UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION OF BOVINE SERUM ALBUMIN-BASED BIOMATERIALS SYNTHESISED BY SUBCRITICAL WATER TECHNOLOGY

ZAHRA MAGHAREH ESFAHAN

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By

ZAHRA MAGHAREH ESFAHAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To my great family: my lovely parents, my unique sister

Words cannot express how grateful I am for all their kindness and support. I am forever in their debt.

- ❖ My lovely parents (Ali Asghar Maghareh Esfahan and Monir Abri), whose smile means a world to me.

My father is my kind-hearted hero. From the day that I remember, he has been trying to make the life better for all of his girls. He is the greatest and kindest dad of all. What can I say about my mother now that truly express my gratitude. She is my beautiful kind-hearted angel, whose biggest wish is the success and happiness of me and my sweet sisters. Her friendly, warm, and welcoming voice is the essential element of my daily life. My parents have always wanted the best for their children and have raised us with pure love. Their only dream is the success and happiness of their children and they have sacrificed everything to make that happen. Words cannot express their pure kindness. My dream has always been to make my parents happy. They're the best and I'm so lucky to be their daughter. Their endless love has always given me the strength no matter how far they were away from me.

- ❖ My lovely sister (Masoueh Maghareh Esfahan), who is not only like my mother, but also she is my kind, and supportive friend

She has always been supportive with her beautiful smile. Her flawless-pure love and support is priceless. She is the best sister and I can't be luckier to have someone like her in my life. I love her like my own mother and her happiness is my happiness. She has always wanted the best for me and has sacrificed a lot for that. I want to express my gratitude for all the sacrifices that she has done. Appreciate all the love and support that she has been given us unconditionally. I hope that someday I can be able to make it up to her for all her kindness

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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August 2019

Chairman : Shamsul Izhar bin Siajam, PhD
Faculty : Engineering

Bovine serum albumin (BSA), is one of the most abundant proteins in mammals' blood, especially from bovine and human. This study investigated the synthesis and characterization of BSA-based biomaterials that was produced by subcritical water technology (SCWT). BSA is freely available and is a cheap source of protein to be recycled into various biomaterials. Using toxin and non-environmentally friendly chemicals is the main drawback of the current methods of biomaterials processes as well as long procedures time. Therefore, the primary goal of this study was to assess whether Subcritical Water can be used as a cheap, clean and simple alternative to the traditional recovery method to produce biomaterials from BSA. In addition, this is the first study ever analyzing 'BSA' biomaterials employing the simple and quick SCWT method without using any catalyst or chemicals. Although BSA solution decomposition at high SCWT temperature (above 250^0C) and long SCWT holding time (5 mins and longer) was investigated wisely, we focused on low SCWT temperature (below 250^0C) which provided indications that it could deliver valuable products as its high temperature treatment. The best conditions of processing biomaterials were identified by assessing the effect of different influential parameters such as SCWT temperature, reaction time, and initial BSA concentration. SCWT was carried out using a batch tube reactor to produce BSA-based biomaterials (BSA-based hydrogel and BSA-based nanoparticles). BSA molecules in temperature range of 80-120^0C accumulate to BSA-based hydrogel. It was found that at SCWT temperature more than 130^0C, due to the water/ethanol mixture behavior of SCW, in very specific SCWT condition, accumulated BSA molecules decomposed to BSA-based nanoparticles and following by decomposing to organic components. The behavior of BSA-based hydrogel was investigated in different media wisely which confirmed that it has an excellent ability to use as drug delivery. By characterization of the BSA-based nanoparticles and liquid-phase components produced were conducted.

In short, the result of this study showed that SCWT of BSA is the best method in terms of time, efficiency and quality of produced biomaterials as well as the method's non-dependence on catalyst or toxic chemicals. Low, medium and high SCWT temperature of BSA (80-200^{0}C) was found to contain beneficial biomaterials useful for medical, especially drug delivery application.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SINTESIS DAN PENCIRIAN BIOBAHAN BERASASKAN BSA OLEH TEKNOLOGI AIR SUBKRITIKAL

Oleh

ZAHRA MAGHAREH ESFAHAN

Ogos 2019

Pengurusi : Shamsul Izhar bin Siajam, PhD
Fakulti : Kejuruteraan

Serum albumin bovin (BSA), yang merupakan salah satu protein yang penting, yang Bovine serum albumin (BSA), adalah salah satu protein yang paling banyak terdapat dalam darah mamalia, terutamanya dari lembu dan manusia. Kajian ini mengkaji sintesis dan pencirian biomaterial berasaskan BSA yang dihasilkan oleh teknologi air subkritikal (SCWT). BSA tersedia secara bebas dan merupakan sumber protein yang murah untuk dikitar semula menjadi pelbagai biobahan. Penggunaan toksin dan bahan kimia yang tidak mesra alam adalah kelemahan utama kaedah semasa memproses biobahan disamping masa prosedur yang panjang. Oleh itu, tujuan utama kajian ini adalah untuk menilai sama ada SWCT dapat digunakan sebagai alternatif yang murah, bersih dan sederhana kepada kaedah pemulihan tradisional untuk menghasilkan biobahan dari BSA. Tambahan lagi, ini adalah kajian pertama yang menganalisis biobahan BSA menggunakan kaedah SCWT yang mudah dan cepat tanpa menggunakan sebarang pemangkin atau bahan kimia.

Oleh sebab BSA terurai pada suhu SCWT yang tinggi (di atas 250C) dan masa proses yang lama (lebih 5 minit), kami menumpukan pada suhu SCWT rendah (di bawah 250C) yang memberikan produk berharga. Keadaan terbaik memproses biomaterial dikenal pasti dengan menilai pengaruh parameter berpengaruh yang berbeza seperti suhu SCWT, masa reaksi, dan kepekatan awal BSA. SCWT dijalankan menggunakan reaktor tabung berasingan untuk menghasilkan biobahan berasaskan BSA (hidrogel berasaskan BSA dan nanopartikel berasaskan BSA). Molekul BSA dalam julat suhu 80-120C terkumpul kepada hidrogel berasaskan BSA. Pada suhu SCWT lebih daripada 130C, oleh kerana sifat SCWT seperti campuran air / etanol dan keadaan SCWT yang sangat spesifik, molekul BSA terurai kepada nanopartikel berasaskan BSA diikuti dengan penguraian ke komponen organik. Sifat hidrogel berasaskan BSA disiasat di dalam media yang berbeza. Hasilnya, kami mengesahkan bahawa ia

mempunyai kemampuan yang sangat baik untuk digunakan sebagai penghantaran ubat. Pencirian komponen nanopartikel dan fasa cair berasaskan BSA telah dilakukan.

Kesimpulannya, hasil kajian ini menunjukkan bahawa pemprosesan BSA dengan SCWT adalah kaedah terbaik dari segi masa, kecekapan dan kualiti biobahan yang dihasilkan. Kaedah SCWT juga tidak bergantung kepada sebarang pemangkin atau bahan kimia toksik. Suhu SCWT yang rendah, sederhana dan tinggi BSA (80-200C) mengandungi biobahan bermanfaat yang berguna untuk perubatan, terutamanya untuk aplikasi penghantaran ubat. Rawatan BSA pada suhu SCWT rendah, sederhana dan tinggi (80-200C) menghasilkan produk yang mengandungi biobahan bermanfaat yang berguna untuk perubatan, terutamanya untuk aplikasi penyampaian ubat.

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This thesis was submitted to the Senate of the Universiti Putraalaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Shamsul Izhar bin Sianjam, PhD
Senior Lecturer, Ir
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Mohd Halim Shah, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Mohd Razif bin Harun PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
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Signature: ____________________

Name of Chairman of Supervisory Committee: Dr. Shamsul Izhar bin Sianjam

Signature: ____________________

Name of Member of Supervisory Committee: Associate Professor Dr. Mohd Halim Shah

Signature: ____________________

Name of Member of Supervisory Committee: Dr. Mohd Razif Bin Harun

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSA	Bovine Serum Albumin
SCWT	Sub-Critical Water Treatment
NPs	Nanpaprticles
ANOVA	Analysis of Variance
CCRD	Central Composite Rotatable Design
FT-IR	Fourier Transform-Infrared
UPLC	Ultra Pressure Liquid Chromatography
HPLC	High Pressure Liquid Chromatography
IC	Insoluble Carbon
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
PDI	Polydispersity index

CHAPTER 1

INTRODUCTION

1.1 Research Background

Biomaterials have been proven to be beneficial to human beings as they have a diverse range of applications in food, medicine, and many other areas (Campoccia and others 2013; Siracusa and others 2008; Williams 2009). For instance, a wide range of proteins has been used as a good source of protein-based biomaterials (Martins and others 2018). It is not surprising that the demand for natural and organic products has increased significantly worldwide as they are less harmful compared to non-organic products. Numerous natural products are currently on the market for cosmetics, food additives, and medicinal purposes (Mie and others 2017). It is vital to identify high-efficiency isolation methods for protein-based biomaterials, as well as understand and identify applications and properties for each protein-based biomaterial.

Subcritical water technology (SCW) is a novel method that has attracted greater attention among researchers to the production of variable biomaterials such as bioplastics and biomass without the use of additives, catalysts or any toxic chemicals (Abdelmoez and Yoshida 2006; Sheehan and Savage 2017). In addition, the promising properties of water at high temperature and pressure along with coagulation/decomposition of the protein structure under SCW conditions have prompted researchers to explore the possibility of replacing time-consuming, toxic methods with clean and safe SCW technology (Garcia-Moscoso and others 2015).

1.2 Problem statement

1.2.1 Why Bovine Serum Albumin is chosen

Bovine Serum Albumin (BSA) has been one of the most extensively studied proteins for several years. It is the most abundant protein in blood plasma with a characteristic concentration of 3-5% (w/v) (Day and Myszka 2003; Roy and others 2018). Animal blood from slaughterhouses is an important source of food ingredients and nutrients that have been underused to date (Baxter and others 2008; Zhu and others 2020). Based on slaughterhouses, waste typically accounts for 45 to 60% of the animal's weight, as shown in Table 1.1 for each of the main species farmed for human consumption (Mcilroy and Roche 2017). Bovine waste is significantly more than that of other animal species.

Table 1.1 : Quantity of waste per species (Mcilroy and Roche 2017)

Species	Weight (kg)	Carcass	Meat (%)	Waste (kg)
Cattle	350	55	40	210.0
Pig	70	72	55	31.5
Sheep/Goat	30	47	40	18.0
Poultry	2	66	56	0.88

Previously, blood was often unused and discharged into drains and local streams, causing contamination with substantial organic content and creating a vector for disease (see Figure 1.1). Nowadays, in large industrial slaughterhouses, animal waste is collected and all variable parts, such as blood, fat, and trimming, are separated as by-products (McCabe and others 2018).

Figure 1.1 : Blood, unborn calves, and other slaughter wastes being discharged into public watercourses

In recent years, BSA has been used as a good source to produce variable bioproducts such as bioplastics, biomass, and nanoparticles (NPs) and hydrogels (Abdelmoez and Yoshida 2006; Baler and others 2014; Sheehan and Savage 2017; Tarhini and others 2018). Apart from being a cheap and abundant source of BSA, Albumin-based biomaterials have been widely accepted for use as drug carriers compared to other proteins because they possess numerous unique properties (Sunphorka and others 2012). As albumin is presented in circulating plasma at 5% (w/v) serum levels, it is not toxic and is well tolerated by the immune system. Albumin has an excellent binding capacity of hydrophobic drugs, as indicated by many drug-binding sites found in protein molecules (Kratz 2008). However, due to their high content of charged amino acids, albumin NPs may permit electrostatic adsorption of positively or negatively charged molecules without the need for other compounds. Albumin molecules have specific functional groups (i.e. carboxylic and amino groups) that are accessible on the surface for covalent drug adhesion or cell-targeting agents (e.g. folate, transferrin, monoclonal antibodies) (Ulbrich and others 2011; Zhang and others 2011). Additionally, albumin has a positive pharmacokinetic profile due to its long half-life in the blood system (19 days) (Elzoghby and others 2012).

The unique potential of BSA is due to its numerous advantages. It is readily available, its degradation metabolites are harmless, water-soluble, and is available in a pure state to produce biomaterials such as hydrogels and NPs (Arabi and others 2018; Tarhini and others 2018). These were the major factors justifying the choice of BSA as the protein role model for this study (Rahimnejad and others 2012).

1.2.2 Why SCW is used to produce BSA-based hydrogel and BSA-based NPs

BSA-based hydrogel and BSA-based NPs are both the most well-known biomaterials that have recently been used in medical applications, especially drug delivery systems that are produced by time-consuming and toxin-based methods that will be briefly mentioned (Agrawal and others 2008; Papagiannopoulos and Pispas 2018).

Continuous efforts have been made to produce strong and biocompatible BSA-based hydrogels with retained protein function, such as the ability to bind and release well-defined molecule types (Clark and others 2001; Iemma and others 2005; Oss-Ronen and Seliktar 2011; Sun and Huang 2016; Sheehan and James 2019). BSA-based hydrogels produced using the thermal method (Figure 1.2) have already been well established, working on the induction gel mechanism for proteins such as BSA (Ferry 1948; Jian and others 2014; Sun and Holley 2011). Initially, the emergence of heat-induced polypeptide chains leads to organizational changes and a modified tertiary protein structure. The next phase of protein-protein interactions leads to the final structure of the gel network (Park and others 2019). The main drawback of the heat-inducing method is broad protein denaturation, with the risk of compromising protein functionality and biocompatibility (Gorobets and others 2019). Moreover, in addition to the disadvantages of the denaturation problem, the time spent during the incubation process is another drawback (Amdursky and others 2018; Baler and others 2018).

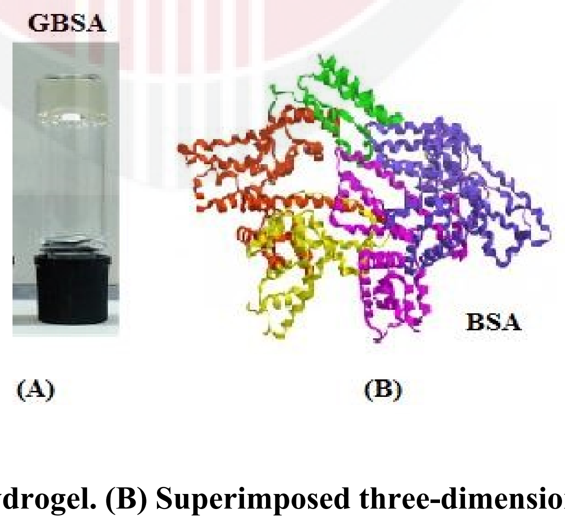

Figure 1.2 : (A) BSA hydrogel. (B) Superimposed three-dimensional structure of BSA (Adapted from Baler and others 2018)

Electrostatic-triggered serum albumin hydrogels are another preparation method of albumin hydrogel introduced very recently (Baler and others 2018). In this method, BSA gel precursor solutions are formed by BSA solution at concentrations ranging from 9 to 20 wt% and stirred at 200−300 RPM until dissolved completely in 2−3 hrs. In order to produce pH-induced BSA gels, the pH of the precursor solution is reduced to pH 3.5 by adding 2 M HCl with continuous stirring and then submerging in a water bath at 37°C for 2 h. In order to produce thermally denatured BSA hydrogels, the precursor solution is neutralized to pH 7.4 by 2 M NaOH and then submerged in a water bath at 80°C for 2 h (Baler and others 2018).

Even though this proposed method reduced time consumption and resolved the denaturation problem, it is still long and needs chemicals that are not environmentally friendly. There is still a need, therefore, for a faster and more environmentally friendly method to replace it. Protein-based biomaterials such as hydrogels, NPs produced with SCW are a new and promising method that is safe, fast, economical, and environmentally friendly compared to other new methods used to produce protein-based biomaterials (Abdelmoez and Yoshida 2006; Sheehan and Savage 2017). SCW treatment of BSA does not use any additive or catalyst; it is cheap and environmentally friendly (Aida and others 2017). Furthermore, SCW has been shown to require a significantly short process time (few minutes) and to use a lower amount of initial protein concentration. Emulsification and coacervation (or anti-solvent precipitation) are the two major approaches to the preparation of BSA NPs. One limitation of the emulsion approach is the need for organic solvents to eliminate both the oily residues and the surfactants needed to stabilize the emulsion (Gunasekaran and others 2007).

Coacervation is a heat-induced self-assembled method for the preparation of protein NPs, which depends on the differential solubility of the protein in aqueous solution and in a desolvating agent, which is usually ethanol. SCW is an attractive method because there is no requirement for any specialized equipment and sophisticated operational situations and the associated costs are acceptably low. According to earlier studies, the major shortcoming of the coacervation method is less than satisfactory. The effect of the preparation conditions on the final size distribution of BSA NPs has been investigated by several authors (Sánchez-Segura and others 2018; Paik and others 2013), but the size variability remains large or inconsistent. Therefore, SCW technology can be a good replacement for the current method of BSA-based NPs by controlling treatment conditions.

According to literature, the BSA solution under SCW is assumed to have an aggregation-decomposition-aggregation pathway by increasing SCW temperature (Aida and others 2017). In this case, this study assumes that BSA first coagulates into a BSA solid network (BSA-based gel-like solid) and then decomposes to BSA-based NPs and then accumulates again to the solid-state and finally decomposes into small organic components such as peptides, amino acids, and organic acids. Thus, based on this assumption, SCW of BSA can be a great, novel method for producing BSA-based hydrogels, NPs without a catalyst or a chemical in a very short time.

1.3 Research Gap

As mentioned earlier, SCW of protein solutions may be used in the medical and food industries. In addition, the assessment of the SCW of the BSA pathway as well as the optimization of the production conditions of biomaterials is very beneficial for controlling and fine-tuning the SCW process. However, to the best of the author's knowledge, no studies have yet been conducted on the optimization and characterization of BSA-based biomaterials produced by SCW technology under the selected range of conditions. In addition, studies on the low-temperature treatment of proteins are very limited (only one study exists, that of (Sunphorka and others 2012)). The biomaterials of BSA, therefore, need further investigated.

There are some indications that SCW of BSA may also be useful in the production of a variety of biomaterials under different SCW conditions, such as protein-based hydrogel (Betz and others 2012). For instance, numerous studies have shown that using supercritical fluid (SCF) technology (Akhtar and others 2016), there is a good chance of producing protein-based hydrogel. These materials have significant applications to the drug delivery system (Guzman-Villanueva and others 2011).

Moreover, there are other indications that the investigation of BSA-based NPs is likely to be successful. For example, Aida and others (2017); Sheehan and Savage (2017) and Abdelmoez and others (2007) noted that low-temperature SCW of BSA significantly aggregated BSA molecules that can be considered as natural hydrogels. Despite all the above indications for possible applications of BSA-based biomaterials, surprisingly, no studies have been conducted on BSA-based hydrogel and swelling behavior prepared by SCW. So, by studying BSA-based hydrogel through characterization, this study attempts to fill this void.

1.4 Significance of the study and Practical Contributions

The significance of this research is threefold. First, by testing SCW of BSA solution in terms of time consumption, SCW temperature, initial BSA solution concentration, and product quality, this study seeks to determine whether SCW, for the first time, can be used as a good method to produce protein-based biomaterials (especially, hydrogels and NPs) from the portion of blood waste protein. By identifying SCW as a better method for BSA, this research can be of significant benefit to the industries in which protein-based biomaterials are used and can help to save a considerable amount of time and money.

Second, by finding useful components in the liquid phase from the SCW post-treatment of BSA solution, such as a peptide, amino acid, and organic acid, which are useful for many industries (e.g. health and medical care), this study takes a major step in providing information on organic products. Finding useful components from blood waste is important as large amounts of blood are traditionally discarded (including protein portion) and can then be used for protein in blood waste.

Finally, by conducting kinetic modeling of processes using BSA solutions, this study provides the necessary information required for better control and optimization. This information can also help organizations to improve process efficiency and consequently improve their revenue (Paunović and others 2014; Sunphorka and others 2012).

1.5 Research Objectives

This study attempts to fill the gaps mentioned above and to solve the problem in three ways. First, for each biomaterial (BSA-based hydrogel and BSA-based NPs), by composing and analyzing biomaterials from different initial concentrations of BSA solutions using SCW, this study attempts to identify the best limitation of SCW conditions in order to provide a basis for further investigations. In addition, by conducting the kinetic modeling for the BSA-based hydrogel swelling process, this study seeks to gain a better understanding of the BSA-based hydrogel diffusion mechanism by SCW and, consequently, to identify the optimum condition in which a higher swelling ratio of BSA-based hydrogel can be achieved. Finally, given the many benefits of finding better protein-based biomaterials from blood waste methods for different industries, this study aims to assess whether the SCW method is a good method for preparing protein-based biomaterials in a short time without any catalysts or additives.

In general, the research objectives are divided in such a way that the main contributions of this study to the existing body of knowledge would be:

1. To investigate the SCW condition to produce BSA-based hydrogel.
2. To optimize the SCW condition to produce BSA-based NPs for the decomposition of accumulated BSA molecules.
3. To assess the effectiveness of SCW on the initial BSA solution under different conditions, from the analysis using SEM, UV-vis spectroscopy, particle sizer.
4. For the provision of a kinetic model from SCW of BSA at temperatures below 200°C using total organic carbon analyzer (TOC), ultra-performance liquid chromatography (UPLC), high-performance liquid chromatography (HPLC).

1.6 Scope of the Study

In order to achieve the objectives; the scope of the study was as follows:

1. The BSA solution preparation and operating parameters (treatment time, treatment temperature, and initial BSA solution concentration) for appearance and swelling ratio were studied and identified for BSA-based hydrogel by SCW.

2. The swelling ratio of BSA hydrogels was measured using the Fickian diffusion model and the Schott's second-order kinetic equation to develop the swelling kinetic model and to evaluate experimental data and to fully understand the swelling mechanism. In addition, the optimum BSA-based hydrogel morphology will be characterized by SEM and FTIR for the test of swelling behavior in different media.
3. The RSM software was used to determine the best SCW condition to produce BSA-based NPs by particle size, zeta potential, and polydispersity responses.
4. UV-vis spectroscopy and SEM were conducted to characterize and approve the optimal NPs by RSM.
5. The presumed decomposition pathway was investigated by the measurement of total organic carbon, total amino acid, and total organic acid concentrations with TOC, UPLC, and HPLC.
6. The decomposition of BSA to amino acid kinetic model was conducted to evaluate the optimum SCW conditions to produce organic components in the SCW temperature range below 200°C.

1.7 Thesis Structure

This thesis is divided into five chapters. Chapter one provided the introduction, problem statements, the study objectives, the scope and the structure of the thesis. Chapter two includes descriptions of BSA and current BSA composition methods and introduces SCW, protein-based biomaterials, and their preparation methods and applications. SCW is introduced and fully described as a possible composition method for the medical industry. Furthermore, this chapter also discusses the kinetic mechanism of SCW of BSA. The theory of multi-objective design optimization using response surface methodology (RSM) is briefly explained. In chapter three, both materials and methods for the biomaterial composition from BSA are elaborated. Moreover, the characterization methods for testing BSA-based biomaterials as well as for the assessment of untreated BSA and SCW-treated BSA are explained. In chapter four, the results of the experiments are explained and discussed in detail. This chapter also presents a preliminary study on the treatment of biomaterials with BSA by SCW under different conditions, which can be found by studying the effect of key parameters and the kinetic modeling of the process. An experimental design using the central composite rotatable design (CCRD) is applied to the SCW of BSA biomaterials at different initial concentrations. It then continues to optimize the operating parameters for each group. Finally, a kinetic study of the SCW process of the BSA biomaterial is presented to obtain a better understanding of the process. This chapter also provides the results of several characterization test reports of different concentration solutions of BSA-based biomaterials (before and after treatment) of SCW in order to better understand the properties of novel biomaterials. Finally, the overview of the study, conclusion, implications, and recommendations for future studies are presented in chapter five.

REFERENCES

Abdelmoez W, Nakahasi T, Yoshida H. 2007. Amino acid transformation and decomposition in saturated subcritical water conditions. Industrial & Engineering Chemistry Research 46(16):5286-5294.

Abdelmoez W, Yoshida H. 2006. Synthesis of a novel protein□based plastic using sub□critical water technology. AIChE journal 52(7):2607-2617.

Abdelmoez W, Yoshida H. 2007. Mechanical and thermal properties of a novel protein-based plastic synthesized using subcritical water technology. Macromolecules 40(26):9371-9377.

Abdelmoez W, Yoshida H. 2013. Production of amino and organic acids from protein using sub-critical water technology. International Journal of Chemical Reactor Engineering 11(1):369-384.

Aguilera JM, Kessler HG. 1989. Properties of mixed and filled□type dairy gels. Journal of Food Science 54(5):1213-1217.

Aida TM, Oshima M, Smith Jr RL. 2017. Controlled Conversion of Proteins into High-Molecular-Weight Peptides without Additives with High-Temperature Water and Fast Heating Rates. ACS Sustainable Chemistry & Engineering 5(9):7709-7715.

Akhtar M, Hanif M, Ranjha N. 2016. Methods of synthesis of hydrogels… A review, Saudi Pharm. J 24(5):554-559.

Akiya N, Savage PE. 2002. Roles of water for chemical reactions in high-temperature water. Chemical reviews 102(8):2725-2750.

Alexis F, Pridgen EM, Langer R, Farokhzad OC. 2010. Nanoparticle technologies for cancer therapy. Drug delivery. Springer. p. 55-86.

Alfrey Jr T, Gurnee E, Lloyd W. Diffusion in glassy polymers. Journal of Polymer Science Part C: Polymer Symposia; 1966: Wiley Online Library. p. 249-261.

Alleoni ACC. 2006. Albumen protein and functional properties of gelation and foaming. Scientia Agricola 63(3):291-298.

Alvarez C, Rendueles M, Diaz M. 2012. The yield of peptides and amino acids following acid hydrolysis of haemoglobin from porcine blood. Animal Production Science 52(5):313-320.

Amdursky N, Mazo MM, Thomas MR, Humphrey EJ, Puetzer JL, St-Pierre J-P, Skaalure SC, Richardson RM, Terracciano CM, Stevens MM. 2018. Elastic serum-albumin based hydrogels: mechanism of formation and application in cardiac tissue engineering. Journal of Materials Chemistry B 6(35):5604-5612.

An F-F, Zhang X-H. 2017. Strategies for preparing albumin-based nanoparticles for multifunctional bioimaging and drug delivery. Theranostics 7(15):3667.

Angra PK, Rizvi SAA, Oettinger CW, D'Souza MJ. 2011. Novel approach for preparing nontoxic stealth microspheres for drug delivery. European Journal of Chemistry 2(2):125-129.

Arabi SH, Aghelnejad B, Schwieger C, Meister A, Kerth A, Hinderberger D. 2018. Serum albumin hydrogels in broad pH and temperature ranges: characterization of their self-assembled structures and nanoscopic and macroscopic properties. Biomaterials science 6(3):478-492.

Araujo L, Löbenberg R, Kreuter J. 1999. Influence of the surfactant concentration on the body distribution of nanoparticles. Journal of drug targeting 6(5):373-385.

Argast A, Tennis III CF. 2004. A web resource for the study of alkali feldspars and perthitic textures using light microscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy. Journal of geoscience education 52(3):213-217.

Ayala RS, De Castro ML. 2001. Continuous subcritical water extraction as a useful tool for isolation of edible essential oils. Food chemistry 75(1):109-113.

Bal A, Özkahraman B, Özbaş Z. 2016. Preparation and characterization of p H responsive poly (methacrylic acid□acrylamide□N□hydroxyethyl acrylamide) hydrogels for drug delivery systems. Journal of Applied Polymer Science 133(13).

Baler K, Michael R, Szleifer I, Ameer GA. 2014. Albumin hydrogels formed by electrostatically triggered self-assembly and their drug delivery capability. Biomacromolecules 15(10):3625-3633.

Banta S, Wheeldon IR, Blenner M. 2010. Protein engineering in the development of functional hydrogels. Annual review of biomedical engineering 12:167-186.

Bantz C, Koshkina O, Lang T, Galla H-J, Kirkpatrick CJ, Stauber RH, Maskos M. 2014. The surface properties of nanoparticles determine the agglomeration state and the size of the particles under physiological conditions. Beilstein journal of nanotechnology 5(1):1774-1786.

Betz M, García-González C, Subrahmanyam R, Smirnova I, Kulozik U. 2012. Preparation of novel whey protein-based aerogels as drug carriers for life science applications. The Journal of Supercritical Fluids 72:111-119.

Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76(5):965-977.

Bouklas N, Huang R. 2012. Swelling kinetics of polymer gels: comparison of linear and nonlinear theories. Soft Matter 8(31):8194-8203.

Boye J, Arcand Y. 2012. Green technologies in food production and processing. Springer Science & Business Media.

Boye JI, Alli I, Ismail AA. 1996. Interactions involved in the gelation of bovine serum albumin. Journal of Agricultural and Food Chemistry 44(4):996-1004.

Bronze-Uhle E, Costa B, Ximenes V, Lisboa-Filho P. 2017. Synthetic nanoparticles of bovine serum albumin with entrapped salicylic acid. Nanotechnology, science and applications 10:11.

Butler MF, Clark AH, Adams S. 2006. Swelling and mechanical properties of biopolymer hydrogels containing chitosan and bovine serum albumin. Biomacromolecules 7(11):2961-2970.

Buzea C, Pacheco II, Robbie K. 2007. Nanomaterials and nanoparticles: sources and toxicity. Biointerphases 2(4):MR17-MR71.

Cahyadi C, Heng PWS, Chan LW. 2011. Optimization of process parameters for a quasi-continuous tablet coating system using design of experiments. Aaps Pharmscitech 12(1):119-131.

Campoccia D, Montanaro L, Arciola CR. 2013. A review of the biomaterials technologies for infection-resistant surfaces. Biomaterials 34(34):8533-8554.

Carter DC, Ho JX. 1994. Structure of serum albumin. Advances in protein chemistry. Elsevier. p. 153-203.

Chakraborty R, Chatterjee S, Sarkar S, Chattopadhyay P. 2012. Study of photoinduced interaction between calf thymus-DNA and bovine serum albumin protein with H2Ti3O7 nanotubes. Journal of Biomaterials and Nanobiotechnology 3(04):462.

Chavanpatil MD, Khdair A, Patil Y, Handa H, Mao G, Panyam J. 2007. Polymer□surfactant nanoparticles for sustained release of water□soluble drugs. Journal of pharmaceutical sciences 96(12):3379-3389.

Chen L, Remondetto GE, Subirade M. 2006. Food protein-based materials as nutraceutical delivery systems. Trends in Food Science & Technology 17(5):272-283.

Cheng H, Zhu X, Zhu C, Qian J, Zhu N, Zhao L, Chen J. 2008. Hydrolysis technology of biomass waste to produce amino acids in sub-critical water. Bioresource technology 99(9):3337-3341.

Clark A, Kavanagh G, Ross-Murphy S. 2001. Globular protein gelation—theory and experiment. Food Hydrocolloids 15(4-6):383-400.

Clark A, Saunderson D, Suggett A. 1981. Infrared and laser□Raman spectroscopic studies of thermally□induced globular protein gels. International journal of peptide and protein research 17(3):353-364.

Clemente A, Vioque J, Sánchez□Vioque R, Pedroche J, Bautista J, Millán F. 2000. Factors affecting the in vitro protein digestibility of chickpea albumins. Journal of the Science of Food and Agriculture 80(1):79-84.

Cortes J, Saura C. 2010. Nanoparticle albumin-bound (nab™)-paclitaxel: improving efficacy and tolerability by targeted drug delivery in metastatic breast cancer. European Journal of Cancer Supplements 8(1):1-10.

Day YS, Myszka DG. 2003. Characterizing a drug's primary binding site on albumin. Journal of pharmaceutical sciences 92(2):333-343.

Desai N, Trieu V, Yao Z, Louie L, Ci S, Yang A, Tao C, De T, Beals B, Dykes D. 2006. Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. Clinical cancer research 12(4):1317-1324.

Dubey A, Boukouvala F, Keyvan G, Hsia R, Saranteas K, Brone D, Misra T, Ierapetritou MG, Muzzio FJ. 2012. Improvement of tablet coating uniformity using a quality by design approach. AAPS PharmSciTech 13(1):231-246.

Ehrick JD, Deo SK, Browning TW, Bachas LG, Madou MJ, Daunert S. 2005. Genetically engineered protein in hydrogels tailors stimuli-responsive characteristics. Nature materials 4(4):298.

El□Zahar K, Chobert JM, Sitohy M, Dalgalarrondo M, Haertlé T. 2003. Proteolytic degradation of ewe milk proteins during fermentation of yoghurts and storage. Food/Nahrung 47(3):199-206.

Elzoghby AO, Elgohary MM, Kamel NM. 2015. Implications of protein-and Peptide-based nanoparticles as potential vehicles for anticancer drugs. Advances in protein chemistry and structural biology. Elsevier. p. 169-221.

Elzoghby AO, Samy WM, Elgindy NA. 2012. Albumin-based nanoparticles as potential controlled release drug delivery systems. Journal of controlled release 157(2):168-182.

Espinoza AD, Morawicki RO, Hager T. 2012. Hydrolysis of whey protein isolate using subcritical water. Journal of food science 77(1):C20-C26.

Esteban M, García A, Ramos P, Márquez M. 2008. Kinetics of amino acid production from hog hair by hydrolysis in sub-critical water. The Journal of Supercritical Fluids 46(2):137-141.

Esteban M, García A, Ramos P, Márquez M. 2010. Sub-critical water hydrolysis of hog hair for amino acid production. Bioresource technology 101(7):2472-2476.

Faisal M, Sato N, Quitain AT, Daimon H, Fujie K. 2005. Hydrolysis and cyclodehydration of dipeptide under hydrothermal conditions. Industrial & engineering chemistry research 44(15):5472-5477.

Fard Masoumi HR, Basri M, Kassim A, Kuang Abdullah D, Abdollahi Y, Abd Gani SS, Rezaee M. 2013. Statistical optimization of process parameters for lipase-catalyzed synthesis of triethanolamine-based esterquats using response surface methodology in 2-liter bioreactor. The Scientific World Journal 2013.

Farris S, Schaich KM, Liu L, Piergiovanni L, Yam KL. 2009. Development of polyion-complex hydrogels as an alternative approach for the production of bio-based polymers for food packaging applications: a review. Trends in food science & technology 20(8):316-332.

Ferry JD. 1948. Protein gels. Advances in protein chemistry. Elsevier. p. 1-78.

Fukushima Y. 1999. Application of supercritical fluids. R&D Review of Toyota CRDL 35(1):1-9.

Galisteo-González F, Molina-Bolívar J. 2014. Systematic study on the preparation of BSA nanoparticles. Colloids and Surfaces B: Biointerfaces 123:286-292.

Galkin AA, Lunin VV. 2005. Subcritical and supercritical water: a universal medium for chemical reactions. Russian Chemical Reviews 74(1):21.

Galooyak SS, Dabir B. 2015. Three-factor response surface optimization of nano-emulsion formation using a microfluidizer. Journal of food science and technology 52(5):2558-2571.

Garcia-Moscoso JL, Teymouri A, Kumar S. 2015. Kinetics of peptides and arginine production from microalgae (Scenedesmus sp.) by flash hydrolysis. Industrial & Engineering Chemistry Research 54(7):2048-2058.

Gault P, Korolczuk J. Heat gelation of whey proteins. Protein and fat globule modifications by heat treatment, homogenization and other technological means for high quality dairy products. IDF Seminar, Munich (Germany), 25-28 Aug 1992; 1993: FIL-IDF.

Gharekhani H, Olad A, Mirmohseni A, Bybordi A. 2017. Superabsorbent hydrogel made of NaAlg-g-poly (AA-co-AAm) and rice husk ash: Synthesis, characterization, and swelling kinetic studies. Carbohydrate polymers 168:1-13.

Gilmour SG. 2006. Response surface designs for experiments in bioprocessing. Biometrics 62(2):323-331.

Global Cancer Observatory. Available from: https://gco.iarc.fr/

Gong Y, Zhang X, He L, Yan Q, Yuan F, Gao Y. 2015. Optimization of subcritical water extraction parameters of antioxidant polyphenols from sea buckthorn (Hippophaë rhamnoides L.) seed residue. Journal of food science and technology 52(3):1534-1542.

Gunasekaran S, Ko S, Xiao L. 2007. Use of whey proteins for encapsulation and controlled delivery applications. Journal of Food Engineering 83(1):31-40.

Guzman-Villanueva D, Smyth HD, Herrera-Ruiz D, El-Sherbiny IM. 2011. A novel aerosol method for the production of hydrogel particles. Journal of nanomaterials 2011:5.

Harvey D. 2000. Modern analytical chemistry. Boston: McGraw-Hill Companies, Inc.

Hawkins MJ, Soon-Shiong P, Desai N. 2008. Protein nanoparticles as drug carriers in clinical medicine. Advanced drug delivery reviews 60(8):876-885.

Hietala DC, Faeth JL, Savage PE. 2016. A quantitative kinetic model for the fast and isothermal hydrothermal liquefaction of Nannochloropsis sp. Bioresource technology 214:102-111.

Horn D, Rieger J. 2001. Organic nanoparticles in the aqueous phase—theory, experiment, and use. Angewandte Chemie International Edition 40(23):4330-4361.

Hu H, Yang H, Huang P, Cui D, Peng Y, Zhang J, Lu F, Lian J, Shi D. 2010. Unique role of ionic liquid in microwave-assisted synthesis of monodisperse magnetite nanoparticles. Chemical Communications 46(22):3866-3868.

Huang BX, Kim H-Y, Dass C. 2004. Probing three-dimensional structure of bovine serum albumin by chemical cross-linking and mass spectrometry. Journal of the American Society for Mass Spectrometry 15(8):1237-1247.

Huang P, Li Z, Hu H, Cui D. 2010. Synthesis and characterization of bovine serum albumin-conjugated copper sulfide nanocomposites. Journal of Nanomaterials 2010:33.

Huang Y, Zhang B, Xu G, Hao W. 2013. Swelling behaviours and mechanical properties of silk fibroin–polyurethane composite hydrogels. Composites Science and Technology 84:15-22.

Iemma F, Spizzirri U, Puoci F, Muzzalupo R, Trombino S, Picci N. 2005. Radical crosslinked albumin microspheres as potential drug delivery systems: preparation and in vitro studies. Drug delivery 12(3):179-184.

Irache J, Merodio M, Arnedo A, Camapanero M, Mirshahi M, Espuelas S. 2005. Albumin nanoparticles for the intravitreal delivery of anticytomegaloviral drugs. Mini reviews in medicinal chemistry 5(3):293-305.

Itoh T, Wada Y, Nakanishi T. 1976. Differential thermal analysis of milk proteins. Agricultural and Biological Chemistry 40(6):1083-1086.

Jahanshahi M, Babaei Z. 2008. Protein nanoparticle: a unique system as drug delivery vehicles. African Journal of Biotechnology 7(25).

Jayasinghe P, Hawboldt K. 2012. A review of bio-oils from waste biomass: Focus on fish processing waste. Renewable and sustainable energy reviews 16(1):798-821.

Jian H, Xiong YL, Guo F, Huang X, Adhikari B, Chen J. 2014. Gelation enhancement of soy protein isolate by sequential low□and ultrahigh□temperature two□stage preheating treatments. International journal of food science & technology 49(12):2529-2537.

Jithan A, Madhavi K, Madhavi M, Prabhakar K. 2011. Preparation and characterization of albumin nanoparticles encapsulating curcumin intended for the treatment of breast cancer. International journal of pharmaceutical investigation 1(2):119.

Joye IJ, McClements DJ. 2013. Production of nanoparticles by anti-solvent precipitation for use in food systems. Trends in food science & technology 34(2):109-123.

Jun JY, Nguyen HH, Chun HS, Kang B-C, Ko S. 2011. Preparation of size-controlled bovine serum albumin (BSA) nanoparticles by a modified desolvation method. Food chemistry 127(4):1892-1898.

Kang K-Y, Chun B-S. 2004. Behavior of amino acid production from hydrothermal treatment of fish-derived wastes. Korean Journal of Chemical Engineering 21(6):1147-1152.

Kang K, Quitain AT, Daimon H, Noda R, Goto N, Hu HY, Fujie K. 2001. Optimization of amino acids production from waste fish entrails by hydrolysis in sub and supercritical water. The Canadian Journal of Chemical Engineering 79(1):65-70.

Katime I, Velada J, Novoa R, de Apodaca ED, Puig J, Mendizabal E. 1996. Swelling kinetics of poly (acrylamide)/poly (mono□n□alkyl itaconates) hydrogels. Polymer international 40(4):281-286.

Khanbabaie R, Jahanshahi M. 2012. Revolutionary impact of nanodrug delivery on neuroscience. Current neuropharmacology 10(4):370-392.

Kim M, Tang S, Olsen BD. 2013. Physics of engineered protein hydrogels. Journal of Polymer Science Part B: Polymer Physics 51(7):587-601.

Kim TH, Jiang HH, Youn YS, Park CW, Tak KK, Lee S, Kim H, Jon S, Chen X, Lee KC. 2011. Preparation and characterization of water-soluble albumin-bound curcumin nanoparticles with improved antitumor activity. International journal of pharmaceutics 403(1-2):285-291.

Kommareddy S, Amiji M. 2007. Poly (ethylene glycol)–modified thiolated gelatin nanoparticles for glutathione-responsive intracellular DNA delivery. Nanomedicine: nanotechnology, biology and medicine 3(1):32-42.

Kou L, Sun J, Zhai Y, He Z. 2013. The endocytosis and intracellular fate of nanomedicines: Implication for rational design. Asian Journal of Pharmaceutical Sciences 8(1):1-10.

Kouchakzadeh H, Shojaosadati SA, Maghsoudi A, Farahani EV. 2010. Optimization of PEGylation conditions for BSA nanoparticles using response surface methodology. Aaps Pharmscitech 11(3):1206-1211.

Kratz F. 2008. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. Journal of controlled release 132(3):171-183.

Kronholm J, Hartonen K, Riekkola M-L. 2007. Analytical extractions with water at elevated temperatures and pressures. TrAC Trends in Analytical Chemistry 26(5):396-412.

Labhasetwar V, Song C, Humphrey W, Shebuski R, Levy RJ. 1998. Arterial uptake of biodegradable nanoparticles: effect of surface modifications. Journal of pharmaceutical sciences 87(10):1229-1234.

Lad MD, Birembaut F, Matthew JM, Frazier RA, Green RJ. 2006. The adsorbed conformation of globular proteins at the air/water interface. Physical Chemistry Chemical Physics 8(18):2179-2186.

Lamoolphak W, De-Eknamkul W, Shotipruk A. 2008. Hydrothermal production and characterization of protein and amino acids from silk waste. Bioresource technology 99(16):7678-7685.

Langer K, Anhorn M, Steinhauser I, Dreis S, Celebi D, Schrickel N, Faust S, Vogel V. 2008. Human serum albumin (HSA) nanoparticles: reproducibility of preparation process and kinetics of enzymatic degradation. International journal of pharmaceutics 347(1-2):109-117.

Langer K, Balthasar S, Vogel V, Dinauer N, Von Briesen H, Schubert D. 2003. Optimization of the preparation process for human serum albumin (HSA) nanoparticles. International journal of pharmaceutics 257(1-2):169-180.

Langton M, Hermansson A-M. 1992. Fine-stranded and particulate gels of β-lactoglobulin and whey protein at varying pH. Food Hydrocolloids 5(6):523-539.

Lee SH, Heng D, Ng WK, Chan H-K, Tan RB. 2011. Nano spray drying: a novel method for preparing protein nanoparticles for protein therapy. International journal of pharmaceutics 403(1-2):192-200.

Lewis LM, Johnson RE, Oldroyd ME, Ahmed SS, Joseph L, Saracovan I, Sinha S. 2010. Characterizing the freeze–drying behavior of model protein formulations. Aaps Pharmscitech 11(4):1580-1590.

Li D, Kaner RB. 2006. Shape and aggregation control of nanoparticles: not shaken, not stirred. Journal of the American Chemical Society 128(3):968-975.

Li H, Zhao L, Chen XD, Mercadé-Prieto R. 2016. Swelling of whey and egg white protein hydrogels with stranded and particulate microstructures. International journal of biological macromolecules 83:152-159.

Li S-D, Huang L. 2010. Stealth nanoparticles: high density but sheddable PEG is a key for tumor targeting. Journal of controlled release: official journal of the Controlled Release Society 145(3):178.

Lim J, Yeap SP, Che HX, Low SC. 2013. Characterization of magnetic nanoparticle by dynamic light scattering. Nanoscale research letters 8(1):381.

Lin H, Sritham E, Lim S, Cui Y, Gunasekaran S. 2010. Synthesis and characterization of pH□and salt□sensitive hydrogel based on chemically modified poultry feather protein isolate. Journal of applied polymer science 116(1):602-609.

Lin V, Koenig J. 1976. Raman studies of bovine serum albumin. Biopolymers: Original Research on Biomolecules 15(1):203-218.

Liu Q, Wang X, De Kee D. 2005. Mass transport through swelling membranes. International Journal of Engineering Science 43(19-20):1464-1470.

Liu WR, Langer R, Klibanov AM. 1991. Moisture□induced aggregation of lyophilized proteins in the solid state. Biotechnology and bioengineering 37(2):177-184.

Liu Z, Chen X. 2016. Simple bioconjugate chemistry serves great clinical advances: albumin as a versatile platform for diagnosis and precision therapy. Chemical Society Reviews 45(5):1432-1456.

Lomis N, Westfall S, Farahdel L, Malhotra M, Shum-Tim D, Prakash S. 2016. Human serum albumin nanoparticles for use in cancer drug delivery: process optimization and in vitro characterization. Nanomaterials 6(6):116.

Lundstedt T, Seifert E, Abramo L, Thelin B, Nyströma Å. 1998. Experimental design and optimization Chemometrics and Intelligent Laboratory Systems 42_. Volume 42:1-2.

Ma J, Guan R, Chen X, Wang Y, Hao Y, Ye X, Liu M. 2014. Response surface methodology for the optimization of beta-lactoglobulin nano-liposomes. Food & function 5(4):748-754.

Manzano M, Vallet-Regí M. 2018. Mesoporous silica nanoparticles in nanomedicine applications. Journal of Materials Science: Materials in Medicine 29(5):65.

Martins JT, Bourbon AI, Pinheiro AC, Fasolin LH, Vicente AA. 2018. Protein-based structures for food applications: from macro to nanoscale. Front. Sustain. Food Syst 2:77.

Masoumi HRF, Kassim A, Basri M, Abdullah DK. 2011. Determining optimum conditions for lipase-catalyzed synthesis of triethanolamine (TEA)-based esterquat cationic surfactant by a Taguchi robust design method. Molecules 16(6):4672-4680.

McCabe BK, Harris PW, Schmidt T, Antille DL, Lee S, Hill A, Baillie CP. Bioenergy and bioproducts in the Australian red meat processing industry: A case study. 2018 ASABE Annual International Meeting; 2018: American Society of Agricultural and Biological Engineers. p. 1.

McMillan J, Batrakova E, Gendelman HE. 2011. Cell delivery of therapeutic nanoparticles. Progress in molecular biology and translational science. Elsevier. p. 563-601.

Mercadé-Prieto R, Coignaud M, Jin Y, Jeantet R, Chen XD. 2018. Dissolution of bovine serum albumin hydrogels in alkali. Food Hydrocolloids 77:598-606.

Micic M, Stamenic D, Suljovrujic E. 2012. Radiation-induced synthesis and swelling properties of p (2-hydroxyethyl methacrylate/itaconic acid/oligo (ethylene glycol) acrylate) terpolymeric hydrogels. Radiation Physics and Chemistry 81(9):1451-1455.

Mie A, Andersen HR, Gunnarsson S, Kahl J, Kesse-Guyot E, Rembiałkowska E, Quaglio G, Grandjean P. 2017. Human health implications of organic food and organic agriculture: a comprehensive review. Environmental Health 16(1):111.

Mohtashamian S, Boddohi S, Hosseinkhani S. 2018. Preparation and optimization of self-assembled chondroitin sulfate-nisin nanogel based on quality by design concept. International journal of biological macromolecules 107:2730-2739.

Moumita K, Ray RR. 2011. Optimization of production conditions of extra cellular β-glucosidase in submerged fermentation of water hyacinth using response surface methodology by Rhizopus oryzae MTCC 9642. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2(2):299-308.

Muralidhar RV, Chirumamila R, Marchant R, Nigam P. 2001. A response surface approach for the comparison of lipase production by Candida cylindracea using two different carbon sources. Biochemical Engineering Journal 9(1):17-23.

Nagesh B, Jeevani E, Sujana V, Damaraju B, Sreeha K, Ramesh P. 2016. Scanning electron microscopy (SEM) evaluation of sealing ability of MTA and EndoSequence as root-end filling materials with chitosan and carboxymethyl chitosan (CMC) as retrograde smear layer removing agents. Journal of conservative dentistry: JCD 19(2):143.

Naveenraj S, Anandan S. 2013. Binding of serum albumins with bioactive substances–nanoparticles to drugs. Journal of Photochemistry and Photobiology C: Photochemistry Reviews 14:53-71.

Nguyen HH, Ko S. Preparation of size-controlled BSA nanoparticles by intermittent addition of desolvating agent. The Third International Conference on the Development of Biomedical Engineering in Vietnam; 2010: Springer. p. 231-234.

Niemeyer CM. 2001. Nanoparticles, proteins, and nucleic acids: biotechnology meets materials science. Angewandte Chemie International Edition 40(22):4128-4158.

Nitta SK, Numata K. 2013. Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering. International journal of molecular sciences 14(1):1629-1654.

Noorani L, Stenzel M, Liang R, Pourgholami MH, Morris DL. 2015. Albumin nanoparticles increase the anticancer efficacy of albendazole in ovarian cancer xenograft model. Journal of nanobiotechnology 13(1):25.

Nyman DW, Campbell KJ, Hersh E, Long K, Richardson K, Trieu V, Desai N, Hawkins MJ, Von Hoff DD. 2005. Phase I and pharmacokinetics trial of ABI-007, a novel nanoparticle formulation of paclitaxel in patients with advanced nonhematologic malignancies. Journal of clinical oncology 23(31):7785-7793.

Omidian H, Hashemi S, Sammes P, Meldrum I. 1999. Modified acrylic-based superabsorbent polymers (dependence on particle size and salinity). Polymer 40(7):1753-1761.

Oss-Ronen L, Seliktar D. 2011. Polymer-conjugated albumin and fibrinogen composite hydrogels as cell scaffolds designed for affinity-based drug delivery. Acta biomaterialia 7(1):163-170.

Panyam J, Labhasetwar V. 2003. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Advanced drug delivery reviews 55(3):329-347.

Park SE, Nho YC, Lim YM, Kim HI. 2004. Preparation of pH□sensitive poly (vinyl alcohol□g□methacrylic acid) and poly (vinyl alcohol□g□acrylic acid) hydrogels by gamma ray irradiation and their insulin release behavior. Journal of applied polymer science 91(1):636-643.

Patil GV. 2003. Biopolymer albumin for diagnosis and in drug delivery. Drug development research 58(3):219-247.

Patra S, Santhosh K, Pabbathi A, Samanta A. 2012. Diffusion of organic dyes in bovine serum albumin solution studied by fluorescence correlation spectroscopy. RSC Advances 2(14):6079-6086.

Patravale V, Date AA, Kulkarni R. 2004. Nanosuspensions: a promising drug delivery strategy. Journal of pharmacy and pharmacology 56(7):827-840.

Paunović DĐ, Mitić SS, Kostić DA, Mitić MN, Stojanović BT, Pavlović JL. 2014. Kinetics and thermodynamics of the solid-liquid extraction process of total polyphenols from barley. Savremene tehnologije 3(2):58-63.

Peppas N, Huang Y, Torres-Lugo M, Ward J, Zhang J. 2000. Physicochemical foundations and structural design of hydrogels in medicine and biology. Annual review of biomedical engineering 2(1):9-29.

Peppas NA, Hilt JZ, Khademhosseini A, Langer R. 2006. Hydrogels in biology and medicine: from molecular principles to bionanotechnology. Advanced materials 18(11):1345-1360.

Peters T. 1996. All About Albumin Academic Press. Inc. San Diego, CA.

Plaza M, Turner C. 2015. Pressurized hot water extraction of bioactives. TrAC Trends in Analytical Chemistry 71:39-54.

Pogaku R, Bono A, Chu C. 2013. Developments in Sustainable Chemical and Bioprocess Technology.

Pourjavadi A, Barzegar S, Zeidabadi F. 2007a. Synthesis and properties of biodegradable hydrogels of κ-carrageenan grafted acrylic acid-co-2-acrylamido-2-methylpropanesulfonic acid as candidates for drug delivery systems. Reactive and functional Polymers 67(7):644-654.

Pourjavadi A, Mahdavinia GR. 2006. Superabsorbency, pH-sensitivity and swelling kinetics of partially hydrolyzed chitosan-g-poly (acrylamide) hydrogels. Turkish Journal of Chemistry 30(5):595-608.

Pourjavadi A, Salimi H. 2008. New protein-based hydrogel with superabsorbing properties: effect of monomer ratio on swelling behavior and kinetics. Industrial & Engineering Chemistry Research 47(23):9206-9213.

Pourjavadi A, Salimi H, Kurdtabar M. 2007b. Hydrolyzed collagen□based hydrogel with salt and pH□responsiveness properties. Journal of applied polymer science 106(4):2371-2379.

Prokop A, Davidson JM. 2008. Nanovehicular intracellular delivery systems. Journal of pharmaceutical sciences 97(9):3518-3590.

Q. Li JH, B. Lu, H. Yao, WG Zhang, F. 2001. Ciprofloxacin-loaded bovine serum albumin microspheres: preparation and drug-release in vitro. Journal of microencapsulation 18(6):825-829.

Qi W, He Z. 2006. Enzymatic hydrolysis of protein: mechanism and kinetic model. Frontiers of Chemistry in China 1(3):308-314.

Quitain AT, Daimon H, Fujie K, Katoh S, Moriyoshi T. 2006. Microwave-assisted hydrothermal degradation of silk protein to amino acids. Industrial & engineering chemistry research 45(13):4471-4474.

Rahimnejad M, Jahanshahi M, Najafpour G. 2006. Production of biological nanoparticles from bovine serum albumin for drug delivery. African Journal of Biotechnology 5(20).

Rahimnejad M, Najafpour G, Bakeri G. 2012. Investigation and modeling effective parameters influencing the size of BSA protein nanoparticles as colloidal carrier. Colloids and Surfaces A: Physicochemical and Engineering Aspects 412:96-100.

Rajagopal K. 2003. Diffusion through polymeric solids undergoing large deformations. Materials science and technology 19(9):1175-1180.

Reis CP, Neufeld RJ, Ribeiro AJ, Veiga F. 2006. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine 2(1):8-21.

Ritger PL, Peppas NA. 1987. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. Journal of controlled release 5(1):37-42.

Rogalinski T, Herrmann S, Brunner G. 2005. Production of amino acids from bovine serum albumin by continuous sub-critical water hydrolysis. The Journal of Supercritical Fluids 36(1):49-58.

Rogalinski T, Liu K, Albrecht T, Brunner G. 2008. Hydrolysis kinetics of biopolymers in subcritical water. The Journal of Supercritical Fluids 46(3):335-341.

Roser M, Fischer D, Kissel T. 1998. Surface-modified biodegradable albumin nano- and microspheres. II: effect of surface charges on in vitro phagocytosis and biodistribution in rats. European Journal of Pharmaceutics and Biopharmaceutics 46(3):255-263.

Rüegg M, Moor U, Blanc B. 1977. A calorimetric study of the thermal denaturation of whey proteins in simulated milk ultrafiltrate. Journal of Dairy Research 44(3):509-520.

Sadeghi M, Hamzeh A. Novel crosslink graft copolymer of methacrylic acid and collagen as a protein-based sperabsorbert hydrogel with salt and pH-responsiveness properties. AIP Conference Proceedings; 2008: AIP. p. 348-350.

Sadeghi M, Hosseinzadeh H. 2010. Synthesis and super-swelling behavior of a novel low salt-sensitive protein-based superabsorbent hydrogel: collagen-g-poly (AMPS). Turkish Journal of Chemistry 34(5):739-752.

Salata OV. 2004. Applications of nanoparticles in biology and medicine. Journal of nanobiotechnology 2(1):3.

Salt DW, Ajmani S, Crichton R, Livingstone DJ. 2007. An improved approximation to the estimation of the critical F values in best subset regression. Journal of chemical information and modeling 47(1):143-149.

Sánchez-Segura L, Ochoa-Alejo N, Carriles R, Zavala-García LE. 2018. Development of bovine serum albumin–capsaicin nanoparticles for biotechnological applications. Applied Nanoscience 8(8):1877-1886.

Santhi K, Dhanaraj S, Joseph V, Ponnusankar S, Suresh B. 2002. A study on the preparation and anti-tumor efficacy of bovine serum albumin nanospheres containing 5-fluorouracil. Drug development and industrial pharmacy 28(9):1171-1179.

Sato N, Quitain AT, Kang K, Daimon H, Fujie K. 2004. Reaction kinetics of amino acid decomposition in high-temperature and high-pressure water. Industrial & Engineering Chemistry Research 43(13):3217-3222.

Schott H. 1992. Kinetics of swelling of polymers and their gels. Journal of pharmaceutical sciences 81(5):467-470.

Selmane D, Christophe V, Gholamreza D. 2008. Extraction of proteins from slaughterhouse by-products: Influence of operating conditions on functional properties. Meat Science 79(4):640-647.

Severin S, Xia W. 2006. Enzymatic hydrolysis of whey proteins by two different proteases and their effect on the functional properties of resulting protein hydrolysates. Journal of Food Biochemistry 30(1):77-97.

Sheehan JD, Savage PE. 2017. Molecular and Lumped Products from Hydrothermal Liquefaction of Bovine Serum Albumin. ACS Sustainable Chemistry & Engineering 5(11):10967-10975.

Shi J-h, Pan D-q, Wang X-x, Liu T-T, Jiang M, Wang Q. 2016. Characterizing the binding interaction between antimalarial artemether (AMT) and bovine serum albumin (BSA): Spectroscopic and molecular docking methods. Journal of Photochemistry and Photobiology B: Biology 162:14-23.

Simon M. 2003. Concrete mixture optimization using statistical methods. United States. Federal Highway Administration. Office of Infrastructure ….

Siracusa V, Rocculi P, Romani S, Dalla Rosa M. 2008. Biodegradable polymers for food packaging: a review. Trends in Food Science & Technology 19(12):634-643.

Stern T, Lamas M, Benita S. 2002. Design and characterization of protein-based microcapsules as a novel catamenial absorbent system. International journal of pharmaceutics 242(1-2):185-190.

Subia B, Kundu S. 2012. Drug loading and release on tumor cells using silk fibroin–albumin nanoparticles as carriers. Nanotechnology 24(3):035103.

Sun XD, Holley RA. 2011. Factors influencing gel formation by myofibrillar proteins in muscle foods. Comprehensive Reviews in Food Science and Food Safety 10(1):33-51.

Sun Y, Huang Y. 2016. Disulfide-crosslinked albumin hydrogels. Journal of Materials Chemistry B 4(16):2768-2775.

Sunphorka S, Chavasiri W, Oshima Y, Ngamprasertsith S. 2012. Kinetic studies on rice bran protein hydrolysis in subcritical water. The Journal of Supercritical Fluids 65:54-60.

Sykes EA, Dai Q, Sarsons CD, Chen J, Rocheleau JV, Hwang DM, Zheng G, Cramb DT, Rinker KD, Chan WC. 2016. Tailoring nanoparticle designs to target cancer based on tumor pathophysiology. Proceedings of the National Academy of Sciences 113(9):E1142-E1151.

Takakura Y, Fujita T, Hashida M, Sezaki H. 1990. Disposition characteristics of macromolecules in tumor-bearing mice. Pharmaceutical research 7(4):339-346.

Takeda K, Wada A, Yamamoto K, Moriyama Y, Aoki K. 1989. Conformational change of bovine serum albumin by heat treatment. Journal of Protein Chemistry 8(5):653-659.

Taktak F, Yildiz M, Sert H, Soykan C. 2015. A novel triple-responsive hydrogels based on 2-(dimethylamino) ethyl methacrylate by copolymerization with 2-(N-morpholino) ethyl methacrylate. Journal of Macromolecular Science, Part A 52(1):39-46.

Tarhini M, Benlyamani I, Hamdani S, Agusti G, Fessi H, Greige-Gerges H, Bentaher A, Elaissari A. 2018. Protein-based nanoparticle preparation via nanoprecipitation method. Materials 11(3):394.

Tarhini M, Greige-Gerges H, Elaissari A. 2017. Protein-based nanoparticles: From preparation to encapsulation of active molecules. International journal of pharmaceutics 522(1-2):172-197.

Tavakoli O, Yoshida H. 2006. Conversion of scallop viscera wastes to valuable compounds using sub-critical water. Green Chemistry 8(1):100-106.

Teo CC, Tan SN, Yong JWH, Hew CS, Ong ES. 2008. Evaluation of the extraction efficiency of thermally labile bioactive compounds in Gastrodia elata Blume by pressurized hot water extraction and microwave-assisted extraction. Journal of Chromatography A 1182(1):34-40.

Teo W-E, Ramakrishna S. 2009. Electrospun nanofibers as a platform for multifunctional, hierarchically organized nanocomposite. Composites Science and Technology 69(11-12):1804-1817.

Teófilo RF, Ferreira M. 2006. Quimiometria II: planilhas eletrônicas para cálculos de planejamentos experimentais, um tutorial. Química Nova.

Tian Z-Y, Song L-N, Zhao Y, Zang F-L, Zhao Z-H, Chen N-H, Xu X-J, Wang C-J. 2015. Spectroscopic study on the interaction between naphthalimide-polyamine conjugates and bovine serum albumin (BSA). Molecules 20(9):16491-16523.

Traub RE, Rowley GL. 1991. Understanding Reliability: An NCME Instructional Module on. Educational measurement: Issues and practice 10(1):37-45.

Treuheit NA, Komives EA. 2012. Probing the Pro-and Anti-Coagulant Interactions of Thrombin. Biophysical Journal 102(3):50a.

Uematsu M, Frank E. 1980. Static dielectric constant of water and steam. Journal of Physical and Chemical Reference Data 9(4):1291-1306.

Ulbrich K, Michaelis M, Rothweiler F, Knobloch T, Sithisarn P, Cinatl J, Kreuter J. 2011. Interaction of folate-conjugated human serum albumin (HSA) nanoparticles with tumour cells. International journal of pharmaceutics 406(1-2):128-134.

Uthayakumar S. 2013. Biomedical optical spectroscopy techniques for diagnosis of human saliva sample. Asian Journal of Biomedical and Pharmaceutical Sciences 3(24):12.

Van Vlierberghe S, Dubruel P, Schacht E. 2011a. Biopolymer-based hydrogels as scaffolds for tissue engineering applications: a review. Biomacromolecules 12(5):1387-1408.

Van Vlierberghe S, Schacht E, Dubruel P. 2011b. Reversible gelatin-based hydrogels: finetuning of material properties. European Polymer Journal 47(5):1039-1047.

Vetri V, Librizzi F, Leone M, Militello V. 2007. Thermal aggregation of bovine serum albumin at different pH: comparison with human serum albumin. European Biophysics Journal 36(7):717-725.

Viganó J, da Fonseca Machado AP, Martínez J. 2015. Sub-and supercritical fluid technology applied to food waste processing. The Journal of Supercritical Fluids 96:272-286.

Wang G, Siggers K, Zhang S, Jiang H, Xu Z, Zernicke RF, Matyas J, Uludağ H. 2008. Preparation of BMP-2 containing bovine serum albumin (BSA) nanoparticles stabilized by polymer coating. Pharmaceutical research 25(12):2896-2909.

Wang W, Kang Y, Wang A. 2010. Synthesis, characterization and swelling properties of guar gum-g-poly (sodium acrylate-co-styrene)/muscovite superabsorbent composites. Science and technology of advanced materials 11(2):025006.

Williams DF. 2009. On the nature of biomaterials. Biomaterials 30(30):5897-5909.

Yan C, Pochan DJ. 2010. Rheological properties of peptide-based hydrogels for biomedical and other applications. Chemical Society Reviews 39(9):3528-3540.

Yang L, Qu H, Mao G, Zhao T, Li F, Zhu B, Zhang B, Wu X. 2013. Optimization of subcritical water extraction of polysaccharides from Grifola frondosa using response surface methodology. Pharmacognosy magazine 9(34):120.

Yedomon B, Fessi H, Charcosset C. 2013. Preparation of Bovine Serum Albumin (BSA) nanoparticles by desolvation using a membrane contactor: A new tool for large scale production. European Journal of Pharmaceutics and Biopharmaceutics 85(3):398-405.

Yoshida H, Tavakoli O. 2004. Sub-critical water hydrolysis treatment for waste squid entrails and production of amino acids, organic acids, and fatty acids. Journal of chemical engineering of Japan 37(2):253-260.

Yu L, Yan D, Sun G, Gu L. 2008. Preparation and characterization of pH□sensitive hydrogel fibers based on hydrolyzed□polyacrylonitrile/soy protein. Journal of applied polymer science 108(2):1100-1108.

Yu X, Wei M. 2011. Controlling bovine serum albumin release from biomimetic calcium phosphate coatings. Journal of Biomaterials and Nanobiotechnology 2(01):28.

Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, Jain RK. 1995. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. Cancer research 55(17):3752-3756.

Zhang B, Cui Y, Yin G, Li X. 2012. Adsorption of copper (II) and lead (II) ions onto cottonseed protein-PAA hydrogel composite. Polymer-Plastics Technology and Engineering 51(6):612-619.

Zhang J, Lan CQ, Post M, Simard B, Deslandes Y, Hsieh TH. 2006. Design of nanoparticles as drug carriers for cancer therapy. Cancer Genomics-Proteomics 3(3-4):147-157.

Zhang L, Gu F, Chan J, Wang A, Langer R, Farokhzad O. 2008. Nanoparticles in medicine: therapeutic applications and developments. Clinical pharmacology & therapeutics 83(5):761-769.

Zhang Z, Chen L, Deng M, Bai Y, Chen X, Jing X. 2011. Biodegradable thermo□and pH□responsive hydrogels for oral drug delivery. Journal of Polymer Science Part A: Polymer Chemistry 49(13):2941-2951.

Zhao D, Zhao X, Zu Y, Li J, Zhang Y, Jiang R, Zhang Z. 2010. Preparation, characterization, and in vitro targeted delivery of folate-decorated paclitaxel-loaded bovine serum albumin nanoparticles. International journal of nanomedicine 5:669.

Zhu F, Cai YZ, Ke J, Corke H. 2010. Compositions of phenolic compounds, amino acids and reducing sugars in commercial potato varieties and their effects on acrylamide formation. Journal of the Science of Food and Agriculture 90(13):2254-2262.

Zhu J, Marchant RE. 2011. Design properties of hydrogel tissue-engineering scaffolds. Expert review of medical devices 8(5):607-626.

Zu Y, Zhang Y, Zhao X, Zhang Q, Liu Y, Jiang R. 2009. Optimization of the preparation process of vinblastine sulfate (VBLS)-loaded folateconjugated bovine serum albumin (BSA) nanoparticles for tumor-targeted drug delivery using response surface methodology (RSM). International journal of nanomedicine 4:321.

BIODATA OF STUDENT

This thesis has been conducted by Zahra Maghareh Esfahan who was born on 15th January 1979 in Tehran, Iran. She received her high school Diploma in Mathematics-Physics from Noorhedayat High School in 1998. She continued her education by studying in Chemical Engineering at Islamic Azad University of Science and

Research (Tehran, Iran). She received her bachelor degree in 2000. She got her master degree in Chemical Engineering from Islamic Azad University of Science and Research in 2006. Along with her study, she worked as lecturer in Islamic Azad University and R&D manager of Kimia factory (biodegradable granules) in Tehran. During these years she studied effect of SCWT condition on protein-based bioproducts, and published several articles in peer-reviewed journals. In 2012, she started her PhD. at Chemical and environmental Engineering, UPM, under supervision of Ir. Dr. Shamsul Izhar. Her research focuses on biomaterials preparation methods and SCWT method as a novel substitution method. Her area of interest includes optimization and technology development of producing biomaterials.

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