



Synthesis and Swelling Kinetic Study of BSA-based Hydrogel Composite by Subcritical Water Technology

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Abstract

This study, for the first time, aimed to analyse 'Bovine serum albumin' hydrogel composite by simple and quick method (SCWT) since not only there had been some indications that the low temperature subcritical water treatment may be as valuable products as its high temperature treatment, but also, the positive outcome would put a stop to the waste of energy and money. For BSA-based hydrogel at first, the optimum conditions were identified by assessing the effect of different influential parameters (SCWT temperature, time). SCWT was done using a batch subcritical reactor. Additionally, the characterization tests were carried out on the BSA-based hydrogels which was produced by this unique method. BSA-based Hydrogel preparation condition by SCWT accurately was investigated and optimized SCWT condition according to maximum ESR (50%). Schott kinetic swelling model provided evidence to approve two-step water diffusion mechanism in BSA-based hydrogel by SCWT.

Keywords: Bovine Serum Albumin, Subcritical Water, Batch Reactor, Hydrogel

1 Introduction

Biomaterials have been proven to be quiet beneficial to human beings as they have a diverse range of applications in food, medicine, and many other areas (1, 2, 3). For instance, the vast range of proteins has been used as a good source of protein-based biomaterials (4). It is not surprising that the demand for natural and organic products has increased significantly worldwide as they are considered less harmful in comparison with non-organic products. Nowadays, there are numerous natural products in the market for cosmetics, food additives, and medicinal purposes (5). Many of the above-mentioned biomaterials are protein-based (6, 7). Protein-based hydrogels are the most common biopolymers shaped from proteins and have medical applications in the drug delivery system, tissue engineering, ect (8, 9). However, the quality and difficulty of each method varies significantly for different protein sources (10, 11, 12). Therefore, given the aforementioned importance of biomaterials for natural products, finding highly efficient isolation methods for protein-based biomaterials as well as understanding and identifying the applications and properties for each protein-based biomaterial is vitally important. Subcritical water technology is a novel method that recently take an attention of researchers to produced variable biomaterials such as bioplastic and biomass without using additive, catalyst or any toxic chemicals (13, 14). Additionally, promising properties of water in high temperature and pressure range (SCW) and coagulation of the protein structure under subcritical water treatment conditions

motivated researchers to investigate on replacing the time consuming, toxic methods by clean and safe SCW technology. According to the literature, it is assumed that BSA solution under Sub-CW treatment, has aggregation-decomposition-aggregation-decomposition pathway (15). In this case, this study assumes that BSA coagulates to BSA solid network (BSA-based hydrogel). So based on this, Sub-CW treatment of BSA can be a great, novel method for producing BSA-based hydrogel without using any chemicals and crosslinker in a very short time.

Continuous efforts have been made to produce strong and biocompatible bovine serum albumin hydrogels with retained protein function; for example, the ability to bind, and release types of well-defined molecules (16, 17, 18, 19). The BSA-based hydrogels made by the thermal method have already been well established and have been working on the induction gel mechanism of proteins such as BSA (20, 21, 22). The main drawback of heat-inducing method is the broadly protein denaturation, with the risk of compromising protein functionality and biocompatibility (23). Moreover, besides the disadvantages of the denaturation problem, the time consumed by the incubation process is another drawback (24, 25). Electrostatically-triggered serum albumin hydrogels is another preparation method of albumin hydrogels introduced very recently (25). Despite the fact that this proposed method reduced the time consumption and solved the denaturation problem, still it is long and needs chemicals that are not

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environmental friendly. So, there is still a need for a faster and environmental friendly method to replace it. Protein-based biomaterials such as hydrogels produced with Subcritical Water Treatment (SCWT) are a new promising method, which is safe, fast, economical, and environmental friendly in comparison with other new methods that are used to produce protein-based biomaterials. Treatment of BSA by Sub-CWT does not use any additives or catalyst; it is cheap and environmental friendly (15). Furthermore, Sub-CWT treatment has been shown to require significantly short process time (few minutes) and utilises lower amount of raw material.

2 Materials and method

2.1 Materials

In this study, couples of chemical materials were used for different purposes. Bovine serum albumin (BSA, fraction V) was used as a model protein in SCWT experiments provide by Wako, Japan. Chloric acid and sodium hydroxide (Sigma Aldrich) were used to study the effect of pH on BSA-based hydrogel swelling behavior in acidic and base media. Ionic solutions (eg. different concentration of NaCl) was used to discuss the impact of ionic strength on BSA-based hydrogels.

2.2 BSA-based hydrogel preparation

For producing BSA-based hydrogel, 10gr BSA was weighted by AND balance series GR-200 from Japan and was gently dissolved in Milli-Q water in a volumetric flask with shaking. For solution of BSA, subcritical water reaction was done in the range of 80-200°C for 30 seconds to 30 minutes and gel-like bovine serum albumin biopolymer (GBSA) was made in a batch reactor. Hydrogel solid was formed by composition of BSA in sub-critical water process. In high concentration of initial BSA solution, at some temperature range only gel phase was produced depending on the reaction time as well. A batch laboratory-scale sub critical fluid system was used for this research. The reactor was a stainless steel batch reactor (SUS316, i.d 7.5 mm×150.4 mm) length, capped with Swagelok fittings. 3ml BSA solution charged into the reactor tube of which one was side capped with Swagelok fitting. After purging argon gas, Sealing of the reactor with Swagelok fitting was done followed by immersion into a preheated oil bath thermometer inspecting Celsius M type (Thomas Kagaku Co. Ltd., Japan) that contained silicone oil for reaction temperature lower than 200°C. The synthesis reactions were performed in the range of 80-130°C. After the desired reaction time, the reactor was immediately removed from salt bath or oil bath and cooled by immersion in a chilled water (20L) bath.

2.3 Freeze-drying

For removing water from the product the method of Robert et al. was followed (26). After removing water, put samples in the refrigerator (-1°C) over a night and after that kept at -40 °C for further precipitation. After 24 hrs, the precipitate was collected and dried using freeze drying system (FTS) for 72 hr (27).

2.4 BSA-based hydrogel behavior

After reaction, remove whole solid from the reactor carefully and prepare predetermined amounts of solid (present weight of dry sample, W_d). The swollen hydrogels were removed from water and weighed at regular interval times (W_t). The swelling ratios of solid matrices were established from the weight change before and after swelling: swelling ratio

$$(SR) = W_t/W_d \quad (1)$$

The swelling behavior of hydrogel in different temperature range of distilled water was explored. The swelling tests were performed in ionic medium solutions, prepared as explained by Wang et al. (28). In a typical experiment about 0.2 g of sample was weighed (W_1) and immersed in different swelling media for 6 hours, after which they were removed and blotted dry, and their weights were recorded (W_2). The equilibrium swelling ratio (ESR) of the hydrogel in distillate water was computed with the equation below:

$$ESR (\text{gr/gr}) = (W_2 - W_1)/W_2 \quad (2)$$

2.5 BSA-based hydrogel swelling kinetic model

In this study, two kinetic models were used to study the kinetic models and the mechanism of swelling for BSA-based hydrogel by SCWT process. (Table 1).

Table1. Applied swelling kinetic models for BSA-based hydrogels

Model	Equation	Plotting parameters	Kinetic constant(s)
Fickian diffusion	$f = M_t/M_\infty = K_1 t^n$	$\ln f$ vs. t	k_1
Schotts second-order	$dS/dt = k_2 (S_\infty - S)^2$	t/s vs. t	k_2

3 Result and Discussion

BSA-based hydrogels were synthesized by self-coagulation of 'Bovine serum albumin' solutions under SCWT temperature from 80°C and above. Coagulation of BSA started at 90°C and gelation process was continued by rising temperature to 120°C (Figure1). Although by increasing SCWT temperature, more gel network was formed, since at 130°C that the accumulated gel decomposes to the smaller molecules and by increasing temperature. It totally decomposed to smaller molecules that will discuss them in our next paper.

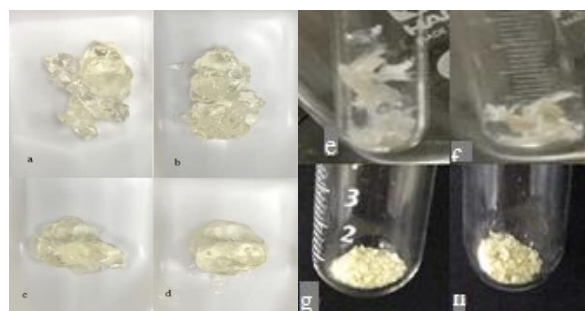


Figure 1: BSA-based hydrogel, prepared at a) 90°C, b) 100°C, c) 110°C, d) 120°C by SCWT, Freezer dried BSA-based hydrogel prepared at e) 90°C, f) 100°C, g) 110°C and h) 120°C

3.1 Effect of coagulation temperature on ESR of hydrogel

The lower range of temperature was the lowest temperature that BSA molecules start to coagulate (i.e. 80°C) that has the ability to bring the water to the boiling point (near subcritical point) (29). The gel preparation temperature increased from 100°C to 120°C, the equilibrium swelling ratio (ESRs) decreased from 49 to 40, demonstrating the swelling behavior was reliant on SCWT temperature (Figure 2). In addition, incomplete gelation at the minimum temperature may be accounted for by the slower BSA coagulation during process which is essential for forming of hydrogel bonds. Similar result

was also reported by other studies that used low temperature range of Sub-CW treatment of BSA. For example, Aida et al. (2017) demonstrated that the lower temperature of BSA Sub-CW treatment at 5 minutes as a result of slow-heating and mass transfer that caused protein aggregation in batch reactor. The decrease of ESR in 120°C accounted for by starting decomposition of the sample as the result of hydrolysis of formed solid.

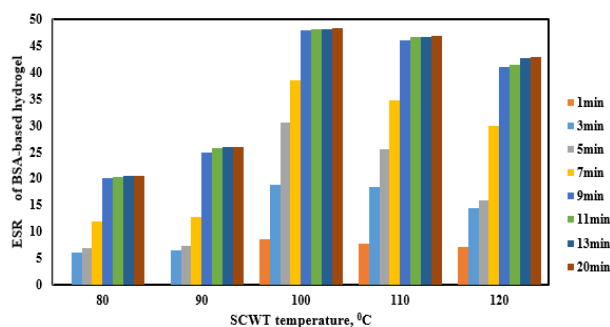


Figure 2: Relation between Equilibrium swelling ratio (ESR) of BSA-based hydrogel and BSA-based hydrogel preparation temperature in different time course

The decomposition, and hydrolysis are the common possible reactions that may happen during SCWT method that perform at high temperatures or long decomposition times (30, 31). So, it is essential to identify the best practical SCWT time and temperature for coagulation the best possible ESR of BSA-based hydrogel occurrence of undesirable reactions. In short, the coagulation process was more efficient at medium temperatures (i.e. 100 or 110°C use the "Insert Citation" button to add citations to this document). As the result, the prepared BSA-based hydrogel was better coagulated from the Bovine serum albumin at SCWT temperature of 100-120°C.

3.2 Effect of Sub-CW coagulation time course on ESR of hydrogel

Duration of Sub-CW treatment is another important parameter in BSA coagulation process. It should be long enough to all protein in the initial solution coagulated and form the gel network. In order to find the optimum time for the complete gelation of BSA, the equilibrium swelling ratio (ESR) of BSA-based hydrogel at coagulation temperature of 80-120°C were measured during the coagulation time until the maximum percentage of swelling ratio was observed (Figure 3).

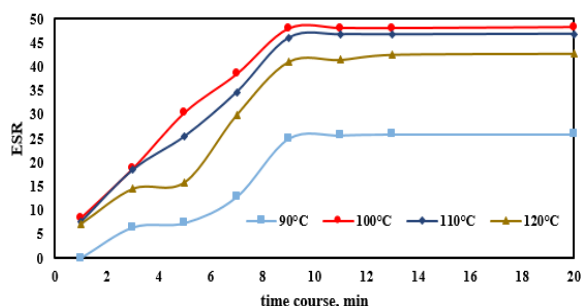


Figure 3: Relation between Equilibrium swelling ratio (ESR) of BSA-based hydrogel and preparation time in different SCWT temperature

The maximum swelling achieved in 100°C after 9 minutes, 49%. BSA-based hydrogels by SCWT have the high swelling at 100, 110 and 120°C after samples reached the equilibrium

point. The equilibrium swelling ratio of BSA-based hydrogel was measured for hydrogels that prepared at 1-20 minutes but ESR% did not change considerably after 9 minutes of Sub-CW treatment. Thus, coagulation and gelation of BSA was considered to be completed after 9 mins reaction time, when the ESR curve reaches the steady state.

3.3 Optimization of SCWT operational condition

The results provided evidence that for reaching the highest percentage of swelling ratio in BSA-based hydrogel, SCWT temperature should be high enough to provide sufficient temperature for coagulating the BSA while not resulting in any undesirable reactions such as decomposition and hydrolysis (they can be undesirable for producing hydrogel). Additionally, the result confirmed the suggested mechanism of Sub-CW treatment of protein that the accumulation of protein is commerce when the SCWT temperature is sufficient for water to be in subcritical region and protect the sample completely from the direct heat and at the same time prevent denaturation(29)(drawback of direct heating gelation of BSA. The result showed that for obtaining highest possible equilibrium swelling ratio, the SCWT time should only be long enough to let all BSA molecules to coagulated (32). Obviously, longer treatment time is not necessary and it is only the wastes of energy since no significant changes in swelling ratio of BSA-based hydrogel.

3.4 Swelling kinetic model

For a better understanding of the swelling model of BSA-based hydrogel prepared by SCWT at different operational conditions, the mechanism of the swelling was assessed using two different models. The swelling behavior of BSA-based hydrogels was analyzed with these models to find the best model for describing the swelling behavior in neutral pH levels.

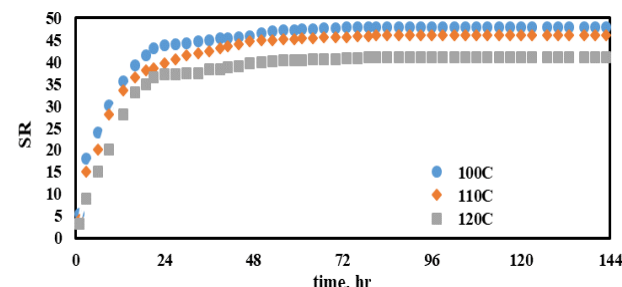


Figure 4: Swelling test of GBSA100, GBSA110, GBSA120 in distilled water

Swelling kinetic of GBSA100, GBSA110, GBSA120 were investigated in distilled water (pH= 7) and result observed in figure 4. The parameters of the two kinetic models and their R^2 (coefficient of determination) were calculated using plotting method. It is obvious that Fickian diffusion model showed the lowest fit to the experiment data compared to Schott model (Table 2). So, it was concluded that this model cannot properly explain the swelling of BSA-based hydrogel by Sub-CW treatment.

For Fickian swelling kinetic model, when gel swells in media, three steps occur: to start with water diffusion into the BSA-based hydrogel structure; then, the swelled chains rest; and finally, the hydrogel grows. $\ln(f)$ versus $\ln(t)$ of selected hydrogels were driven. In distillate water, diffusion exponent n and characteristic constant K were computed from slopes and intercepts of the lines are recorded in Table 2. The n values were all in the range from 0.45– 0.47 territory, showing the swelling conduct at the underlying swelling stage was

overwhelmed by Fickian dispersion. Linear relations of t/S with t are plotted; the kinetic parameters computed from the slopes and intercepts of the lines are listed in Table 2. It can be seen that the related coefficients (R) are all beyond 0.998, indicating the Schott's second-order kinetic equation was appropriate in describing the whole swelling process. The results provided evidence that both models of Fickian diffusion and second-order Schott model fitted with experiment results. As it can be seen in Table 2, The Fickian diffusion model's kinetic constants had increased by the increase of SCWT temperature. Despite the fact that Fickian kinetic model was better fitted to the data, model of second order diffusion had significantly higher R^2 (0.998 and above).

The Fickian diffusion kinetic model describing a swelling mechanism that occurs in fast water diffusion processes using one rate constant (k ; Table 2;(33)) whereas model of second order Schott describes the gelation using a kinetic constant for slow and fast stage of water diffusion process (k and S_{∞} ; Table 2;(34, 35)). Second-order Schott and Fickian diffusion kinetic models were one of the best kinetic models for explaining the protein-based swelling mechanism (35). On the basis of the proposed model, the water mechanism in BSA-based dry hydrogel consists of two stages. First is the fast penetration of the water on or near the particle surface and second is the slow diffusion through the particles.

These two stages can be easily identified in the figure 4. The fast swelling can be identified during the beginning of swelling process (first 3th hours), in which the swelling ratio increases rapidly due to high-capacity pores and mass transfer. As it is clear, swelling in acidic media grows more sharply in gel compare to the pure water. In short, the swelling kinetic model provided evidence that coagulation temperature (i.e. SCWT temperature) affects the gelation process, equilibrium swelling ratio of hydrogel, and the kinetic of swelling hydrogels. The result of swelling kinetic modelling showed that the gelation of BSA-based hydrogel from BSA solution under Sub-CW treatment follows Fickian diffusion model in early hours of swelling. Based on the swelling kinetic modelling

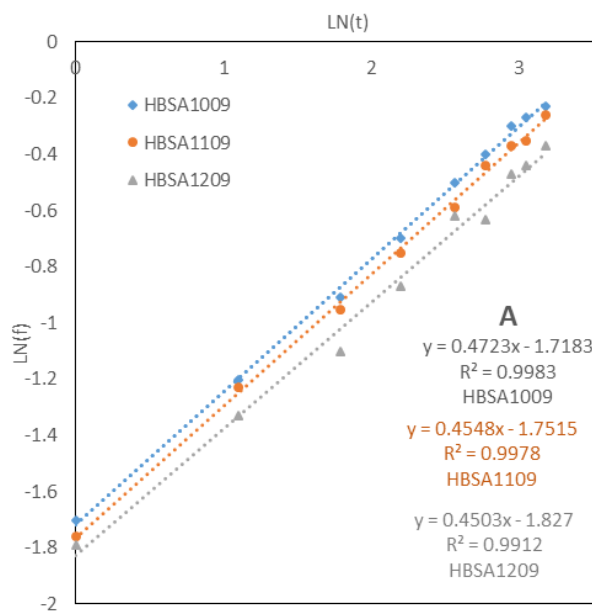
results, the coagulation of BSA-based hydrogel from BSA solution follows second order Schott model through the whole swelling process. For Fickian dispersion, three stages happening in progression are proposed when dry gel swells in water: to start with, water particles diffuse into the polymer structure; second, the hydrated polymer chains rest; and third, the polymer arrange grows to the encompassing. In the first swelling step, ($M_t/M_{\infty} \leq 60\%$), the main equation that describe permeation in the second step of swelling: $f = M_t/M_{\infty} = Kt^n$, where M_t was the mass of water absorbed at time t and M_{∞} was the gel mass at equilibrium, K was a characteristic constant showed network structure of gels and n was the diffusion exponent. For $n < 0.5$, Fickian diffusion would be the best model to explain swelling producer; for $0.5 < n < 1$, the transport would be anomalous (non-Fickian diffusion); and for $n = 1$, the relaxation of polymer chains would control the system. Charts of $\ln(f)$ versus $\ln(t)$ of selected hydrogels were driven in Fig. 5.

Table 2: Values of the kinetic parameters

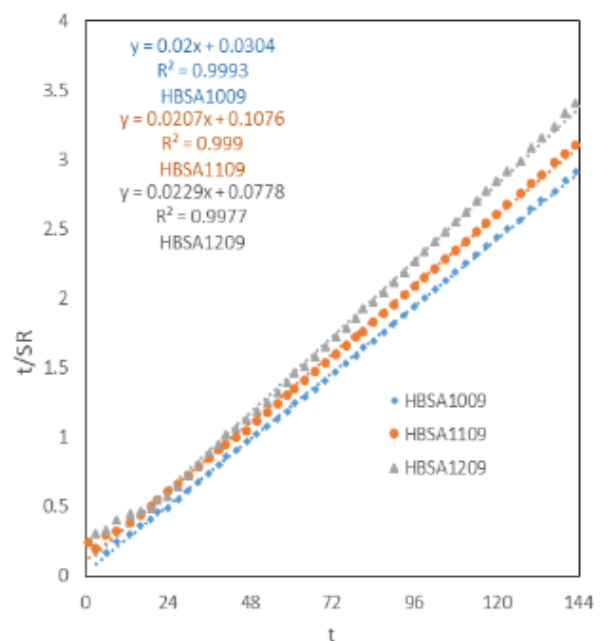
Sample	Fickian diffusion model			Schotts model		
	N	K	R ²	$k_1(\text{min}^{-1})$	R ²	S_{∞}
GBSA100	0.47	0.179	0.998	33.33	0.999	50
GBSA110	0.45	0.177	0.998	9.09	0.999	47.62
GBSA120	0.45	0.159	0.991	12.82	0.998	43.48

4 Conclusion

In the current study BSA-based hydrogels was successfully synthesized with Subcritical water technology. The result in general indicated that subcritical water treatment (SCWT) method is a better alternative as a current method of BSA-based hydrogel preparation since the BSA-based biomaterials using SCWT were obtained in significantly shorter time without adding any reagents or crosslinkers (toxic materials). It was found that temperature was the most influential parameter of preparing BSA-based hydrogel as well as initial BSA solution concentration.



(a)



(b)

Figure 5: (a) kinetic theory of Fickian, (b) Schott's second-order kinetic model

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