two sets of JXD-02 multi-frequencies processing system and the low temperature circulating water tank was employed (JXD-02, Beijing Jinxing Ultrasonic Equipment Technology Co., Ltd., China). The intensity of ultrasound energy could be varied at different levels by adjusting the output of frequency (28, 40, 50, 135 kHz) and power (0.05, 0.15, 0.25, 0.36, 0.45 W/cm^2). The length, width and depth of the ultrasonic bath were 0.2, 0.2, and 0.15 m, respectively. This instrument is shown in Fig. 1.

2.3. Casein entrapment in the alginate–chitosan gel beads

Alginate (0.57 g) and chitosan (0.03 g) were brought into good contact by dissolving them in 20 mL of 0.1 M Tris–HCl buffer solution at pH 7.0. The solutions were heated to ensure complete dispersion and dissolution. The heating temperature is 70 °C in 1 h and then cooled to 40 °C. After that, casein was added to the solution at a concentration of 10 mg/mL. Finally, the mixture was dropped into 0.6 M $CaCl_2$ at 4 °C for 30 min to form the beads.

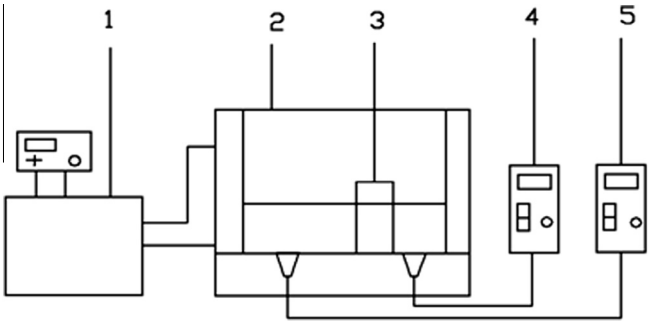

Fig. 1. Plane graph of assemble ultrasonic bath system equipment (1, Thermostatic water; 2, Ultrasonic bath; 3, Sample flask; 4 and 5, Ultrasonic power supply).

2.4. Ultrasonic treatment of casein in the alginate–chitosan gel beads

Casein in the alginate–chitosan gel beads (5 g) was dissolved in 300 mL of 0.1 M Tris–HCl buffer solution at pH 7.0. The solution was then treated by the ultrasonic processor at different frequencies (28, 40, 50, 135 kHz), 0.45 W/cm^2, and at different powers (0.05, 0.15, 0.25, 0.35, 0.45 W/cm^2), 28 kHz, at 50 °C for different times (10, 20, 30, 40, 50, 60 min), respectively. After ultrasonic treatment, casein concentration was determined).

2.5. Ultrasonic pretreatment of casein in the alginate–chitosan gel beads

Alginate–chitosan gel beads which embedded casein were pretreated by ultrasound. The treatment conditions were the same with the above 2.4. Then put these alginate–chitosan gel beads into the new Tris–HCl buffer. Casein concentration was determined every 10 min until it reached 60 min.

2.6. Casein concentration measurement

Casein concentration was measured by fluorescence spectroscopy (RF 5301, Shimadzu, Co., Ltd., Tokyo, Japan). The fluorescence intensity of the sample was determined with an excitation wavelength of 280 nm and an emission wavelength of 341 nm. For a given material, when the excitation wavelength, the emission wavelength and the thickness of liquid layer are fixed, the low concentration of casein can be calculated as the following equation:

$$F = k \cdot C \tag{1}$$

where F is the fluorescence intensity, k is a coefficient and C is the casein concentration.

2.7. The calculation of casein diffusion coefficient

According to Estapé [15], the cumulative diffusion coefficient of casein in the alginate–chitosan gel beads can be expressed by the following equation:

$$\frac{C_{(t)}}{C^{\infty}} = 1 - \sum_{n=1}^{\infty} \frac{6 \cdot \alpha \cdot (1+\alpha)}{9 + 9 \cdot \alpha + \alpha^2} \cdot e^{\frac{D_e \cdot q_n^2 \cdot t}{R^2}} \tag{2}$$

where $C_{(t)}$ is the concentration of release with different diffuse time. C^{∞} means the total casein concentration. D_e is the effective diffusion coefficient.

α is defined as the ratio of buffer volume to the volume of alginate–chitosan gel beads ($\alpha = 45.87$). The number of gel beads is indicated by $n = 100$. R means the radius of each alginate–chitosan gel bead which is 0.25 mm.

$$\alpha = \frac{V}{\frac{4}{3} \cdot \pi \cdot R^3 \cdot n} \tag{3}$$