Sustainable material for urea delivery based on chitosan cross-linked by glutaraldehyde saturated toluene: Characterization and determination of the release rate mathematical model

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Keywords

Chitosan, Cumulative release, Diffusion, Emulsion cross-linking, Release kinetics

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RESEARCH PAPER

Sustainable Material for Urea Delivery Based on Chitosan Cross-linked by Glutaraldehyde Saturated Toluene: Characterization and Determination of the Release Rate Mathematical Model

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Abstract

The aims of this study were to characterize the urea-loaded chitosan microspheres and determine the release kinetic constants and diffusion coefficients. An emulsion cross-linking method was used to prepare the urea-loaded chitosan microspheres. Urea was dissolved in a solution of chitosan then put into vegetable oil and stirred to form an emulsion. Glutaraldehyde saturated toluene (GST) was added into the emulsion dropwise while continuously stirring for the solidification process. Chitosan microspheres filled with urea were washed, dried, and then analyzed. Characterization of the urea-loaded chitosan microspheres was conducted using a scanning electron microscope (SEM), Raman spectroscopy, X-ray diffraction, and particle size distribution. The cumulative release analysis was used to determine the amount of urea released from the chitosan microspheres and determine the release kinetic constants and diffusion coefficients. The chitosan microspheres had a good spherical geometry with a smooth surface and crystallinity of 95.5−98.18%. They had a diameter in the range of 125.31−153.65 μm and a cumulative release value in the range of 38.22−48.06%. Based on the kinetic analysis, the best kinetic models were models of Korsmeyer-Peppas, Peppas-Sahlin, and simple power law with the burst effect resulting in the highest R^2 of 0.99. The diffusion coefficient obtained was in the range of 5.439×10^{-11} - 7.512×10^{-11} cm²/s.

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1. Introduction

Agriculture is one of the most critical sectors for Indonesia and is one of the pillars of the country's life. The agricultural sector provides primary needs and improves the Indonesian economy. The continuous population growth gives a great challenge for the agricultural sector to produce efficient and sustainable products. The main contributors to increase in agricultural productivity are fertilizers [1,2,3].

One of the largest fertilizers used in Indonesia is urea because it is not only cheap but also has a high nitrogen content (about 46%) and is easy to handle [4,5,6]. However, it should be noted that urea is easily soluble in water, and only 30−35% of its nutrients are absorbed by the plants. The unabsorbed nutrients (nitrogen) will be dispersed by rainfall, irrigation, and water flow causing excess nitrogen in the environment [7,8], which contribute to environmental pollution through nitrate contamination of soil and surface water. Furthermore, it leads to

various issues, including eutrophication of ecosystems, degradation of downstream water quality, development of photochemical smog, increase in global concentrations of greenhouse gases, and disruption of food chains in ecosystems [4,9].

One solution to overcome the low efficiency of nutrient absorption from conventional urea and the high accumulation of unabsorbed urea contents in the environment is the controlled release urea fertilizer. This type of fertilizer can control nutrient loss, increase the efficiency of nutrient use, and reduce fertilizer procurement costs as well as the risk of environmental pollution [1,10,11]. Various methods have been developed to produce the controlled release fertilizers, one of them is the coated or trapped fertilizers with natural polymers (biopolymers). Natural materials, which have been developed using various methods, which can be used as the trapping or wrapping materials for the fertilizers are chitosan, alginate, starch, cellulose, lignin, biochar, and polydopamine [12]. Chitosan is one of the most natural polymers widely used as a microcapsule wall material because of its biodegradable and biocompatible properties.

Controlled release urea fertilizer is very useful for agriculture. One of the advantages of this technology is that it is more economical and environmentally friendly compared to the conventional fertilizers, but its use is still limited. The potential for the controlled release urea fertilizer is enormous and can be realized only by solving the problems of its development, production, and application [13]. The production of the controlled release urea fertilizer needs to be continuously developed to get the right method with a low production cost. The emulsion crosslinking method can be considered for the manufacture of the controlled release urea fertilizer because it is simple and very useful for liquid or solid materials.

Several researchers have investigated the chitosan as a carrier for controlling the fertilizer release [4,14,15,16]. Modification of the chitosan was carried out to increase the effectiveness of nutrient absorption by controlling fertilizer release, increase the value of eco-activity, and reduce the production costs. Modification of chitosan by emulsification and cross-linking methods using genipin as a cross-linking agent and then application of that in forming the urea-loaded chitosan microspheres (matrix) were reported by a previous study [4]. In that study, the cumulative release value was in the range of 70–90% for seven days. Genipin is a natural and non-toxic cross-linking agent, but the price of the material is very high [17]. The controlled release potassium nitrate fertilizer was prepared using the chitosan-starch cross-linked with sodium tripolyphosphate (TPP) [18]. The results showed that the cumulative release reached 95% for 14 days using 100% chitosan. When the chitosan/starch ratios were 30/70 and 20/80, the cumulative release reached 73% and 80%, respectively. Nevertheless, the use of TPP as a cross-linking agent is disadvantageous due to the possible lack of mechanical stability and the risk of system dissolution, due to swelling which was very sensitive to pH [19,20].

Modification of chitosan by combining it with inorganic materials aims to increase the physical and retained nutritional capacity, for examples, chitosan has been combined with montmorillonite to form microspheres [21] and combined with clay and paraffin wax for hydro-soluble diammonium phosphate fertilizer [22]. The fertilizer resulted in a cumulative release of less than 40% for 30 days. Meanwhile, urea coating with sepiolite-chitosan nanocomposite could achieve a cumulative release of 65% for the same period [22].

The current study aimed to continue our previous study. In the previous study, the urea-loaded chitosan microspheres were prepared at different ratios of the dispersed and continuous phases and at different stirring speeds. Chitosan microspheres had diameters from 153.66 ± 26.35 to 179.39 ± 34.95 μm. Although not uniform, the size of the chitosan microspheres showed a good spherical geometry. The urea release mechanism from chitosan microspheres was anomaly behavior (non-Fickian kinetics) with a cumulative release of 32.38–37.69% [23]. The diffusion coefficient calculated using the reservoir systems with a non-constant activity source equation was from 1.180×10^{-14} to 1.433×10^{-14} cm^2/s [24].

The emulsion crosslinking method is a simple and versatile method. One of the influential parameters in the synthesis of chitosan microspheres is the concentration of chitosan. The rigidity/density of the microcapsule wall directly affects the amount of urea released. The release rate is very important to study because the release rate of the controlled release fertilizers is designed in a synchronized pattern to meet the changing nutritional needs of plants. Mathematical models such as release kinetic models are very useful for studying the release systems more deeply. The mathematical models are expected to be able to predict the cumulative release profiles as a function of time accurately and result in some important physical parameter values such as diffusion coefficient, thereby providing a better understanding of the topic being studied. The chitosan microspheres characterization analysis will strengthen the mathematical modeling data. The

combination of the experimental and calculated data is very important for the optimization of the formula process before it was applied on the commercial scale in the future.

The preparation of chitosan microspheres as a urea carrier with the difference in the concentration of chitosan and the initial weight of urea loaded in chitosan microspheres needs to be carried out. Therefore, the goal of this study was to study the characteristics of the chitosan microsphere structural properties such as the particle size distribution, morphology, structure of molecules, and crystalline and amorphous structures. Furthermore, mathematical modeling was conducted to simulate the urea release profile from the chitosan microspheres by determining the release kinetic constant and diffusion coefficient values.

2. Material and methods

2.1. Materials

Preparation of the chitosan microspheres filled with urea used the following materials: urea (produced by PT Pupuk Sriwidjaja), glutaraldehyde (25% aqueous solution, from Merck), acetic acid (glacial) (purity of 100%, from Merck), toluene (technical grade, purity of 95%, from CV. Tri Jaya Dinamika), chitosan (with a degree of deacetylation of 87.2% and a viscosity of 37.10 cps, from PT. Biotech Surindo), vegetable oil (from PT Sarwana Nusantara), chemicals such as petroleum ether and n-hexane (technical grade, from CV. Labora).

2.2. Chitosan microspheres preparation using the emulsion cross-linking technique

Our previous studies reported that the urea-loaded chitosan microsphere was produced using the emulsion cross-linking technique [23]. The chitosan as much as 4% w/v was dissolved in a 2% (v/v) acetic acid solution to form a chitosan solution. After that, urea fertilizer as much as 2.5 g was put into 50 mL of chitosan solution and then stirred until dissolved completely. Then, the solution was put into 400 mL of palm oil. The mixture was stirred using a homogenizer at 10,000 rpm for 1 h to form an emulsion. Furthermore, the glutaraldehyde saturated toluene (GST) solution as a cross-linker was added into the emulsion dropwise. After the GST addition was complete, the mixture continued to be stirred for 15 min, followed by the addition of 2 mL of 25% aqueous solution of glutaraldehyde before being stirred again for 2 h. The chitosan microspheres were separated (using a centrifuge),

filtered, washed (using petroleum ether and hexane), and dried at 65 °C. The urea-loaded chitosan microspheres were analyzed to determine yield, water absorbency, and characterization of urea fertilizer microcapsules.

2.3. Characterization of the chitosan microspheres containing urea fertilizer

2.3.1. Morphological analysis using scanning electron microscopy (SEM)

Chitosan microspheres filled with urea were analyzed for their morphology using a SEM (model of JSM-6510 L A, from JEOL Ltd., Japan). In the process, chitosan microspheres were coated with platinum. Secondary electron resolution was 3.0 nm (accelerating voltage of 30 kV, high vacuum mode) and backscattered electron resolution was 4.0 nm (accelerating voltage of 30 kV, low vacuum mode). The voltage acceleration ranged from 0.5 to 30 kV.

2.3.2. Analysis of Raman spectroscopy

Analysis of the interaction between functional groups of chitosan and glutaraldehyde cross-linked chitosan was observed using a LabRAM HR Evolution Raman spectroscopy (Horiba Scientific, Japan) with a laser wavelength of 785 nm and the objective lens of $100\times$.

2.3.3. X-ray diffraction analysis

The crystal of chitosan microspheres was analyzed using powder X-ray diffraction (XRD, Shimadzu 7000 Maxima-X). Samples were analyzed using Cu-Kalpha radiation, and the scan rate was $2°/min$ at 2θ from $2°$ to $90°$ with a step size of $0.02°$.

2.3.4. Particle size distribution analysis

Analysis of particle distribution was carried out by measuring the diameter of chitosan microspheres using a digital microscope. Calibration of the diameter determination was done by determining the correction factor, namely comparing the actual size using a calliper with the size of a digital microscope. The size of the digital photo microspheres was multiplied by a correction factor to obtain the accurate diameter. The diameter of the microspheres was determined using fitted Gaussian functions.

2.4. Mathematical models for urea release

2.4.1. Determination of the cumulative release

The cumulative release was determined from the amount of urea released from the chitosan microspheres with the following steps. Firstly, at

atmospheric temperature, 0.2 g of urea-loaded chitosan microsphere was immersed in 50 mL of water for 1, 3, 7, 14, 21, and 30 days. Then, the urea concentration in the medium release (water) was analyzed using a Nessler's reagent spectrophotometry method.

2.4.2. Release kinetics model

The release kinetics of chitosan microspheres was determined using equations (1)–(5) [24,25].

1. Higuchi model

$$\frac{M_t}{M_\infty} = k_H t^{1/2} \tag{1}$$

2. Korsmeyer-Peppas model

$$\frac{M_t}{M_\infty} = k_{K-P} t^n \tag{2}$$

3. Peppas-Sahlin model

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \tag{3}$$

4. Modified hyperbola formula

$$\frac{M_t}{M_\infty} = \frac{at}{1+bt} \tag{4}$$

5. Modification of power law with the burst effect

$$\frac{M_t}{M_\infty} = kt^n + b \tag{5}$$

where $\frac{M_t}{M_\infty}$ is the cumulative release of urea, k_H, k_{K-P}, (k_1 and k_2), a, and b are release kinetic constants, n is release mechanism, m is the diffusional exponent, and t is release time.

2.4.3. The determination of the diffusion coefficient

The diffusion coefficient was determined using the reservoir systems with non-constant activity sources, monolithic solutions [26], and equations of diffusion in the reservoir system [27].

1. Equations for reservoir systems with non-constant activity sources

$$\frac{M_t}{M_\infty} = 1 - \exp\left(-\frac{3R_0 DKt}{R_i^2 R_0 - R_i^3}\right) \tag{6}$$

2. Equations for monolithic solutions

$$\frac{M_t}{M_\infty} = 6\left(\frac{Dt}{\pi R^2}\right)^{1/2} - \frac{3Dt}{R^2} \tag{7}$$

3. Equations of diffusion in the reservoir system [27]

$$C_{Aw} = \frac{C_{A0}}{\left(1 + \frac{V_m}{V_w}\frac{H_l}{H_d}\right)}\left\{\frac{V_m}{V_w}\frac{H_l}{H_d} + \exp\left[-A_m\frac{D}{\delta}\left(\frac{H_d}{V_m} + \frac{H_l}{V_w}\right)t\right]\right\} \tag{8}$$

The wall thickness was determined using equation (9)

$$\delta = \frac{d\left[1 - \sqrt[3]{\frac{\rho_{urea}}{\rho_{chit} + \rho_{urea}\left(\frac{1}{x}-1\right)}}\right]}{2} \tag{9}$$

The nitrogen (urea) concentration obtained in the release medium was used to determine the cumulative release through equations (10) and (11) [28].

$$\text{Amount of nitrogen release} = \frac{concentration \times dissolution\ bath\ volume \times dilution\ factor}{1000} \tag{10}$$

$$Cumulative\ release\ (\%) = \frac{Volume\ of\ sample\ withdrawn\ (mL)}{Bath\ volume} \times P(1-t) + Pt \tag{11}$$

3. Results and discussion

The urea-loaded chitosan microspheres were prepared using the emulsion cross-linking method. The basic principle of the method was a cross-linking reaction between the aldehyde group of glutaraldehyde saturated toluene (GST) and the amine of chitosan. The process began with forming emulsion droplets between a chitosan solution containing urea in the vegetable oil (water in oil), followed by slowly dripped GST to cross-link. As a result, it would gradually harden to form chitosan microspheres.

3.1. Characterization of urea-loaded chitosan microspheres

A digital microscope was used to determine the particle size of chitosan microspheres as a control of

the urea release. The particle size distribution of the chitosan microspheres at various chitosan solution concentrations is shown in Fig. 1. The particle size histogram of chitosan microspheres was equipped with Gaussian curve fitting.

The Fig. 1 shows that the diameter of chitosan microspheres prepared with 2% (w/v) chitosan solution was 70−220 μm with an average diameter of 138.86 μm. Meanwhile, the chitosan microspheres prepared with 3% and 4% (w/v) chitosan solution had a diameter in the range of 100−220 μm (with an average diameter of 148.35 μm) and that in the range of 100−230 μm (with an average diameter of 153.65 μm), respectively. Furthermore, the particle size of the chitosan microspheres was calculated using the Gaussian normal distribution function equation [29]. The results demonstrated that the average size of chitosan microspheres prepared with 2% (w/v) chitosan solution was 125.31 μm, and those prepared with 3% and 4% (w/v) chitosan solution had an average size of 141.53 μm and 145.03 μm, respectively.

The preparation of the chitosan microspheres as a carrier for the release of urea was conducted using the emulsion cross-linking method. An essential step of this method was the formation of the emulsion droplets. The urea was coated with the chitosan solution on the droplet. An increase in the chitosan concentration enhanced the thickness of the emulsion droplet layer and increased the droplet diameter. The high viscosity of the chitosan solution contributed to a coarser emulsion with large droplets. After the addition of a cross-linker, chitosan microspheres with a large diameter were formed. Similar results have been reported by previous studies [30,31]. The increase in the diameter of the chitosan microspheres was caused by agglomeration or aggregation due to interactions between unstable droplets, causing the diameter of the microspheres to become larger [32,33] The increase in the chitosan concentration produced a large size of the microspheres because the intermolecular interactions between chitosan molecules became stronger with the shorter distance. This condition caused the chitosan molecules to become entangled and when cross-linked with glutaraldehyde would form single large particles [34,35].

3.1.1. Cross-linking reaction obtained from FT-Raman spectroscopy

Raman spectroscopy provides information about the structure of molecules containing ethylene bonds. Analysis of the cross-linking reaction
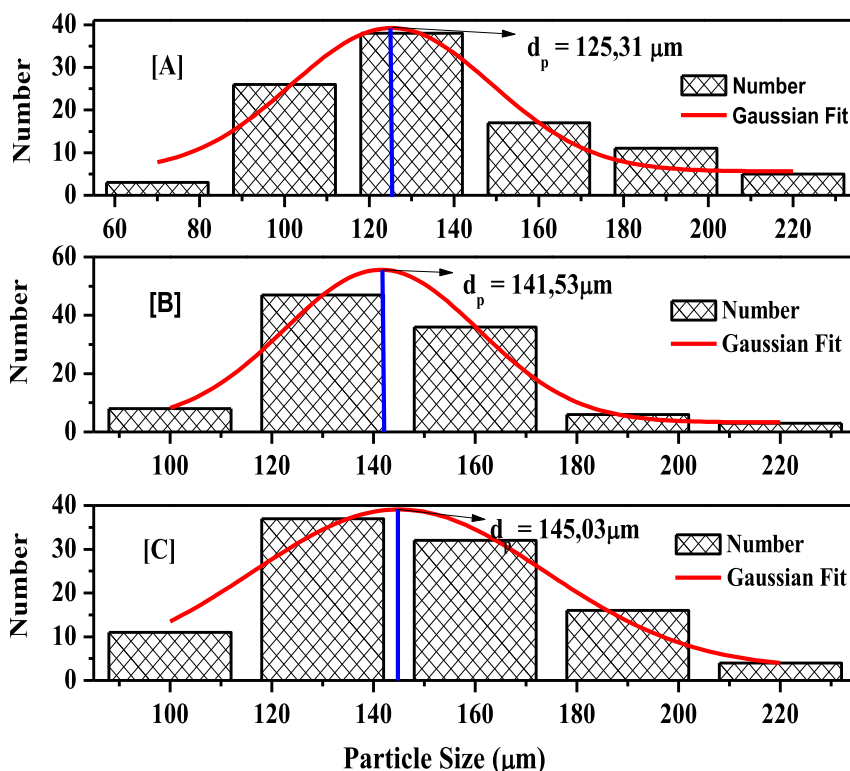

Fig. 1. The particle size distribution of chitosan microspheres was affected by differences in the concentration of the chitosan solution. The particle size distribution was determined by a digital microscope for the concentration of chitosan solution: [A] 2% (w/v), [B] 3% (w/v), and [C] 4% (w/v). All samples together with fitted Gaussian functions.

between chitosan and glutaraldehyde in the chitosan microsphere is shown in Fig. 2.

The Fig. 2A shows Raman spectra for chitosan with the following results. The peak for the C–H stretching vibration appeared at 2886 cm^{-1}. The peak at 1667 cm^{-1} correspondedto the C=O (amide I) stretching vibration. The peak for the N–H (amide II) stretching vibration appeared at 1592 cm^{-1}. The cross-linking reaction between the chitosan and the glutaraldehyde is shown in Fig. 2B. Glutaraldehyde (aldehyde group) reacted to chitosan (amine group) to form a stable imine bond called Schiff base. Aldol condensation/polymerization reaction forming an oligomeric product with a group of aldehydes, which the chitosan amine group catalyzed, reacted to other adjacent amine groups. The conjugate system with adjacent ethylenic (C=C) double bonds was formed by cross-linking aldol condensed glutaraldehyde oligomers with multiple branched imine bonds [36]. Analysis of Raman spectra for chitosan cross-linked with glutaraldehyde in Fig. 2B showed four double bonds stretching: (1) the peak at 1712 cm^{-1} was assigned to the carbonyl stretch (C = O), (2) the C=N stretch occurred at the peak at 1653 cm^{-1} but in unconjugated form, (3) the peak at 1633 cm^{-1} was assigned to the Schiff base (C=N stretch), forming the conjugate system with an adjacent ethylenic double bond, and (4) the peak at 1591 cm^{-1} was assigned to the C=C stretch. This peak confirmed the formation of aldol-condensed oligomers [36].

3.1.2. Characterization of crystallinity using X-ray diffraction (XRD)

XRD analysis is intended to identify crystalline and amorphous structures in a material. XRD diffractograms of pure chitosan and chitosan microspheres containing urea are shown in Figs. 3 and 4.

In this study, the diffraction for urea-loaded chitosan microsphere prepared with a chitosan concentration of 4% (w/v) had crystalline peaks at 22.63°, 25.01°, 29.65°, 32.00°, 35.85°, 37.52°, 41.17°, 41.95°, 49.75°, and 55.19°. Whereas, the crystalline
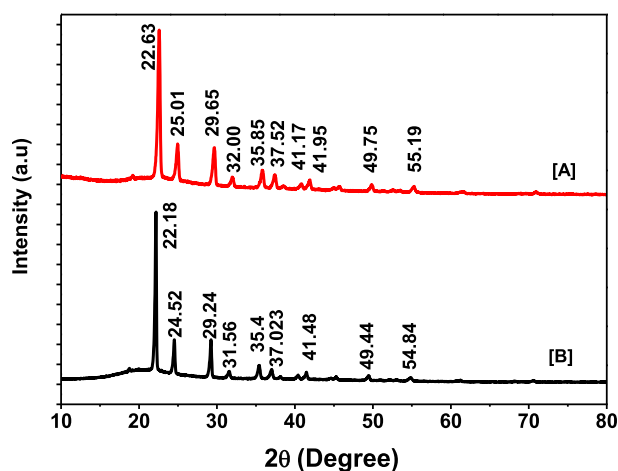

Fig. 3. XRD analysis diffractogram graph for urea loaded chitosan microsphere prepared with chitosan solution concentration of [A] 4% (w/v) and [B] 2% (w/v).
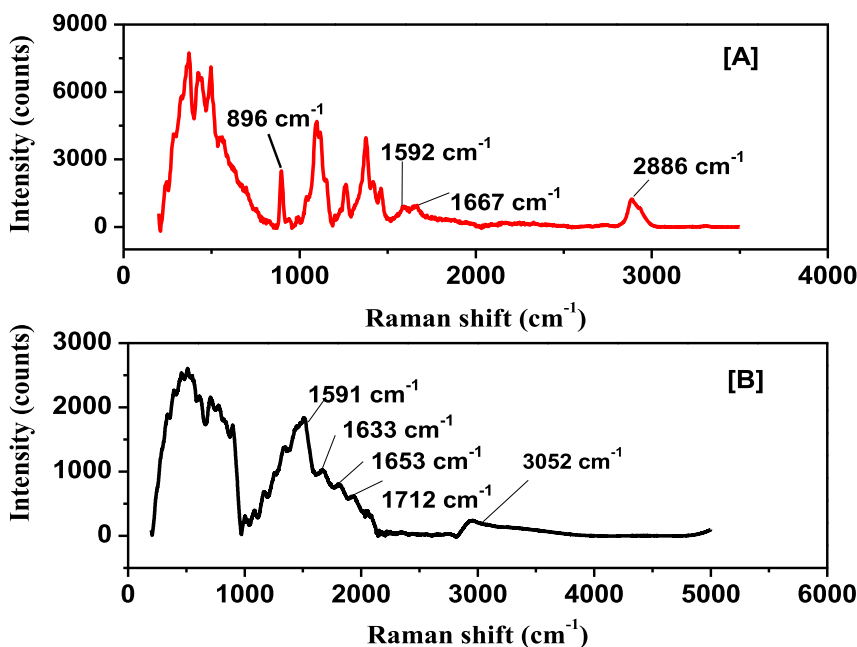

Fig. 2. FT-Raman spectra of (A) chitosan and (B) chitosan microspheres. Chitosan was cross-linked with glutaraldehyde saturated toluene.
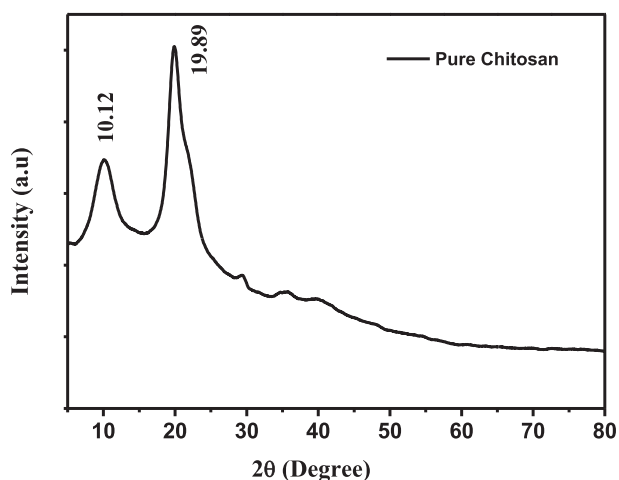
Fig. 4. XRD analysis diffractogram graph for pure chitosan.

peak for urea-loaded chitosan microspheres prepared with 2% (w/v) chitosan concentration was at 22.18°, 24.52°, 29.24°, 31.56°, 35.4°, 37.02°, 41.48°, 49.44°, and 54.84°, as well as a relatively higher intensity. This abundance of energy at the 2θ position can be observed when the x-rays pass through the crystal [38].

The Fig. 4 shows that the pure chitosan used in this study had two crystalline diffraction peaks at the 2θ which were 10.12° and 19.89°. A previous study [37] reported relatively similar results which were 9.89° and 19.93°.

From Fig. 3, using a peak and base analyzer, the crystalline percentage of urea-loaded chitosan microspheres prepared with 4% (w/v) and 2% (w/v) chitosan concentrations was 98.18% and 95.5%. The crystallinity of chitosan microspheres affected the release rate of urea because the monomer composition regulated the crystallinity and affected the flexibility, swelling, solubility, and degradation rates. High crystallinity levels caused the release rate to be lower or reduced [39].

3.1.3. Scanning electron microscope (SEM)

The shape and surface morphology of chitosan microspheres containing urea were analyzed using a scanning electron microscope (SEM) and the results are shown in Fig. 5.

The Fig. 5 shows that the chitosan microspheres had a good spherical geometry with a smooth surface. The chitosan microspheres without urea had a smoother surface than the chitosan microspheres filled with urea. The geometric shape of chitosan microspheres was perfectly spherical due to the uniform and slow cross-linking of the droplet surface [30]. The chitosan microsphere morphology in
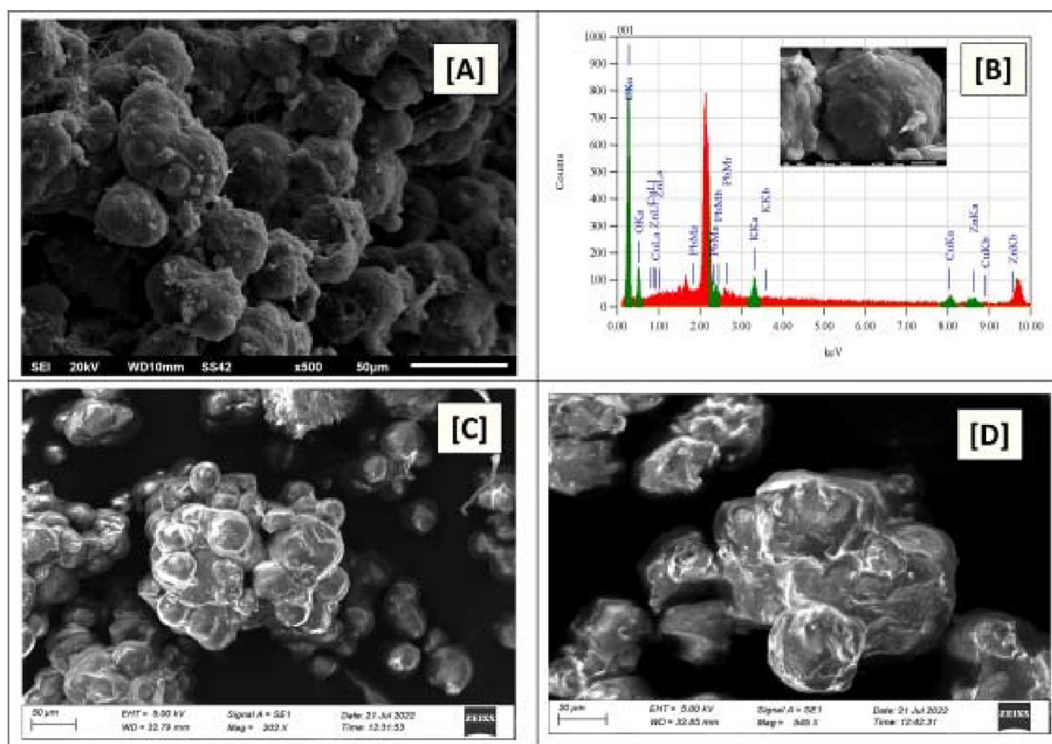

Fig. 5. SEM image for chitosan microspheres filled with and without urea. [A] urea-loaded chitosan microspheres with a magnification of 500×, [B] SEM-EDX analysis for urea-loaded chitosan microspheres, [C] chitosan microsphere without urea with 200× magnification, and [D] chitosan microsphere without urea with 500× magnification.

this study was similar to that in the previous studies in which the chitosan microspheres also produced a good spherical geometry. Moreover, the SEM-EDX analysis showed that the largest components of the chitosan microspheres were oxygen (20.93%) and carbon (75.39%) [30] because the materials used were chitosan and glutaraldehyde, which mainly contained carbon and oxygen. Meanwhile, other components with low composition were impurities that come from raw materials [23].

3.2. Cumulative release analysis

The Figs. 6 and 7 show the cumulative release based on the changes in the ratio of urea weight to the chitosan solution volume and the changes in the chitosan solution concentration. The changes in the
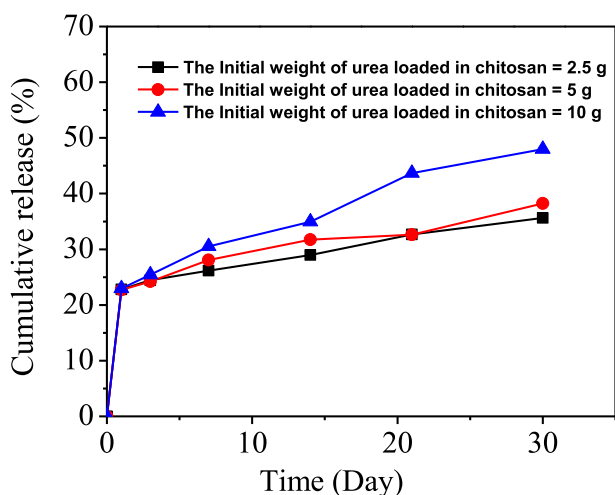

Fig. 6. Cumulative nitrogen release based on changes in the ratio of the initial urea weight to the chitosan solution volume.
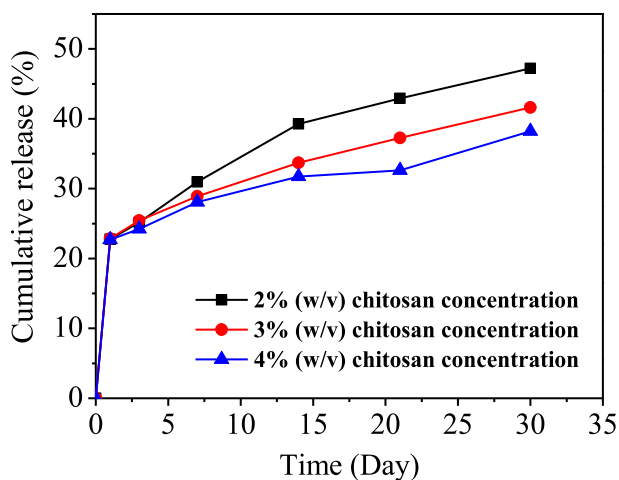

Fig. 7. Fig. 6. Effect of the difference in chitosan solution concentration on the cumulative nitrogen release.

chitosan concentration and the amount of urea filled in the chitosan microspheres affected the amount of urea released from the microspheres because it was related to the density of the microcapsule wall material.

The urea release rate value was determined by analyzing the nitrogen released from the chitosan microspheres. The nitrogen release was monitored for up to 30 days. The Fig. 6 shows the profiles of cumulative nitrogen release from the 1st to the 30th day of immersion time at the difference in the initial urea weight loaded in chitosan microspheres. Analysis of nitrogen release from the chitosan microspheres for 24 h of immersion resulted in almost the same cumulative release. The chitosan microspheres with an initial urea weight of 2.5, 5, and 10 g produced a cumulative release of 22.78, 22.69, and 22.93% respectively. The low nitrogen release was caused by the diffusion barrier of the hydrophobic chitosan layer. After immersion for 3−14 days, there were no significant differences in nitrogen release from the chitosan microspheres. The chitosan microspheres with initial urea weight of 2.5, 5, and 10 g produced cumulative nitrogen release of 28.9, 31.74, and 34.95% respectively. However, after 30 days, there was a significant difference in cumulative nitrogen release in which the chitosan microspheres with an initial urea weight of 10 g urea resulted in 48.06%, while those with an initial weight of 2.5 and 5 g resulted in only 35.68 and 38.3% respectively.

The increase in nitrogen release likely resulted from the amount of urea in the chitosan microspheres. The significant difference in the amount of nitrogen released from the chitosan microspheres filled with 10 g of urea was probably due to a large amount of urea not being adsorbed and attached to the chitosan microspheres. When the amount of urea filled into the chitosan microspheres was lower, the walls of the chitosan microspheres became thicker. The thickening of the matrix wall slowed the urea dissolution rate due to a longer diffusion pattern [40].

The Fig. 7 shows the behavior of nitrogen released from the chitosan microspheres with the difference in the chitosan solution concentration. It can be seen that there was a difference in the percentage of cumulative release from the beginning to the end of the immersion time. From the 1st to the 3rd day, no significant difference was observed because the percentage of cumulative release was relatively the same. From the 7th to the 30th day, however, the percentage of cumulative release was significantly different. The chitosan microspheres prepared with 2% (w/v) chitosan concentration produced the highest cumulative release of 47.21%, while those

prepared with 4% (w/v) chitosan concentration produced the lowest cumulative release of 38.22%. The chitosan solution was a microcapsule wall material for encapsulating urea. This coating process occurred in the emulsion process where urea was coated with a chitosan solution, followed by the addition of GST as a cross-linker for the solidification process.

The chitosan concentration affected the size of the emulsion droplet diameter, which directly influenced the diameter of the chitosan microspheres. An increase in the chitosan solution concentration increased the solution viscosity and caused the microsphere wall thicker, thereby lowering the cumulative nitrogen release. Furthermore, an increase in the chitosan solution concentration resulted in a more rigid microsphere wall, which was responsible for lowering the rate of urea diffusion from the microsphere core to the outside.

3.3. Release kinetics

The cumulative urea release from the chitosan microspheres was modeled using the Higuchi, Korsmeyer-Peppas, Peppas-Sahlin, Modified hyperbola formula, and Modification of power law with the burst effect models in which their equations were shown in equations (1)–(5). The release kinetic analysis was conducted using the experimental data at the variation of the chitosan solution concentration because this factor affected the pattern and amount of urea released from the chitosan microspheres. The Table 1 and Fig. 7 show the results of the release kinetic constants and the comparison between experimental data and calculated data using the release kinetic models.

The Table 1 shows the kinetic constant values from each release kinetic model. The Higuchi model described the diffusivity constant which was applicable to release without significant swelling when the microspheres were in contact with water. Peppas-Sahlin model and Simple power law with the burst effect had kinetic constants of m and n respectively to explain the release mechanism. The Table 1 shows that the n value in Korsmeyer-Peppas model was 0.157–0.242, while the m value in Peppas-Sahlin model was 0.118–0.142. The two release kinetic models produced a high R^2 value which was 0.99. The constant values of the two models indicated that the release mechanism occurred by diffusion without swelling. Fickian diffusion was characterized by a high rate of solvent diffusion into the interior of the matrix and a low rate of polymer relaxation. The Table 1 shows that all the release kinetic models had high R^2 values which were 0.89–0.99,

and the highest R^2 value (0.99) was obtained from the Korsmeyer-Peppas, Peppas-Sahlin, and Simple power law with the burst effect models. Based on the n value of the Korsmeyer-Peppas model and the m value of the Peppas-Sahlin model, the urea release mechanism from the chitosan microspheres can be categorized as a Fickian diffusion mechanism due to the value of n < 0.43.

Application of the simple power law with the burst effect model for chitosan microspheres prepared with 2% and 4% (w/v) chitosan concentrations resulted in the value of n < 0.5, in which the mechanism of urea release was categorized as the Fickian diffusion mechanism, while that for chitosan microspheres prepared with 3% (w/v) chitosan concentration resulted in the value of n in the range of 0.5 < n < 1, which suggested the case II transport, which was purely controlled relaxation [41]. The two release kinetics models (the Korsmeyer-Peppas and Peppas-Sahlin) showed that the urea release occurred through diffusion.

3.4. Determination of the diffusion coefficient

The diffusion coefficient was calculated using the three different models which were shown in equations (6)–(8). The equation (6) (for reservoir systems with non-constant activity sources) was used to determine the diffusion coefficient under perfect sink conditions in the surrounding bulk fluid based on the Fick's law. Urea concentration at the inner membrane surface decreased with time. The equation (7) was used to determine the diffusion coefficient under a monolithic system because urea was molecularly dispersed in the matrix forming. After the penetration of water into the system, urea dissolved completely in a rapid manner. The equation (7) was based on Fick's second law of diffusion for spherical geometry [26].

Similarly, equation (8) was an equation based on the Fick's law. The system assumed that the urea in the core was covered by a chitosan membrane with a certain thickness. Another assumption was that the wall thickness was much lower than the diameter of the microspheres; therefore, the mass transfer of two solid–liquid phases was approximated by a mathematical model through a slab. The comparison of the three equations was used to determine which system was appropriate for this study based on the highest R^2 value which showed a good fitting.

The diffusion coefficient was calculated using the three mathematical models, which resulted in different values (Table 2 and Fig. 8). Model of Reservoir systems with non-constant activity

Table 1. Kinetic constant values of various release kinetic models.

Chitosan concentration (% w/v)	Higuchi		Korsmeyer-Peppas			Peppas-Sahlin			
	k_H	R^2	K_{K-P}	n	R^2	k_1	k_2	m	R^2
2	9.875	0.95	20.449	0.242	0.99	7.905	12.777	0.143	0.99
3	8.791	0.92	21.091	0.188	0.99	15.267	5.890	0.142	0.99
4	8.113	0.89	21.168	0.157	0.99	15.121	6.086	0.118	0.99

Chitosan concentration (% w/v)	Modified hyperbola formula			Simple power law with the burst effect			
	a	b	R^2	k	b	n	R^2
2	25.877	0.570	0.97	5.909	16.131	0.494	0.99
3	38.349	1.012	0.97	2.625	20.244	0.616	0.99
4	47.181	1.381	0.97	19.867	1.314	0.165	0.99

Table 2. Diffusion coefficient calculated by some mathematical models.

No	Model	Diffusion coefficient (cm^2/sec)		
		2% (w/v) chitosan	3% (w/v) chitosan	4% (w/v) chitosan
1	Reservoir systems with non-constant activity sources	1.169×10^{-14}	1.228×10^{-14}	1.144×10^{-14}
2	Monolithic solutions	9.275×10^{-14}	9.376×10^{-14}	7.352×10^{-14}
3	Diffusion in the reservoir system	7.512×10^{-11}	5.718×10^{-11}	5.439×10^{-11}

sources produced smaller diffusion coefficient values than models of Monolithic solutions and Diffusion in the reservoir system. This was because, in the model of reservoir systems with non-constant activity sources, urea was located in the core of the microspheres and then diffused out through the surrounding membrane with a spherical shape.

The model of monolithic solutions showed that urea was molecularly dispersed in the matrix, so the urea was easily dissolved after the release medium diffused into the microspheres. Meanwhile, the model of diffusion in the reservoir system produced the highest diffusion coefficient values of all models because the model was approached with a slab
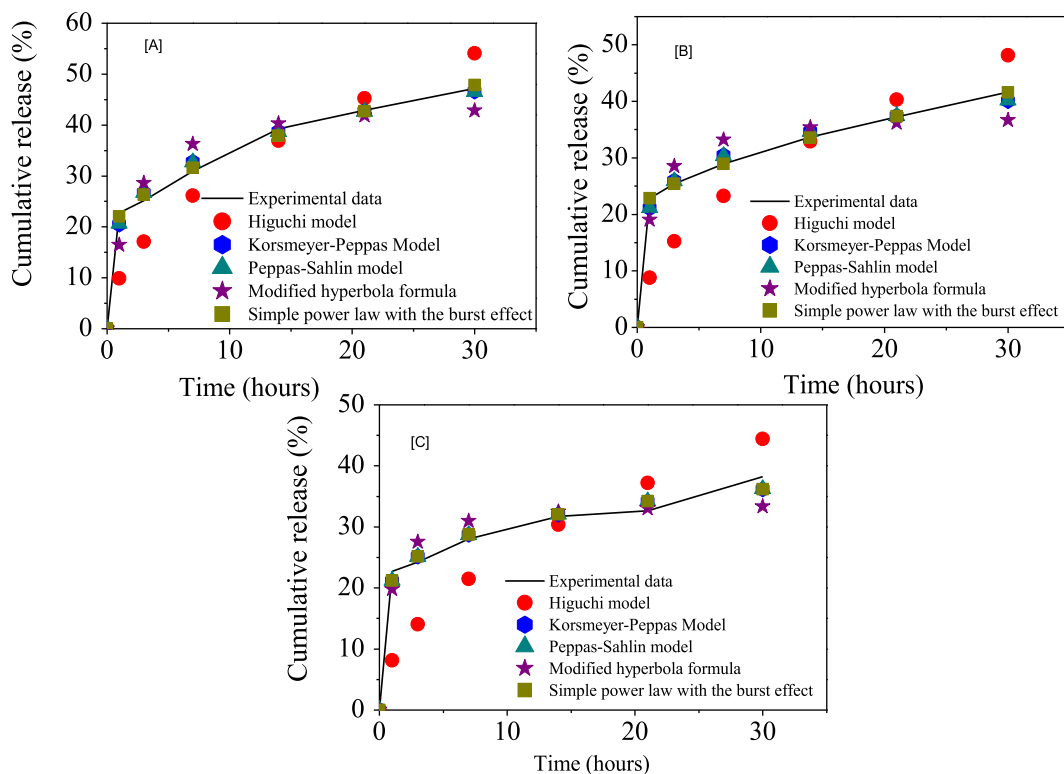

Fig. 8. Calculation results of cumulative release using various release kinetic models and compared with the experimental data at chitosan concentration of: [A] 2% (w/v), [B] 3% (w/v), and [C] 4% (w/v).

system. Diffusion across the membrane was affected by the membrane permeability and related to the thickness of the microcapsule wall.

The Table 2 shows that the diffusion coefficient values of chitosan microspheres prepared with 4% (w/v) chitosan concentration was lower than those with 3% (w/v) and 2% (w/v) chitosan concentrations. This was due to the higher wall thickness and more rigidity, resulting in lower urea release. The Table 2 shows that an increase in the chitosan concentration decreased the diffusion coefficient value. The lower the diffusion coefficient value, the stiffer the microsphere walls would be. The diffusion coefficient affected the cumulative urea release from the chitosan microspheres, in which an increase in the diffusion coefficient increased the cumulative urea release.

The Fig. 9 compares the experimental data with the calculated data obtained from some models. The best fitting was obtained from the model of diffusion in the reservoir system with a high R^2 value of 0.86–0.98, which was higher than that in the model of reservoir systems with non-constant activity sources (R^2 value of 0.7–0.88) and model of monolithic solutions (R^2 value of 0.82–0.95). Based on the fitting results, the pattern of urea release from

chitosan microspheres was the diffusion in the reservoir system model.

4. Conclusions

In this study, chitosan microspheres prepared using the cross-linking emulsion method were characterized to be applied to control the urea release. The chitosan microspheres containing urea showed a smooth surface, which means that the microspheres had a well-rounded geometry. The interaction of the chitosan functional group (amine group) with the glutaraldehyde (aldehyde) to form a stable imine group (Schiff base) indicated that a cross-linking reaction occurred. An increase in the chitosan concentration increased the crystalline content and decreased the cumulative urea release. Based on the kinetic analysis, the urea release from the chitosan microspheres followed the Fickian diffusion mechanism, and the models with the best fitting were Korsmeyer-Peppas, Peppas-Sahlin, and Simple power law with the burst effect models. An increase in the chitosan concentration decreased the diffusion coefficient, which means that the released urea was lower. The model to determine the diffusion coefficient with the best fitting was the model of
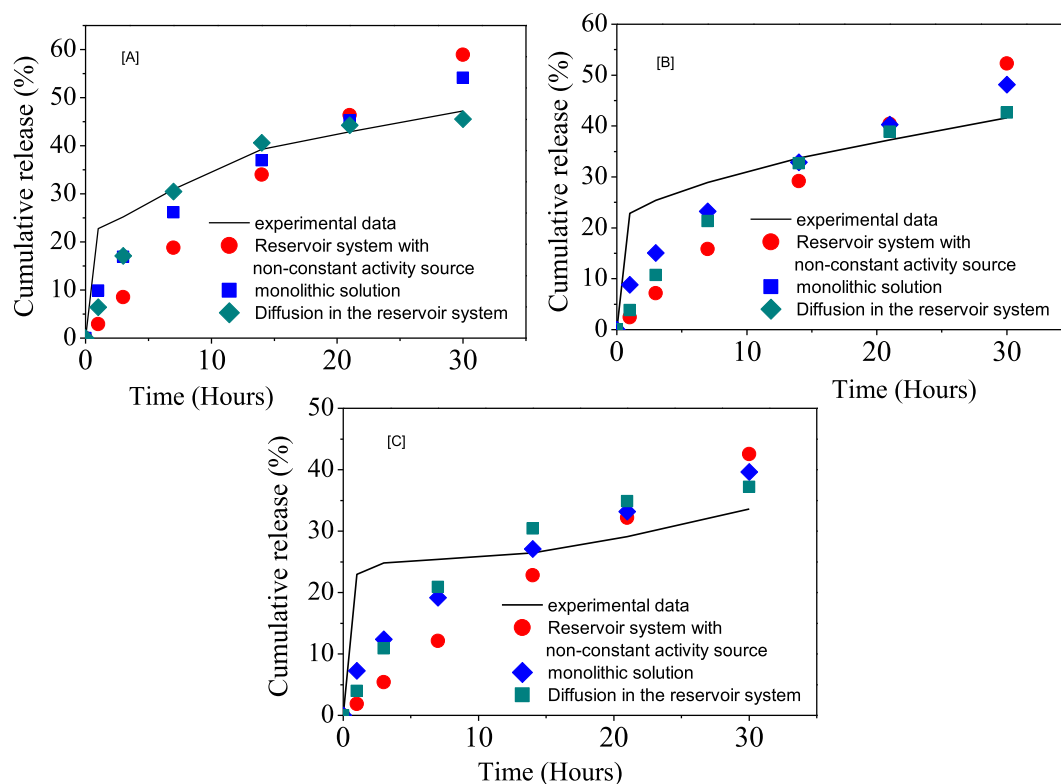

Fig. 9. Cumulative release comparison of experimental data and calculation using several models based on changes in chitosan concentration: [A] 2% (w/v), [B] 3% (w/v), and [4% (w/v).

diffusion in the reservoir system with a high R^2 of 0.86–0.98. The diffusion coefficient value obtained from the model was in the range of 5.439×10^{-11} -7.512×10^{-11} cm^2/s.

Acknowledgments

Appendix. List of symbols

$\frac{M_t}{M_\infty}$	Cumulative release of urea (%)
k_H, k_{K-P}	(k_1 and k_2), a, and b Release kinetics constants
n	Release mechanism
m	The diffusional exponent
t	Release time (hours)
R_0	The outer radius (μm)
R_i	The inner radius (μm)
K	The partition coefficient
C_{Aw}	Nitrogen (Urea) concentration in water [ppm]
C_{A0}	nitial concentration of nitrogen (urea) [ppm]
V_m	Total volume of nitrogen in microcapsule [cm3]
H_l	Equilibrium constant in phase I
H_d	Equilibrium constant in phase II
V_w	Total volume of water [cm3]
A_m	Total surface area of microcapsule [μm]
D	The diffusion coefficient $\left[\frac{cm^2}{sec}\right]$
δ	Thickness of microcapsule wall [μm]
ρ_{urea}	Urea density $\left[\frac{g}{cm^3}\right]$
ρ_{chit}	Chitosan density $\left[\frac{g}{cm^3}\right]$
x	Fraction
$P(1-t)$	Percentage release previous to 't'
Pt	Percentage release at time t

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