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Proceeding Paper

Chitosan-SDS Coacervates for Encapsulating Amyloglucosidase: A Study on Structured Capsule Formation and Enzymatic Performance †

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Abstract: This study investigated the use of chitosan-SDS coacervates for encapsulating amyloglucosidase, an essential amylolytic enzyme applied in food and beverage production. Various chitosan solutions (1.25–2% *w*/*w*) with slightly acidic pH (5.04–5.87) were added dropwise into a 50 mM SDS solution under gentle magnetic stirring. The ionic interaction between chitosan and the anionic surfactant yielded self-supporting capsules characterised by a liquid core and a gelled chitosan-SDS membrane. This encapsulation methodology efficiently immobilised amyloglucosidase with a 71% yield. The chitosan-SDS coacervates open new possibilities for enzyme incorporation in the food and beverage industry, enhancing product quality and process efficiency.

Keywords: chitosan; coacervates; enzyme immobilisation; encapsulation; amyloglucosidase; encapsulated enzyme; sodium dodecyl sulfate (SDS)

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1. Introduction

Chitosan, a cationic polymer, is extracted from chitin found in crustacean exoskeletons or fungal cell walls. It is available in various degrees of deacetylation and molecular weights, influencing its viscosity and capability to form gels. With its exceptional biodegradability and biocompatibility, chitosan is widely used in food applications and enzyme immobilisation due to its distinctive properties [1–3]. In an aqueous acidic solution, modifiable amino and hydroxyl groups can be easily altered by chemical treatments. Furthermore, the high porosity, high hydrophilicity, broad adhesion area, and low mass transfer resistance contribute to its value in enzyme immobilisation and have proven as an effective support for various enzyme immobilisation systems [2,4].

While chitosan is advantageous for the immobilisation of proteins and cells, its extensive application and excellent quality were studied and explored mainly as support for the adsorption and covalent attachment of enzymes [5–8]. In addition, the commercial use of chitosan hydrogel beads formed via alkaline precipitation has limitations due to their low mechanical strength and acid stability. Therefore, sodium dodecyl sulfate (SDS) offers a promising method to enhance the chitosan hydrogel beads resilience [9,10]. Chitosan-SDS interaction can form hydrogel capsules and be an attractive alternative for drug delivery, cosmetics, and food applications [11]. Studies have also shown potential for wastewater treatment, particularly in the adsorption of coloured compounds from textile dyeing wastewater [10,12].

Chitosan can form coacervates with anionic surfactants like SDS through a simple coacervation phenomenon [11,13]. A spontaneous liquid/liquid phase separation yields a

dense coacervate phase and a dilute equilibrium phase. The coacervates can form below the critical micelle concentration of SDS and may be stabilised by electrostatic attraction between oppositely charged molecules [13–15]. Prior research has not explored anionic surfactant-based chitosan hydrogel capsules for enzyme immobilisation. Therefore, this study investigated chitosan-SDS coacervates for encapsulating amyloglucosidase, a crucial amylolytic enzyme in food and beverage production, considering morphological characteristics, immobilisation yield and enzyme efficiency to convert corn starch into glucose.

2. Materials and Methods

2.1. Materials

Food-grade chitosan (deacetylation degree 96% and molecular weight of 210 kDa based on supplier data) was acquired from Primex ehf (Siglufjordur, Iceland). Glacial acetic acid, sodium dodecyl sulfate (BioUltra, form molecular biology, purity ≥ 99.0%), sodium hydroxide, 3,5–dinitro salicylic acid, sodium-potassium tartrate heptahydrate, liquid amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger* (260 U/g), corn starch were obtained from Sigma Aldrich.

2.2. Methods

2.2.1. Production of Chitosan-SDS Capsules Containing Amyloglucosidase through Simple Coacervation

Various chitosan concentrations (1.25%, 1.50%, 1.75%, and 2% *w*/*v*) were dissolved in 0.4% (*v*/*v*) acetic acid solution at room temperature with a magnetic stirrer (IKA® C-MAG MS, Ika-Werke, Staufen, Germany). The overnight refrigerated solutions were then extruded dropwise, through a pipette tip (200µL), into a sodium dodecyl sulfate (50 mM) solution under gentle magnetic stirring. The process employed a peristaltic tubing pump with a flow rate of around 50 drops per minute (Fisher Scientific FH10, Fisherbrand™, Waltham, MA, USA). The obtained coacervates remained under stirring for 30 min to ensure stable structure formation. Subsequently, the microspheres were washed with distilled water to remove excess SDS. For amyloglucosidase immobilisation, 240 mg of amyloglucosidase was mixed with 10 mL of 2% chitosan solution, resulting in an enzyme concentration of 24 g/L in the gel-forming solution prior to dropwise extrusion into the SDS solution, as described above.

2.2.2. Morphological Characteristics

The morphological characteristics (average capsule and core diameter, as well as the membrane size) of chitosan-SDS coacervate were evaluated and recorded using a Ceti SI-3 high-definition digital camera (Medline Scientific, Chalgrove, UK) equipped with XliCap® image software from Xl Imaging Ltd. (Southampton, UK). The camera was coupled with a stereo microscope with a zoom capacity of x7 (Leica Zoom 2000, Feasterville, PA, USA). The degree of swelling or shrinkage was also determined as per the Equation (1).

$$Swelling\ or\ shrinkage\ degree\ = \frac{(Capsule\ weight - Drop\ weight)}{Drop\ weight} \times 100 \quad (1)$$

2.2.3. Enzymatic Activity Assay

Amyloglucosidase enzymatic activity assay was conducted by the hydrolysis of gelatinised corn starch solution. 0.5 g of corn starch was added to 100 mL of sodium citrate buffer (50 mM) at pH 4.8. The solution was heated to 80 °C using a water bath (DMS360, Fisher Scientific, Leicestershire, UK) and shaken at 30 rpm for 2 min to aid solubilisation. The glucose production was evaluated for free, immobilised and released enzymes. Either 24 mg enzyme solution or 1.3 g capsules containing amyloglucosidase were introduced to a 100 mL substrate solution to give an overall enzyme concentration of 240 mg/L. The

mixture was maintained at a temperature of 60 °C for 300 min. Aliquots were withdrawn at various time points to monitor the progress of the enzymatic hydrolysis reaction and the resulting glucose production. The determination of reducing sugars was performed using the 3,5-dinitro salicylic acid (DNSA) method proposed by Robyt and Whelan [16].

2.2.4. Immobilisation Yield

Immobilisation yield describes the percentage of total enzymatic activity that has been encapsulated. To calculate it, the enzyme activity that has been efficaciously encapsulated was divided by the enzymatic activity of the free form of enzyme used in the encapsulation process, as Sheldon and van Pelt [36] suggested and described in Equation (2).

$$Immobilisation\ Yield\ (\%) = \frac{Immobilised\ activity}{Initial\ activity} \times 100 \quad (2)$$

Classically, this parameter is evaluated by measuring the residual enzymatic activity not encapsulated in the gel-forming solution as suggested by Sheldon and van Pelt [36], but none was detected in our case. Therefore, the enzymatic activity of the immobilised enzyme was measured by releasing the enzyme mechanically from the capsules by cutting the beads open with a scalpel and contrasting it against the initial activity of the free enzyme before immobilisation.

3. Results and Discussion

Images of chitosan-SDS beads produced by varying chitosan solution concentrations (1.25, 1.50, 1.75, and 2.0%) are shown in Figure 1.

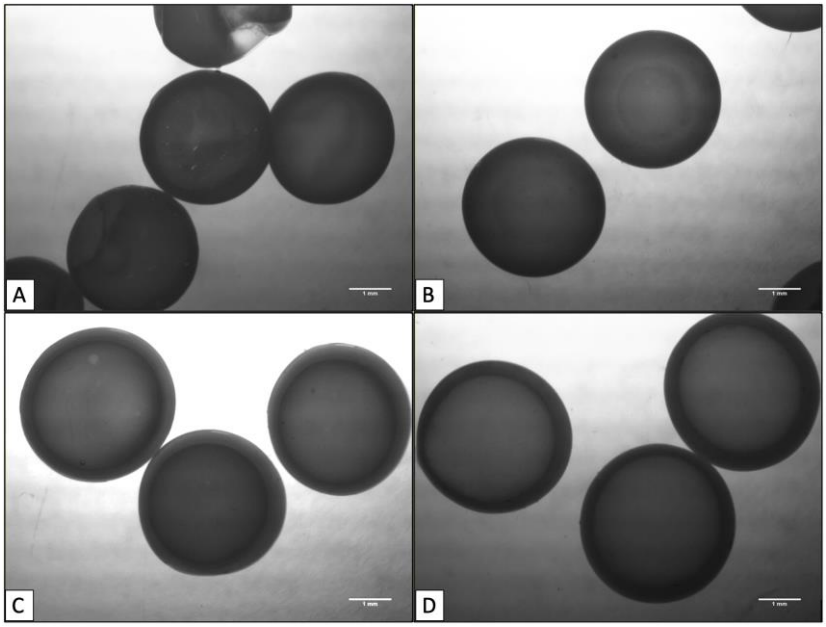

Figure 1. Images of chitosan-SDS coacervate capsules obtained from stereo microscope zoom x7 and obtained from 1.25% (**A**), 1.5% (**B**), 1.75% (**C**) and 2% (**D**) chitosan solutions.

The capsule-like beads exhibit distinctive characteristics depending on the chitosan concentration. As chitosan concentration increased, the outer layer membrane became thicker, and the central core more transparent. In Figure 1C,D, chitosan-SDS coacervates produced with 1.75 and 2.0% chitosan solution were characterised by a well-defined membrane and core with smooth surfaces. While those coacervates produced with 1.25% and 1.50% chitosan solutions (Figure 1A,B) showed a slightly dark membrane and rough surfaces. Higher chitosan concentrations might have allowed a more effective and improved ionic interaction between chitosan and the anionic surfactant (SDS), resulting in spherical

and self-supporting capsules. Upon cutting these capsules with the aid of a scalpel, it was observed a liquid core enclosed by a gel-like membrane.

The average capsule and core diameters, as well as the membrane size and the degree of swelling or shrinkage, were evaluated and shown in Table 1. Higher chitosan concentration resulted in heavier, larger, and more stable capsules. Statistical analysis has shown a significant difference in the membrane size, capsule, and core diameters between treatments between lower chitosan concentrations (1.25 and 1.50%) and higher concentrations (1.75 and 2.00%), which can be correlated with the stability and shape of the capsules shown in Figure 1. Lower concentrations of chitosan (1.25 and 1.50%) resulted in shrunk and weak capsules. Low chitosan concentration might be not enough to form a substantial coacervate membrane, and the electrostatic force be not strong enough to form well-structured gel capsules [17].

The increase in the size, core diameter and membrane size can also be observed in Figure 2. Through the bar graphs, it is possible to observe a slight increase in the core diameter and membrane size. The difference is more evident by increasing the chitosan concentration from 1.5 to 1.75%, as confirmed by the statistical analysis. Despite the significant difference, all treatments presented large particle sizes stemming from the orifice through which the chitosan solution was extruded, a 200 µL pipette tip, resulting in large chitosan pre-gel solution drops (drop weight of 19 mg ± 0.04). The large size of coacervates can be considered an advantage for enzyme immobilisation application, it facilitates enzyme recovery, enzymatic reaction control and termination.

Table 1. Capsule/drop swelling degree, average capsule, core diameters, and membrane size as a function of chitosan concentration.

Chitosan Concentration (%)	Capsule Diameter (mm)	Core Diameter (mm)	Membrane Size (mm)	Capsule/Drop Swelling %
1.25	3.04 ± 0.05 ^{A}	2.30 ± 0.05 ^{A}	0.74 ± 0.10 ^{A}	−32.13 ± 1.2%
1.50	3.13 ± 0.02 ^{A}	2.35 ± 0.04 ^{A}	0.78 ± 0.09 ^{A}	−21.47 ± 0.4%
1.75	3.48 ± 0.04 ^{B}	2.58 ± 0.07 ^{B}	0.89 ± 0.07 ^{B}	+2.51 ± 0.2%
2.00	3.50 ± 0.02 ^{B}	2.59 ± 0.10 ^{B}	0.91 ± 0.10 ^{B}	+9.65 ± 0.5%

Different uppercase letters present significant differences by Tukey posthoc test at the 5% significance level.

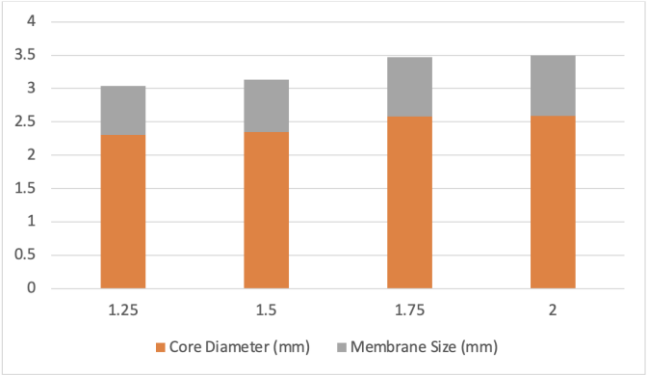

Figure 2. Representation of chitosan-SDS coacervates sizes through the core diameter and membrane size as a function of chitosan concentration. Axis-*x* is the concentration of chitosan solution (1.25, 1.50, 1.75, and 2.00%) while Axis-*y* contains the size measurements in millimetres.

As the 2% chitosan-SDS coacervates presented as the best treatment, it was selected to immobilise amyloglucosidase. The effectiveness of chitosan-SDS coacervates as an immobilisation system of enzymes was assessed by evaluating immobilisation yield and enzyme efficiency. In addition, the glucose production curve of free, released, and encapsulated enzymes was also performed, and it is demonstrated in Figure 3.

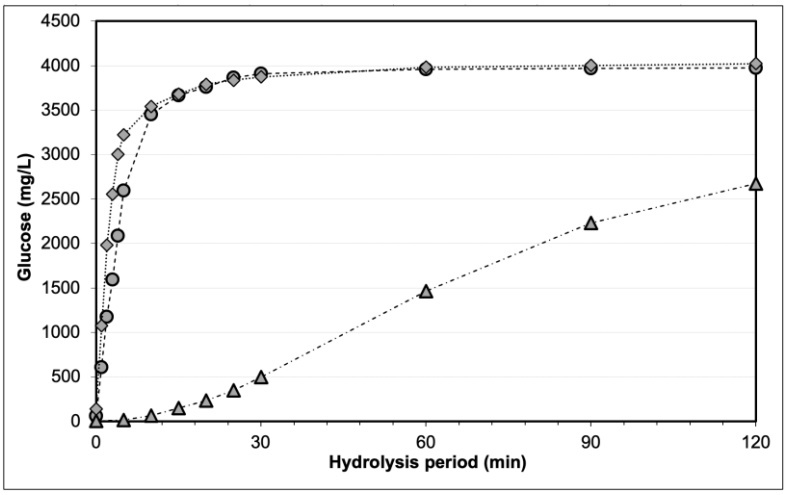

Figure 3. Glucose production during gelatinised corn starch hydrolysis carried out at 60 °C for 120 min with free (◊), released (○), and encapsulated (Δ) amyloglucosidase.

Figure 3 shows the glucose production curve during the hydrolysis of gelatinised corn starch solution (5 g/L) at 60 °C over 120 min of reaction, employing free, released, and encapsulated enzymes. Notably, the glucose production profiles for free and released enzymes exhibited striking similarities. In the initial phase, the glucose production increased linearly with hydrolysis time, followed by a plateau where the glucose production remained constant. At the beginning of the reaction, the free enzyme presented slightly higher activity and a steeper linear slope than the released one, indicating higher initial reaction velocity. However, both free and released enzymes reached identical performance levels, in terms of glucose production, within only 10 min of reaction time. This observation suggests that the encapsulation process retained a substantial portion of the enzyme in a free form inside the capsules (71%), probably within the liquid core, without interaction with the chitosan-SDS membrane. The encapsulated enzyme exhibited a distinct behaviour. A lag phase was evident in the first 10 min of the reaction, where the glucose production was prolonged and only reached a maximum between 30 and 90 min. The encapsulated enzyme displayed a much slower slope, suggesting that the encapsulation process has vastly reduced the enzymatic reaction velocity.

Although the enzymatic reaction velocity had been reduced with the encapsulation process, the corn starch to glucose conversion rate was still high (81 ± 0.43%) with prolonged hydrolysis reaction (300 min) and the immobilisation yield was determined to be (71.3 ± 2.6%). These findings highlight that the encapsulation method preserved enzymatic activity and enabled efficient substrate catalysis. Therefore, the simple coacervation methodology did not induce denaturation or inactivation of the biocatalytic compound. Moreover, this suggests its potential for encapsulating not only enzymes but also various other proteins and compounds.

4. Conclusions

This study introduces a novel enzyme immobilisation system through the simple coacervation of chitosan and SDS. The investigation encompassed various chitosan concentrations, assessing capsule formation structure in terms of morphology and size. Results indicated that higher chitosan concentration led to larger and more stable capsules, with thicker membranes. Coacervates produced with 1.75 and 2.0% chitosan solutions exhibited well-defined and smooth surfaces, being ideal for enzyme immobilisation. The mild and food-grade encapsulation process effectively retained enzyme activity and demonstrated successful starch-to-glucose conversion. The chitosan-SDS coacervates represent an innovative technique for encapsulating diverse enzymes, offering improved stability and broader applicability by enabling precise product release control. The development

opens new possibilities for enzyme incorporation in the food and beverage industry, enhancing product quality and process efficiency.

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