การปลดปล่อยแบบควบคุมของลิโดเคนและไดโคลฟีแนกจากอนุภาคไคโตซาน

นางสาวรัตนา ไม้โภคทรัพย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2550

CONTROLLED RELEASE OF LIDOCAINE AND DICLOFENAC FROM CHITOSAN PARTICLES

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2007

Thesis Title	CONTROLLED RELEASE OF LIDOCAINE AND DICLOFENAC FROM CHITOSAN PARTICLES
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รัตนา ไม้โภคทรัพย์ : การปลดปล่อยแบบควบคุมของลิโดเคนและไดโคลฟีแนกจากอนุภาคไคโตซาน (CONTROLLED RELEASE OF LIDOCAINE AND DICLOFENAC FROM CHITOSAN PARTICLES) อ. ที่ปรึกษา: รศ. ดร. มงคล สุขวัฒนาสินทธิ์, 63 หน้า.

ไคโตซานถูกนำมาใช้ในการศึกษาการควบคุมการปลดปล่อยยา 2 ชนิด คือ ลิโดเคน และ ไดโคลฟีแนก อนุภาคไคโตซานถูกเตรียมขึ้นโดยใช้พอลิแอนไอออนเป็นสารเชื่อมขวางด้วยวิธีตกตะกอน อนุภาคของไคโตซานที่มีการบรรจุยาแล้วถูกศึกษาในด้านของขนาดอนุภาค,สรีระวิทยา,ประสิทธิภาพในการบรรจุยา และการปลดปล่อยยาในภาวะจำลอง ซึ่งขึ้นอยู่กับหลายปัจจัย คือ ความเข้มข้นของไคโตซานและยา รวมถึงชนิดของพอลิแอนไอออน ขนาดของอนุภาคไคโตซานเพิ่มขึ้นจากความเข้มข้นของไคโตซาน ในช่วง 10-30 ไมโครเมตร วิธีการบรรจุยาที่แตกต่างกันทั้ง 2 วิธีมีประสิทธิภาพในการบรรจุยาต่างกันคือ การบรรจุยาหลังจากเกิดการเชื่อมขวางไคโตซานแล้วมีประสิทธิภาพมากกว่าการบรรจุยาก่อนเกิดการเชื่อมขวาง ประสิทธิภาพในการบรรจุยาสูงสุดของลิโดเคนคือ 80% และไดโคลฟีแนกคือ 70% ด้วยวิธีบรรจุยาแบบหลังจากเกิดการเชื่อมขวางไคโตซานแล้ว เมื่อเพิ่มความเข้มข้นของยาพบว่าประสิทธิภาพในการบรรจุยาลดลงอย่างมีนัยสำคัญ ในการศึกษาการปลดปล่อยยาที่ pH 7.4 แสดงให้เห็นว่ารูปแบบการปลดปล่อยยาของทั้ง 2 ชนิดประกอบด้วยการปลดปล่อยยาอย่างรวดเร็วในช่วง 30 นาทีแรก และปลดปล่อยยาอย่างช้า ๆ ต่อเนื่องถึง 8 ชั่วโมง

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต รัตนา ไม้โภคทรัพย์

ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา

4773427123: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEY WORD: CHITOSAN, LIDOCAINE, DICLOFENAC, PARTICLES, CONTROLLED RELEASE

RATTANA MIPHOKASAP : CONTROLLED RELEASE OF LIDOCAINE AND DICLOFENAC FROM CHITOSAN PARTICLES. THESIS ADVISOR: ASSOC. PROF. MONGKOL SUKWATTANASINITT, Ph.D., 63 pp.

Chitosan was used for controlled release of drug models, lidocaine and diclofenac. Chitosan/surfactant/polyanion particles were prepared with polyanion as the coacervation crosslink agent. The drug loaded chitosan particles were characterized for particle size, morphology, drug loading efficiency and in vitro release, which were found dependent on the factors of chitosan concentration, drug concentration and polyanion types. The particle size of chitosan/surfactant/polyanion particles were increased with the chitosan concentration in range of 10-30 micrometers. Two different methods were used for drug loading, incubation can loaded drug more than incorporation. The maximum loading efficiencies of drug were 80% for lidocaine and 70% for diclofenac, using incubation method. The loading efficiency was significantly decreased by the increase of initial drug concentration. In vitro release study at pH 7.4 showed a release profile consisting of an initial burst in the first 30 minutes and a continuous slow release for 8 hours for both drug models.

Field of study Petrochemistry and Polymer Science Student's signature Rattana Miphokasap
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ACKNOWLEDGEMENTS

I would like to express gratitude and deep appreciation to my thesis advisor, Associate Professor Dr. Mongkol Sukwattanasinitt for his invaluable suggestion, guidance and kindness throughout this work. Gratefully thanks to Associate Professor Dr. Supawan Tantayanon, Assistant Professor Dr. Warinthorn Chavasiri, Associate Professor Dr. Supason Wanichwecharungruang and Dr. Pranee Lertsuthiwong for their invaluable comments and suggestions as the committee members.

Moreover, I am thankful for Unilever Thai Tradings Co.,Ltd, distinguished Scholar, for some financial support to study master program. I also thankful for supports from my boss and all colleagues including the supports of experimental testing facilities at the R&D laboratory of Unilever Thai Tradings Co.,Ltd.

I am gratefully thank staff of The National Metal and Materials Technology Center , Scientific and Technological Research Equipment Center Chulalongkorn University and Science-Center for Analytical Service (Sci-CAS) for assistance with many instruments. In addition, I would not actually forget to thank my friends especially Mongkol's group members for their supports and encouragement.

Finally, I would like to thank my family and friends for their love and encouragement throughout my entire study.

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LIST OF ABBREVIATIONS

MW	:	Molecular weight
mM	:	Millimolar
mV	:	Millivolt
kV	:	Kilovolt
^{o}C	:	Degree celcius
rpm	:	Round per minute
μm	:	Micrometer
nm	:	Nanometer
g	:	Gram
min	:	Minute
w/o	:	Water in oil
mL	:	Millilitre
kDa	:	Kilo Dalton
[η]	:	Intrinsic viscosity
η_{sp}	:	Specific viscosity
TEM	:	Transmission electron microscopy
SEM	:	Scanning electron microscopy
PVSK	:	Potassium polyvinylsulfate
CPC	:	Cetyl pyridinium chloride
DT	:	Diphtheria toxoid
AOT	:	Sodium bis (ethylhexyl) sulfosuccinate
SDS	:	Sodium dodecyl sulfate
DCA	:	Sodium deoxycholate
BSA	:	Bovine serum albumin
SOD	:	Superoxide dismutase
PEG	:	Polyethyleneglycol
PLLA	:	Poly-L-lactic acid
DLS	:	Dynamic light scattering

CHAPTER I

INTRODUCTION

Chitosan is currently receiving a great deal of interest for medical and pharmaceutical applications. The main reasons for this increasing attention are certainly its interesting properties. Indeed, chitosan is known for being biocompatible allowing its use in various medical applications [1-3]. Moreover, chitosan is metabolized by certain human enzymes, and is considered as biodegradable [4-5].

There are a wide variety of techniques available for producing particles including solvent evaporation, emulsion crosslinking, or spray drying. The most of the processes frequently need the use of orgnanic solvents or heat, which are undesirable process steps which may affect drug efficiency [6]. Therefore, in this work select to use coacervation technique is usually a simpler and milder loading process than above mentioned techniques in order to separate. Chitosan able to form particles with polyanions that is physical crosslinking by electrostatic interaction through inter or intramolecular linkages.

1.1 Theory

1.1.1 Chitosan

Chitosan, a natural linear biopolymer is obtained by alkaline deacetylation of chitin, which is the second abundant polysaccharide next to cellulose.[7] Chitin is the principal component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as *aspergillus* and *mucor.*

Chitosan is a cationic copolymer of glucosamine and N-acetyl-D-glucosamine linked together by B-1-4 glycosidic linkages. Chitosan has one primary amino and two free hydroxyl groups for each C6 building unit as showed in Figure 1.1. Due to the easy availability of free amino groups in chitosan, it carries the positive charged surfaces/ polymers and thus in turn reacts with many negatively charged surfaces/polymers.

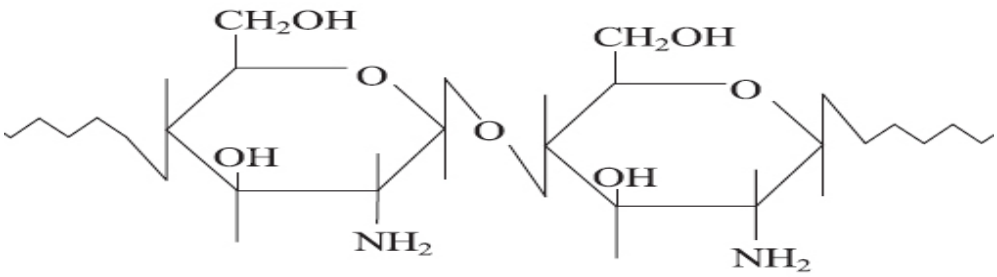


Figure 1.1 Chemical structure of chitosan

A great advantage of chitosan relative to other loading polymers is its high hydrosolubility at pH lower than 6.4 due to the protonation of the glucosamine groups. Chitosan is a weak base and is insoluble in water and organic solvents, however, it is soluble in dilute aqueous acidic solution (pH < 6.5) , which can convert the glucosamine units into a soluble form $R\text{-}NH_3^+$[8]. It gets precipitated in alkaline solution or with polyanions and forms gel at lower pH. These properties enable to interact with negatively charged polymers, macromolecules and polyanions in aqueous environment. So the amine groups of chitosan become positively charged. For interaction with polyanion ,microparticles are formed immediately on mixing the two aqueous phases (one containing chitosan and the other containing sodium sulphate or tripolyphosphate) through inter and intramolecular linkages created between phosphate and chitosan amino group [9].

1.1.2 Methods for preparation of chitosan-drug delivery particles

Various methods have been used to form chitosan particles. Selection of any of the methods depends upon affects such as particle size, thermal and chemical stability of the active agent, reproducibility of the release profiles. Reacting chitosan with controlled amounts of multivalent anion results in crosslinking between chitosan molecules. The crosslinking may be achieved in acidic, neutral or basic environments depending on the method applied. The different processes that have been used for the preparation of the chitosan particles based on drug delivery system are given below.

1.1.2.1 Interaction with anions

(a) Ionotropic gelation

The use of complexation between oppositely charged macromolecules to prepare chitosan particles has attracted much attention. Because this process is reversible physical crosslinking by electrostatic interaction , instead of chemical crosslinking , has been applied to avoid the possible toxicity of reagents and other undesirable effects. The counterions

used for ionotropic gelation can be divided into three categories as low molecular weight counterions (e.g. pyrophosphate, tripolyphosphate, tetrapolyphosphate), hydrophobic counterions (e.g. alginate, k-carragenan) , high molecular weight ions (e.g. octyl sulphate, lauryl sulphate) [10].

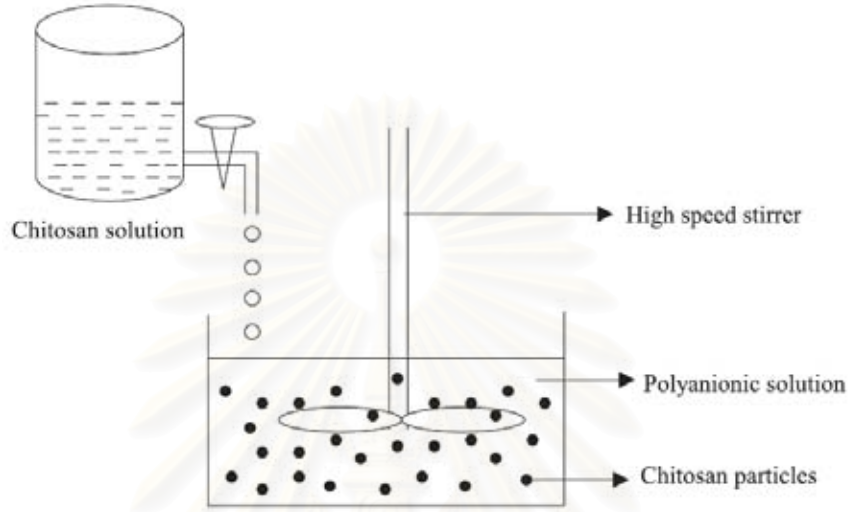


Figure 1.2 Preparation of chitosan particulate systems by ionotropic gelation method

(b) Coacervation / precipitation

In this method, the polymer is solubilized to form a solution. This is followed by addition of a solute, which forms insoluble polymer derivative and precipitates the polymer. This process is usually a simpler and milder encapsulation process because it avoids the use of toxic organic solvents and glutaraldehyde used in the other methods of preparation of chitosan particles such as solvent evaporation, emulsion polymerization methods. This approach generally employs water-soluble polymers and is a straightforward method for the induction of the complex formation. Coacervates are formed as a result of electrostatic interaction between two aqueous phase [11]. Coacervation of chitosan can be done with several anions, namely sulfate, citrate, alginate and tri-polyphosphate.

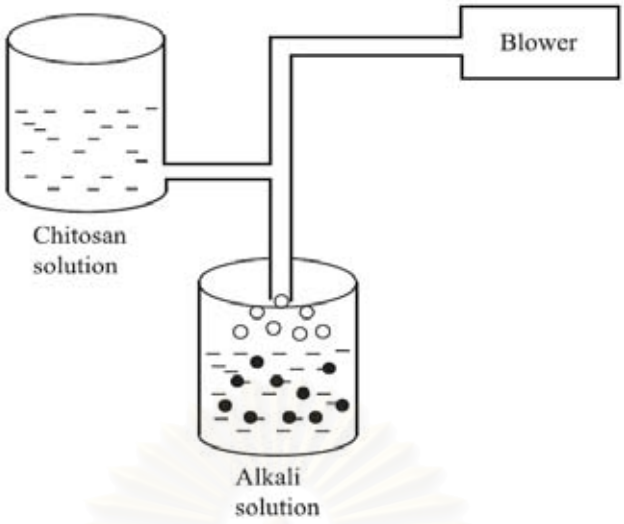


Figure 1.3 Preparation of chitosan particulate system by coacervation/precipitation method

1.1.2.2 Crosslinking with other chemical

(a) Emulsion crosslinking

This process uses the reactive functional amine group of chitosan to crosslink with aldehyde groups other crosslinking agent [12]. In this method, a water-in-oil (w/o) emulsion is prepared by emulsifying the chitosan aqueous solution in the oil phase. Aqueous droplets are stabilized using a suitable surfactant. The stable emulsion is crosslinked by using an appropriate crosslinking agent such as glutaraldehyde to harden the droplets. The chitosan particles are filtered and washed repeatedly with n-hexane followed by alcohol and then dried. However, complete removal of the un-reacted crosslinking agent may be difficult in this process.

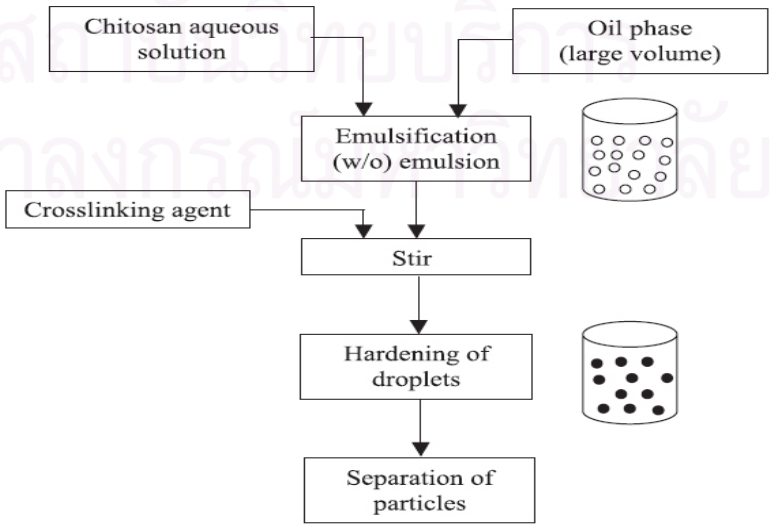


Figure 1.4 Preparation of chitosan particulate system by emulsion crosslinking method

1.1.2.3 Miscellaneous methods

(a) Spray drying

This method is a well-known technique to produce powders, granules or agglomerates from the mixture of drug as well as suspensions. The method is based on drying of atomized droplets in as stream of hot air. Drug may be dissolved or dispersed in the chitosan solution and then, a suitable cross-linking agent is added. This solution or dispersion is then atomized in a stream of hot air. Various process parameters are to be controlled to get the desired size of particles. This method of microencapsulation is particularly less dependent on the solubility characteristics of the drug and polymer and is simple, reproducible, and easy to scale up.

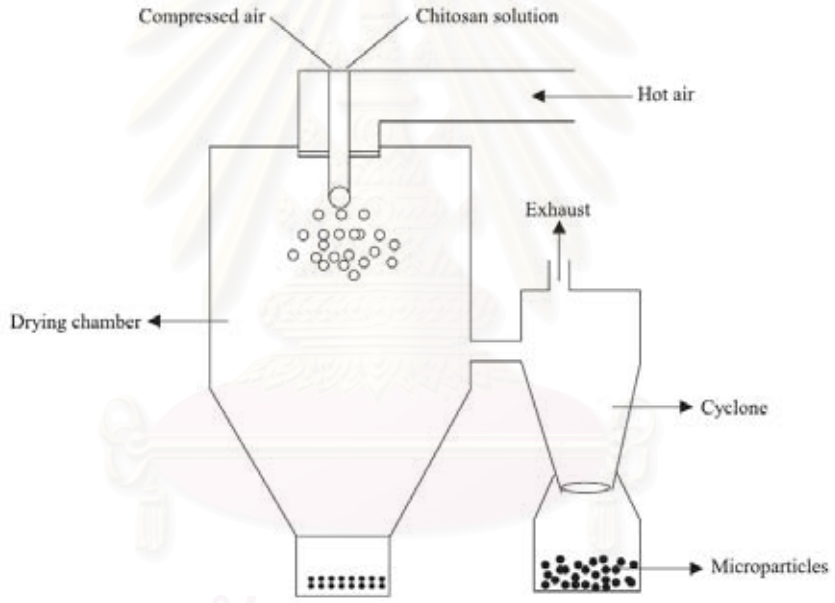


Figure 1.5 Preparation of particulate system by spray drying method

(b) Solvent evaporation method

It is a non-aqueous method, drug is dispersed or dissolved in a solution of the water labile polymers in a volatile organic solvent. This mixture is then suspended in span 85 and methylene chloride. The resulting particles can then be dried in vacuum.

(c) Thermal crosslinking

This method using citric acid by chitosan solution of varying concentration were prepared maintaining a constant molar ratio between chitosan and citric acid. The above

chitosan crosslinker solution was then cooled at 0 ^{o}C and added to corn oil followed by thermal crosslinking at 120 ^{o}C [13].

1.1.3 Drug Loading

Drug loading can be done by two methods i.e. during at the time of particles production (incorporation method) and absorbing the drug after formation of particles by incubating the carrier with a concentrated drug solution (incubation method). Drug is physically encapsulated into the matrix or adsorbed onto the surface in these systems. Various factors affect the entrapment efficiency of the drugs in the chitosan particles, e.g. nature of the drug, chitosan concentration, drug polymer ratio, stirring speed, etc [14-15].

Both water-soluble and water-insoluble drugs can be loaded into chitosan-based particulate systems. Water-soluble drugs are mixed with chitosan solution to form a homogeneous mixture, and then, particles can be formed by any of the methods. Water-insoluble drugs and drugs that can precipitate in acidic pH solutions can be loaded after the production of particles by incubation the performed particles with the saturated solution of drug. The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption [16]. The small molecules, studies show the use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading.

1.1.4 Drug release

Release of the drug from chitosan particles is typified by an initial rapid release (burst effect). It is generally recognized that drug loading in biodegradable polymeric matrices are released by three mechanisms taking place in sequence : (i) drug desorption from the surface of particles, (ii) diffusion and readsorption of drug through the swollen polymer matrix and (iii) degradation and erosion of the polymeric network [17] as showed in Figure 1.6.

Drug release follows more than one type of mechanism [18]. The higher release rates are observed due to the dissolution of surface-adhered drug. At longer time, drug release is due to the diffusion process, which is much slower when compared to the initial release. The particles have showed 100% release and complete release of the drug from the matrix occurs only after complete erosion or degradation of the chitosan matrix. Initial burst effect is a common phenomenon with chitosan-based delivery systems when loaded with water-soluble drug.

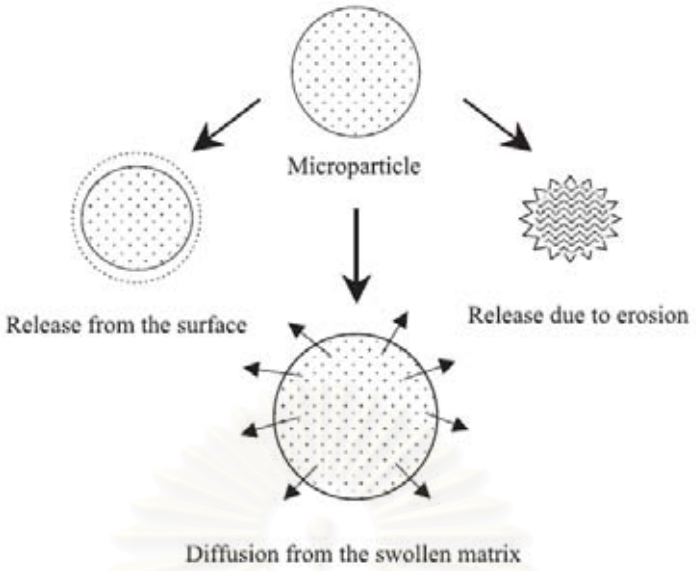


Figure 1.6 Mechanism of drug release from particles

Most of the drug content is released soon after administration, causing drug levels in the body to rise rapidly, peak and then decline sharply. Controlled release technology regulate the drug release rate and reduce the frequency of drug administration to encourage patients to comply with dosing instructions. The release of drugs, absorbed or encapsulated by polymers, involves their slow and controllable diffusion from/through polymeric materials [19].

1.1.5 The model drug for this study with cationic and anionic drug.

Lidocaine hydrochloride is the most commonly used local anesthetic in intradermal infiltration, topical anesthesia, and peripheral nerve blocks [20]. The mechanism of this drug alters depolarization in neurons, by blocking the fast sodium (Na^+) channels in the cell membrane. With sufficient blockade, the membrane will not depolarize and so not transmit an action potential, leading to its anesthetic effects. This drug was selected as a model drug because its use is limited by the short duration of its effects. It chemically known as 2-(diethylamino)-N-(2-6-dimethylphenyl) acetamide as showed the structure in appendix.

Diclofenac sodium **,** the sodium salt of o-(2,6-dichlorophenylamino)-phenylacetic acid is one of the most useful non-steroidal anti-inflammatory (NSAID) agents taken to reduce inflammation and an analgesic reducing pain in conditions such as in arthritis or acute injury [21]. However, the downsides of this drug are the short half life and side effects that include gastrointestinal ulcer and bleeding. To overcome the problem, chitosan was

selected to help the controlled release of diclofenac sodium. It chemically known as 2-[2-(2,6-dichlorophenyl) aminophenyl] ethanoic acid as showed the structure in appendix.

1.1.6 Molecular weight determination of chitosan by intrinsic viscosity

Molecular weight is one of the most important structural parameters of polymers. The average molecular weight of a polymer can be determined by various methods such as mass spectroscopy, light scattering, gel permeation chromatography and viscometry. Among these methods, viscometry is the most economical and convenient method for determination of chitosan.

A polymer chain contains many single bonds around which rotation is possible. If the configurations around successive carbon atoms are independent and unreleated, it will be seen that two parts of the polymer chain more than a few carbon atoms apart are essentially uncorrelated in regard to direction in space. The polymer chain is then 'statistically coiled' and resembles a loose tangle of yarn. When the polymer chains behave as statically coils, the molecular weights of the polymers are related to the intrinsic viscosity [η] according to Mark-Houwink equation, $[\eta] = kM_v^a$, where K and a are empirical parameters characteristic for both the polymer itself and the solvent. At 25 ^oC , $K= 1.8 \times 10^{-3}$ and a = 0.93.

The intrinsic viscosity, denoted by [η] is defined as the ratio of the specific viscosity (η_{sp}) to the weight concentration of the polymer (C) at the limit of zero concentration ($C \rightarrow 0$) experimentally obtained from a Y-intercept in the plot of η_{sp}/C against C ,

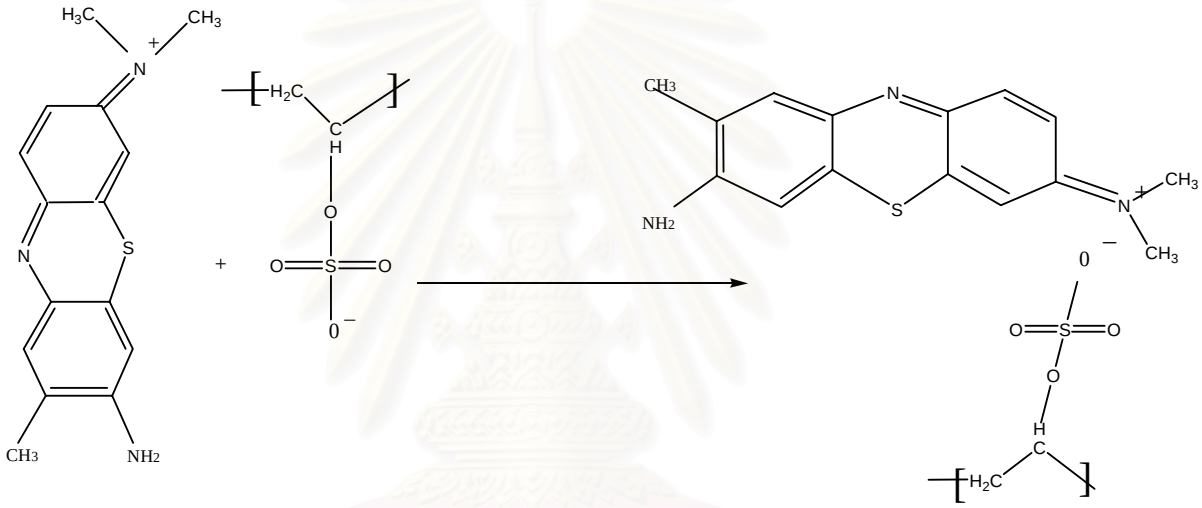
$[\eta] = \lim (\eta_{sp}/C)$, $(C \rightarrow 0)$

The defining equation of specific viscosity (η_{sp}) can be shown as $\eta_{sp} = \eta_r - 1$ where η_r is the relative viscosity, which defined as the ratio of the viscosity of the solution to the viscosity of the solvent (η/η_0) or the ratio of the falling time of the solution to the falling time of solvent (t/t_0) , $\eta_r = \eta/\eta_0 = t/t_0$

The molecular weight determination of a polymer by viscometry gives the most accurate result when a polymer whose molecules are all of same molecular weight is said to be monodisperse. Furthermore, the analysis should be done on low concentration solutions in order to decrease interaction between particles of polymer. Viscosity of a dilute polymer solution is usually determined by using an Ubbelohde viscometer tube.

1.1.7 Colloid Titration

The colloid titration method is one way to estimate the net charge density of surfaces, polyelectrolytes, and the charge demand of colloid materials in an aqueous mixture. In the titration of amino groups in chitosan, it was treated with excess acetic acid to produce highly cationic polymer. Then it is filtered to remove solid. A small amount of indicator dye (usually toluidine blue-O) is added to a known volume of the filtrate. The blue solution is back-titrated with an anionic polyelectrolyte such as potassium polyvinylsulfate (PVSK) to a purple-pink end point. Complexation between the dye and the negatively charged polymer caused the color change.



Toluidine blue PVSK Pink complex (end point)

Figure 1.7 Reaction of PVSK and toluidine blue at the end point of colloid titration of chitosan

1.2 Literature Review

1.2.1 Different technique of chitosan particles preparation.

In 1996, Berthold et al. [22] prepared chitosan microspheres by using sodium sulfate as a precipitant. The particle size and the morphological characteristics found to be spherical and smooth surface with a size between 1.5 and 2.5 μm. Side-by-side diffusion cells having a dialysis membrane was used to measure the release of prednisolone sodium phosphate from the microsphere formulation that was the highest loading as up to 30.5%. The drug/polymer ratio was a significant factor that affected the release of the drug from the microspheres. As the chitosan/drug ratio increased, the release rate decreased.

In 2001 , Van der Lubben et al. [23] studied chitosan microparticles were made according to a precipitation / coacervation method. The size of the prepared non-loaded chitosan microparticles were 4.3 $\pm$ 0.7 μm. SEM demonstrated the morphology of the chitosan microparticles that pores are present at the rough surface. Loading studies with the model compound ovalbumin resulted in loading capacities of about 40%. Subsequent release studies showed only a very low release of ovalbumin within 4 h and most of the ovalbumin (about 90%) remained entrapped in the microparticles.

In 2001, Mao et al. [24] prepared chitosan-DNA nanoparticles using a complex coacervation process with sodium sulfate and heated condition at 50-55 °C. The important parameters for preparation including the concentration of DNA, chitosan and sodium sulfate, temperature of the solutions, pH of the buffer. At an amino group of phosphate group ratio (N/P ratio) between 3 and 8 and a chitosan concentration of 100 μg/ml, the size of particles was optimized to 100-250 nm with a narrow distribution.

In 2002, Ko [25] prepared chitosan microparticles with tripolyphosphate (TPP) by ionic crosslinking. The particle sizes were in range from 500-710 μm and encapsulation efficiencies of drug were more than 90%. The morphologies of TPP-chitosan microparticles had more spherical shape and smooth surface. With lower pH and higher concentration of TPP solution resulted in slower felodipine release. The release of drug from TPP-chitosan microparticles decreased when cross-linking time increased.

In 2002, Hejazi et al. [26] prepared chitosan microspheres by ionic cross-linking and precipitation with sodium sulfate. Microspheres with a spherical shape and an average diameter of 2.0-3.0 μm were formed. Only 8% of tetracycline was incorporated when the drug was added to the chitosan before crosslinking and precipitation. On the other hand, a maximum of 69% could be loaded when the drug was incubated with the pre-formed microspheres. The drug was stable for up to 12 h even under acidic condition.

In 2003 , Van der Lubben et al. [27] Chitosan microparticles was prepared by the coacervation method with sodium sulfate solution. Loading capacities of about 25% and loading efficacies of about 100% were obtained after loading the chitosan microparticles with diphtheria toxoid (DT). It is found that chitosan microparticles also show excellent loading and release characteristics for the vaccine DT, suggesting that these microparticles could be used for multiple vaccines.

In 2004 , Dastan [28] prepared chitosan-DNA microparticles were prepared by the coacervation method with sodium sulfate solution by using different concentrations of chitosan and plasmid DNAs. Approximately 75-85% of DNA was encapsulated into the

chitosan-DNA microparticles. The average size of microparticles was found to be around 2 μm. Chitosan microparticles provide a sustained release of plasmid DNA for a long period. However, transfection efficiency of chitosan-DNA microparticles is low and dependent on the cell type.

In 2005, Saratid [29] studied the effect of different surfactant on the formation of chitosan nanoparticles found that only anionic surfactant, sodium bis (ethylhexyl) sulfosuccinate (AOT) and sodium dodecyl sulfate (SDS) can formed the chitosan nanoparticles in aqueous media at chitosan concentration of 2.5 mM. The size and zeta potentials of the particles were 110 $\pm$ 10 nm and -47.42 $\pm$ 5 mV, respectively. The chitosan/AOT nanoparticles obtained can be loaded with tetracycline with 50% entrapment efficiency. The suspensions of the nanoparticles were stable at ambient condition for at least 30 days.

In 2006 Lameiro et al. [30] developed a mucosal delivery system based on chitosan by anionic precipitation / coacervation is accomplished by the addition of sodium deoxycholate (DCA). These microparticles were prepared under mild conditions, where bovine serum albumin (BSA) and DCA were simply dipped into a chitosan solution under stirring. Platelet-like and /or spherical microparticles, having high protein loading efficiency and relatively low protein external exposure, are obtained. To achieve a better compaction of the microparticles matrix, block copolymers and other non-ionic surfactants are added to the formulation.

In 2007, Akbuga et al. [31] investigated chitosan microspheres was encapsulated superoxide dismutase (SOD) is the most potent antioxidant enzyme. Protein-loaded chitosan microspheres with various formulations were prepared based on complex coacervation process. 70-80% protein encapsulation efficiency was obtained. The addition of PEG to the protein solution enhanced the encapsulation efficiency also. Mean sizes of microspheres were between 1.38 and 1.94 μm. Factors affecting the release behaviour of SOD from microspheres have been studied. They included pH values of chitosan solution , addition of PEG to the protein solution and the use of adsorption technique.

1.2.2. Different technique of chitosan particles preparation to load lidocaine hydrochloride and diclofenac sodium

In 2001, Chiou [32] investigated the effect of post-coating PLLA (poly-L-lactic acid) microspheres with different chitosan on the initial burst and controlling the drug release of the microspheres. Coating by chitosan retarded the burst release of lidocaine

hydrochloride. Without chitosan, 19.25% of encapsulated lidocaine would release from PLLA microsphere within the first hour (R1) and the time of 50% release (T50) was 25 hours after the microsphere were coated with chitosan of viscosity 384±10 cps, R1 and T50 could be reduced and prolonged to 14.6% and 90 hour, respectively. The study indicated that manipulating the viscosity of chitosan solution was the most important factor in contributing to controlling the drug release of chitosan post-coated PLLA microsphere.

In 2002, Kumbar [33] prepared chitosan microspheres of diclofenac sodium with three different crosslinking methods, as glutaraldehyde, sulphuric acid and heat treatment. Chitosan microspheres were produced in a w/o emulsion followed by crosslinking using one of the above methods. Among all the system studied, the 32% glutaraldehyde crosslinked microspheres showed the slowest release 41% at 420 min, and a fastest release of 81% at 500 min was shown by heat crosslinking for 3 h.

In 2002, Gonzalez-Rodriguez [34] studied alginate/chitosan particles were prepared by ionic gelation (Ca^{2+} and Al^{3+}) for the sodium diclofenac release. The ability to release the active substance was examined as a function of some technological parameters and pH of dissolution medium. The release of sodium diclofenac was prevented at acidic pH, while was complete in a few minutes when pH was raised up to 6.4 and 7.2. The alginate/chitosan ratio and the nature of the gelifying cation allow a control of the release rate of the drug.

In 2005, Praphairaksit et al. [35] investigated the optimal ratio of chitosan and diclofenac sodium which also include the optimal temperature and the solvent for diluting chitosan. The result show that when used acetic acid as the solvent for chitosan, the optimal ratio of diclofenac sodium : chitosan is 1:1 prepared at room temperature. The release of diclofenac sodium from bead in buffer solution of pH 1.2 is 3.42 – 8.33%, 2.98 – 29.17% in pH 6.6 and 36.61 – 94.05% in pH 7.4. When citric acid was used as the solvent for chitosan, the release of diclofenac sodium from bead in buffer solution of pH 1.2 is 7.63 – 18.93%, 7.34 – 17.23% in pH 6.6 and 49.44 – 98.87 in pH 7.4. When compare between the two solvents, the result shows that acetic acid yield the better result in controlled release of diclofenac sodium from diclofenac-chitosan bead.

From the previous study of Saratid could be prepared nanoparticles with chitosan/surfactant as the main reference of this study. In this study, the objective of working were find to stabilize particles for further study of drug loading efficiency by including determine the mild and non toxic technique as coacervation with polyanion. In addition, in vitro release was studied with different conditions in different type of drugs.

CHAPTER II

EXPERIMENTS

2.1 Instruments and apparatus

1. UV-Visible spectrophotometer (Lambda 35, Perkin Elmer)
2. Mastersizer 2000S (Malvern instruments Ltd., UK)
3. Autosizer S4700 (Malvern instruments Ltd., UK)
4. Transmission electron microscopy (TEM) (JEOL, JEM-2100 EM)
5. Scanning electron microscopy (SEM) (JEOL-JSM-6480LV)
6. Optical microscopy (Axioskop-type microscope , Zeiss Weesp, The Netherland)
7. Freeze-dryer (Freezone 77520, Benchtop, Labconco)
8. Centrifuge (IEC Multi Refrigerated Centrifuge)
9. Blade stirrer (IKA-Werk, Germany)
10. Dessicator
11. Ubbelohde viscometric tube
12. Titrate tube
13. Stopwatch
14. pH meter (pH 3 scan, Eutech Instruments)
15. Hot-plated magnetic stirrer (Coning)
16. Magnetic bar
17. Spectrapore membrane dialysis bag
18. Disposable syringe 10 ml (Nipro)
19. Syringe filter (0.45 μm PTFE, Alltech)
20. Pipette man (P200 and P1000, Gilson)
21. Solvent membrane filters (0.45 μm cellulose, Millipore)
22. Membrane filter (whatmann no. 41)
23. Vial bottle 10 ml
24. Beaker
25. Cylinder
26. Spatula
27. Plastic dropper

2.2 Chemicals

1. Chitosan (M_v~130000, 84% DD from Chemicals, Ltd, Fluka, Switzerland)
2. Glacial acetic acid, analytical grade (Merck, Germany)
3. Citric acid, analytical grade (Merck, Germany)
4. Sodium bis (ethylhexyl) sulfosuccinate (AOT) (Cognis, Germany)
5. Diclofenac sodium (Fluka, Switzerland)
6. Lidocaine hydrochloride (Aldrich)
7. Phosphotungstic acid (Fluka Chemicals, Ltd., Switzerland)
8. Sodium sulfate anhydrous (Riedel-de Haen)
9. Sodium citrate (Riedel-de Haen)
10. Sodium tripolyphosphate (Fluka Chemicals, Ltd. Switzerland)
11. Potassium chloride
12. Sodium hydrogen phosphate
13. Potassium hydrogen triphosphate
14. Sodium chloride
15. Potassium polyvinyl sulfate solution (PVSK)
16. Cetyl pyridinium chloride (CPC)
17. Toluidine blue

2.3 Determination of molecular weight of chitosan by using intrinsic viscosity

The solvent : 0.1 M acetic acid and 0.2 M NaCl in water

Dried chitosan sample (30 mg) was weighed accurately in a 50 ml volumetric flask, dissolved in 1 M aqueous acetic acid solution (5 ml) and water (30 ml) and stirred with a magnetic stirrer overnight. After that 1 M aqueous NaCl solution (10 ml) was added and stirred overnight, then made up the volume to the mark with distilled water (C1). A portion of solution (10 ml) was taken and diluted with the solvent (0.2 M NaCl/ 0.1 M acetic acid, 10 ml) (C4). The temperature of a thermostatic water bath was maintained at 25 ^{o}C.

Measurement of solvent viscosity : The solvent (0.2 M NaCl/0.1 M acetic acid, 10 ml) was put into an Ubbelohde viscometric tube as shown in Figure 2.1. The liquid was pushed up above the upper level (a) by a balloon pump. The time of falling between the upper (a) and lower mark (b) was measured by a stopwatch in triplicate.

Measurement of chitosan solution viscosity: The solution (C1, 10 ml) was put into a viscometer. The falling time was measured by a stopwatch in triplicate. The solvent (2 ml) was added through tube L and mixed well to give C2. The falling time was measured again.

The solvent (2 ml) was added again to give C3 and the falling time was measured. The viscometer was rinsed with the solvent (15 ml), water (15 ml) and solution C4 (15 ml). The solution C4 (10 ml) was put into the viscometer. The falling time was measured as described above. The solution was diluted (C5 and C6) and the falling time was measured according to the procedure described for C2 and C3. Plot a graph of η_{sp} / C and against C (concentration) , and draw a straight line using the linear least square. The intrinsic viscosity [η] was obtained from the Y-intercept. Viscosity-average molecular weight can be calculated as follows :

$$[\eta] = kM_v^a \quad (k = 1.8 \times 10^{-3},\ a = 0.93) \qquad (2.1)$$

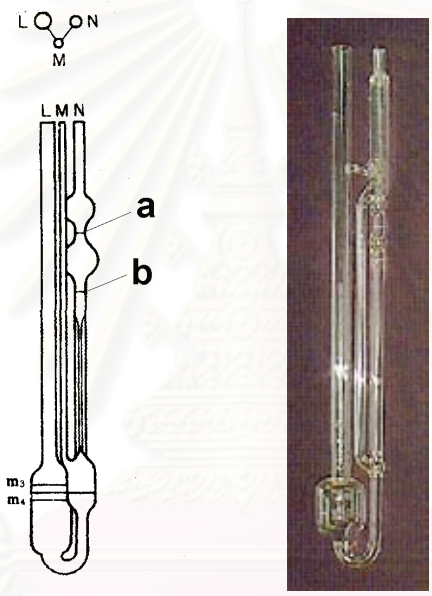


Figure 2.1 Ubbelohde viscometric tube

2.4. Determination of degree of deacetylation of chitosan by using colloid titration method

At first the solution of patassium polyvinyl sulfate solution (PVSK) must be titrated to determine the concentration. Cetyl pyridinium chloride (CPC, 12.5 mg) was weighed precisely into a 25 mL volumetric flask and the volume was made up to the mark with 0.1 M aqueous acetic acid solution. The solution (precise 5 mL) was placed in a 25 mL beaker, three drops of 0.1% toluidine blue were added as an indicator, and the mixture was continuously stirred with a magnetic bar. The PVSK solution was added titrimetrically from a burette until the blue color of the indicator changed into reddish purple. The titration was

repeated three times. In a blank titration, 0.1 M aqueous acetic acid solution was used in place of the CPC solution. The other procedures are the same as the above.

For the determination of the concentration of PVSK (N, equiv./L) :

$$N = \frac{50C'}{358D} \quad (2.2)$$

When ; C' = concentration of CPC (% w/v)

D = The difference between CPC and blank titration volumes (mL)

Dried chitosan (8.7 mg) was weighed precisely in a 25 mL volumetric flask and the volume was made up to the mark with 0.1 M aqueous acetic acid solution. The solution (precise 5 mL) was pipetted and placed in a 25 mL beaker and titrated as described above.

For the determination of the degree of *N*-acetylation (DA):

$$\%DA = \frac{[(50C-161ND)*100}{(42ND+50C)} \quad (2.3)$$

When: N = concentration of PVSK (equiv./L)

D = The difference between chitosan and blank titration volumes (mL)

C = concentration of chitosan (%)

For the determination of the degree of deacetylation (DD) :

DD (%) = 100-DA (%) (2.4)

2.5 Preparation of chitosan/surfactant particles

2.5.1 Chitosan/surfactant particles without polyanion

Chitosan/surfactant particles were prepared by micelle system of surfactant in an aqueous medium. The chitosan solution (1.0-5.0 mM) in an aqueous solution of acetic acid (1%v/v) was slowly added into an equal volume of 50 mM anionic surfactant sodium bis (ethylhexyl) sulfosuccinate (AOT) under continuous stirring of magnetic stirrer at 250 rpm at room temperature.

The mean particle size of the chitosan/surfactant particles was determined by dynamic light scattering (DLS) or photon correlation spectroscopy using the Autosizer S4700 (Malvern Instruments Ltd., UK). This instrument used an air-cooled argon ion laser at 488 nm as the light source with 128 channel correlator. The samples were diluted prior to

measuring to obtain optimal signals and measurements were performed at 90 degree to the incident light and data were collected over a period of 3 min.

The morphology of the chitosan/surfactant particles was investigated using transmission electron microscope (TEM) (JEOL, JEM-2100 EM). Freshly made particles were diluted with deionized water and one-drop sample was syringe placed on a carbon coated film 300 mesh copper grid, allowing sitting for 10 min until air-dried. The sample was stained with 1%phosphotungstic acid solution for 5 min and any excess stained dye was removed with filter paper before viewing on the TEM instrument.

2.5.2 Chitosan/surfactant/polyanion particles

Chitosan/surfactant/polyanion particles were prepared by coacervation/precipitation method. The chitosan solution in range 5.0-25.0 mM was dissolved in 2% aqueous solution of citric acid and slowly added into equal volume of aqueous surfactant solution as anionic surfactant sodium bis (ethylhexyl) sulfosuccinate (AOT) during stirring with a blade stirrer at 400 rpm for 5 min. Then, a solution of polyanion as sodium sulfate, sodium citrate or sodium tripolyphosphate at 0.6 M was added dropwise with continuously stirring at 400 rpm for 15 min. The chitosan/AOT/polyanion particles were collected by centrifugation for 15 min at 3000 rpm. The pellet was resuspended in deionized water to wash the particles and centrifuged again. These two purification steps were repeated twice.

2.6 Physicochemical characterization of chitosan/AOT/polyanion particles

2.6.1 Determination of the particle size of chitosan/AOT/polyanion particles

The average particle size and size distributions of chitosan/AOT/polyanion particles was determined by laser light scattering technique using a Mastersizer 2000 instrument (Malvern Instruments Ltd., UK). The proper amounts of the particles were resuspended with distilled water and suspended completely by the magnetic stirrer. The suspension was then placed in the laser particle counter. The medium particle size (D50, the particle size when cumulative value is 50% by volume in the particle size cumulative distribution profile) and particle size distribution were measured for three times.

2.6.2 Determination of the morphology of chitosan/AOT/polyanion particles

Optical microscopy (Axioskop-type microscope , Zeiss Weesp, The Netherland) was performed for initial visualization of the chitosan/AOT/polyanion particles. Morphology was evaluated using the following items as approximate size, form, and uniformity.

Chitosan/AOT/polyanion particles were analyzed after resuspension in water without staining or fixation.

Further morphology studies were carried out by scanning electron microscopy (SEM). The chitosan/AOT/polyanion particles suspension was freeze dried and the dried powder sample was spreaded on a carbon tape and attached on the gold disk after that these chitosan/AOT/polyanion particles were coated with gold film under vacuum using a gold-sputter coater (SPI-module Sputter Coater) and studied with a JEOL-JSM-6480LV scanning electron microscope at 15 kV.

2.7 Determination of drug loading of chitosan/AOT/polyanion particles

Drug loading in chitosan/AOT/polyanion particles system can be done by one of two methods of incorporation and incubation.

2.7.1 The incorporation method

Drug was premixed with sodium sulfate solution after that was added dropwise to the chitosan solution was premixed with surfactant. The chitosan particles suspension was gently stirred on a magnetic stirrer for 1 hour. The drug loaded particles were collected by centrifugation for 15 min at 3000 rpm.

2.7.2 The incubation method

Chitosan particles were formed first through polyanion crosslinking, and the particles were then mixed with drug solution at predetermined concentrations. The mixed solutions were gently stirred for 1 hour to allow drug adsorption on the chitosan particles. The drug loaded particles were collected by centrifugation for 15 min at 3000 rpm.

The amount of lidocaine hydrochloride or diclofenac sodium associated with the chitosan particles was determined spectrophotometrically at 262 nm and 276 nm, respectively. It was calculated by the difference between the total amount used to prepare the particles and the amount of drug present in the aqueous phase after centrifugation for 15 min at 3000 rpm. The loading efficiency was determined indirectly applying the following equation :

$$\% \text{ loading efficiency} = \frac{(\text{total amount of drug} - \text{free amount drug in supernatant})}{\text{total amount of drug}} \times 100$$

2.8. In vitro release of drug from the chitosan/AOT/polyanion particles

Drug loaded chitosan particles (0.2 g) were incubated with 20 ml of 0.2 mol/l phosphate buffer saline solution of pH 7.4 and pH 5.5 by using magnetic stirring at 37$\pm$1 ^{o}C 150 rpm in water bath. At specified collection times, samples were centrifuged at 4000 rpm 5 min. The supernatant,4 ml, was taken and replaced by an equal volume of fresh PBS solution to maintain a constant volume. The amount of drug released was evaluated by UV-visible spectrophotometer at 262 nm for lidocaine hydrochloride and 276 nm for diclofenac sodium.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Molecular weight and degree of deacetylation of chitosan

Using intrinsic viscosity and colloid titration methods, the molecular weight and degree of deacetylation of chitosan were determine as 130 kDa and 84%, respectively.

3.2 Chitosan particles prepared from chitosan / AOT system

Mixing of various concentration of chitosan solution with sodium bis(ethylhexyl) sulfosuccinate solution (AOT, 50 mM) gave milky white colloids. Dynamic light scattering (DLS) analysis of these colloids showed the average particle size increasing from 60 to 110 nm when the chitosan concentration increased from 1.0-5.0 mM (Figure 3.1).

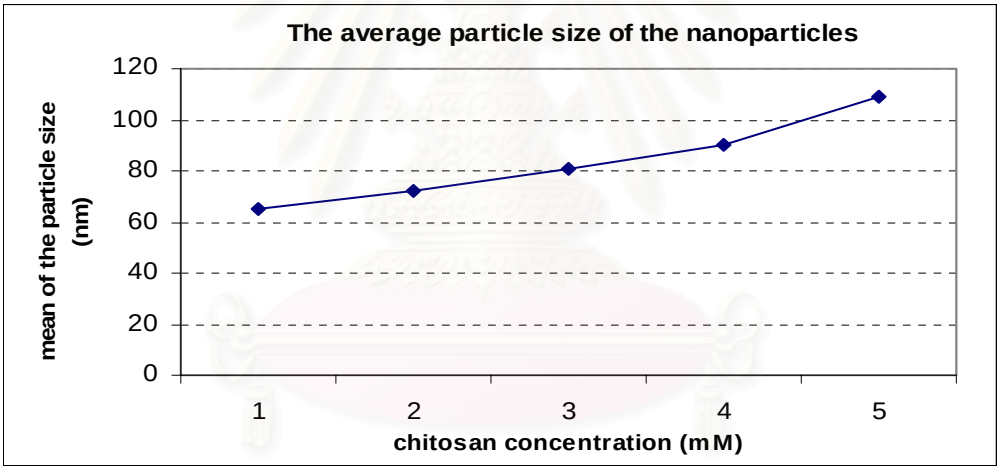
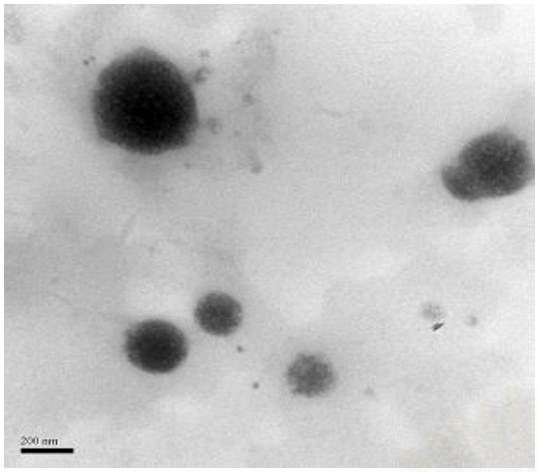
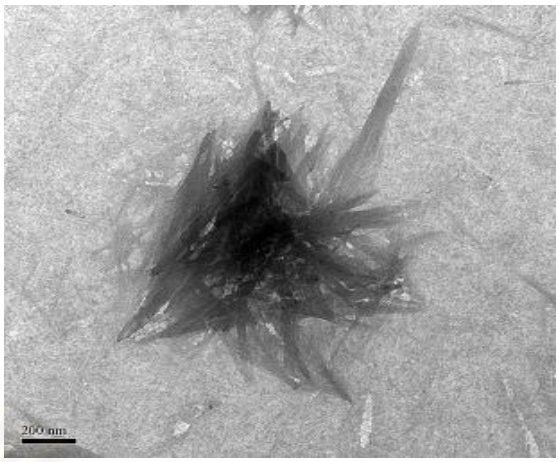


Figure 3.1 The average particle size measured from the chitosan/AOT mixtures by DLS

The particle morphology of the chitosan/AOT colloidal system was studied by a transmission electron microscope (TEM). TEM images of the air dried sample of chitosan/AOT solution revealed the existence of spherical particles, with diameter of 100-200 nm, which were not observed in the air dried sample of chitosan solution in 1%w/v acetic acid (Figure 3.2). The images confirm that the anionic surfactant, such as AOT, can induce the formation of submicron particles of chitosan in an aqueous medium probably through the packing chitosan polymeric chains inside the surfactant shell.





(a) (b)

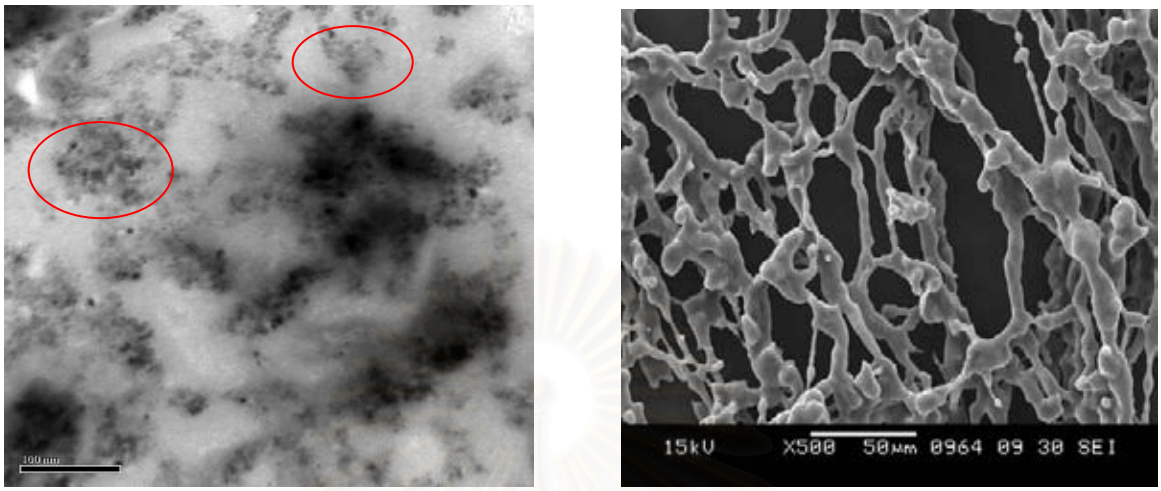
Figure 3.2 TEM image of air dried sample of (a) 2 mM chitosan/50 mM AOT (b) 2 mM chitosan solution in 1% AcOH

In an attempt to be remove excess surfactant, the chitosan/AOT colloidal system was dialyzed against excess water for 8 hours with the water being refreshed every 2 hours using a Spectrapore membrane dialysis bag (12 kDa cut off). After dialysis, white precipitates were clearly observed in dialysis bag (Figure 3.3). The results suggested some sort of aggregation of the chitosan particles probably due to the lost of the surfactant molecules from their surface.

Figure 3.3 Color pictures of chitosan/AOT colloidal systems: (a) prior to the dialysis and (b) after dialysis through 12 kDa membrane against excess water

The TEM and SEM images of these precipitates showes a network which is probably formed from the fusion of particles during the surfactant removal (Figure 3.4). The results confirmed that the chitosan particles formed in the chitosan/AOT aqueous system are not stable upon surfactant removal. In the subsequent preparation of chitosan particles,

polyanions were introduced into the system to serve as coacervative crosslinking agents between polycationic chitosan chains.



(a) (b)

Figure 3.4 (a) TEM and (b) SEM images of dry sample chitosan/AOT particles after surfactant removal by dialysis method.

3.3 Chitosan particles prepared from chitosan/AOT/polyanion

To enhance the stability of the particles, the chitosan and AOT solutions were mixed in the presence of a polyanion such as sulfate, citrate and tripolyphosphate. The mixture turned cloudy and gradually precipitated upon standing overnight. The polyanions presumably serve as a crosslinking and charge neutralization agents to induce coacervation of the polycationic chitosan chains into particles. The precipitate was collected by centrifugation and washed twice with copious amount of DI water prior to further analysis and drug loading study.

3.3.1 Laser light scattering analysis

The particle size was measured using a laser light scattering technique (Mastersizer 2000, Malvern, UK). The particle size distribution curve of the chitosan/AOT/polyanion particles shows the average particle size of the unloaded and loaded particles in the range of 10 – 30 µm (Figure 3.5).

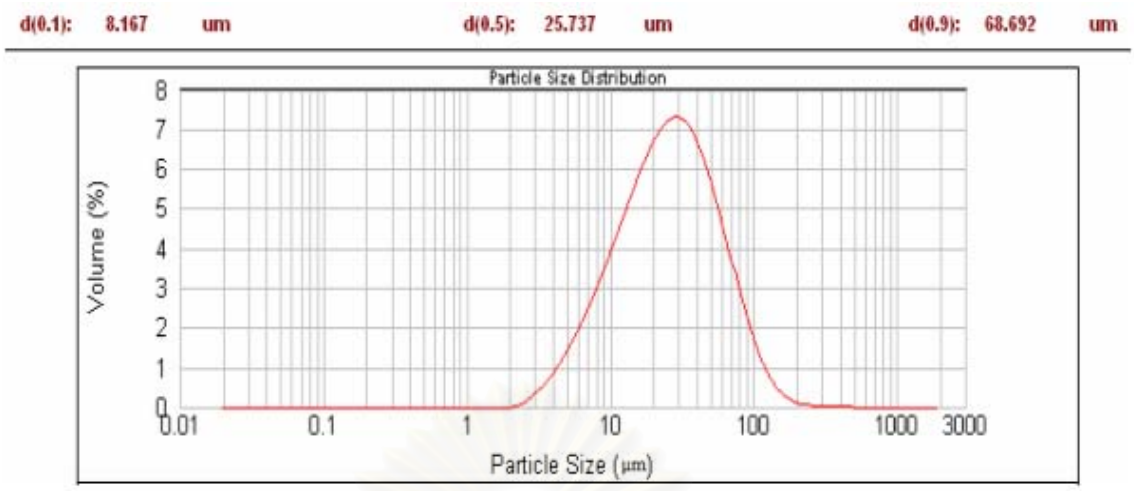
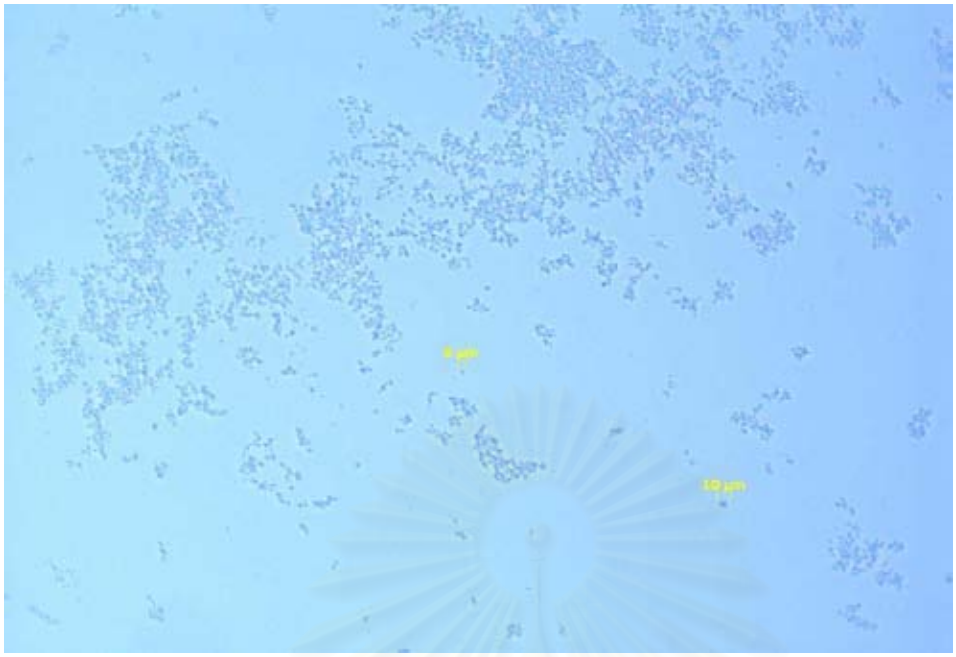


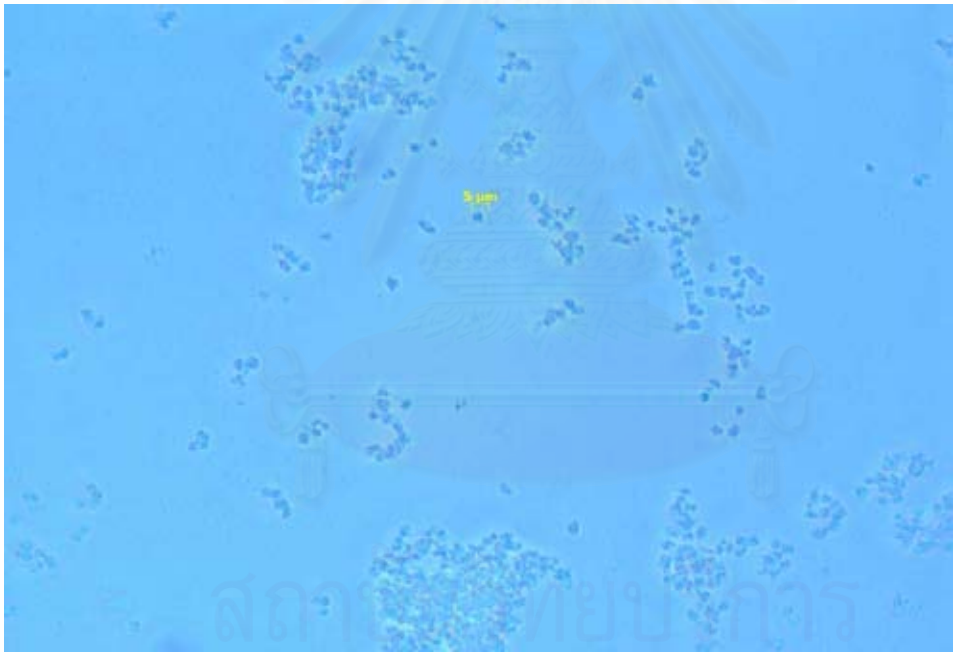
Figure 3.5 Chromatogram of the chitosan/AOT/sodium sulfate particle size distribution measured from laser light scattering.

3.3.2 Optical microscopy and scanning electron microscopy

A light microscopic images of the precipitate, obtained from the chitosan/AOT/polyanion system, show particles with sphere-like shape with an average particle size in the range of 5-10 µm (Figure 3.6). The particles sizes and shapes are reasonably uniform.



(a)

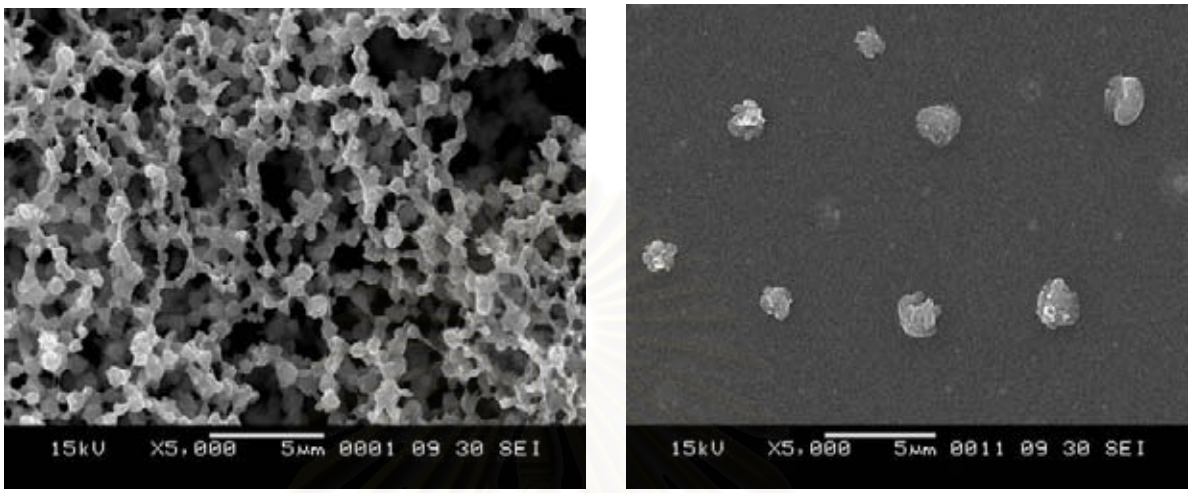


(b)

Figure 3.6 Light microscopic image of chitosan particles (a) 400X and (b) 1000X

Higher resolution of morphology obtained from SEM image of the air dry sample reveals that each particle has rough surface looked more like a cluster of smaller particles (Figure 3.7a). Unlike the air dry sample, the image obtained from the freeze dry sample showed net work of bead string indicating the some sort of association between the particles during the freeze drying process (Figure 3.7b). The average particle sizes observed from

the dried samples are 1-2 μm that is smaller than those measured by laser light scattering analysis and optical microscope which are measured from the wet samples. The results indicate considerable swelling of the particles in the wet state.



(a) (b)

Figure 3.7 SEM micrographs of chitosan/AOT/sodium sulfate precipitates dried by (a) air dry (b) freeze dry

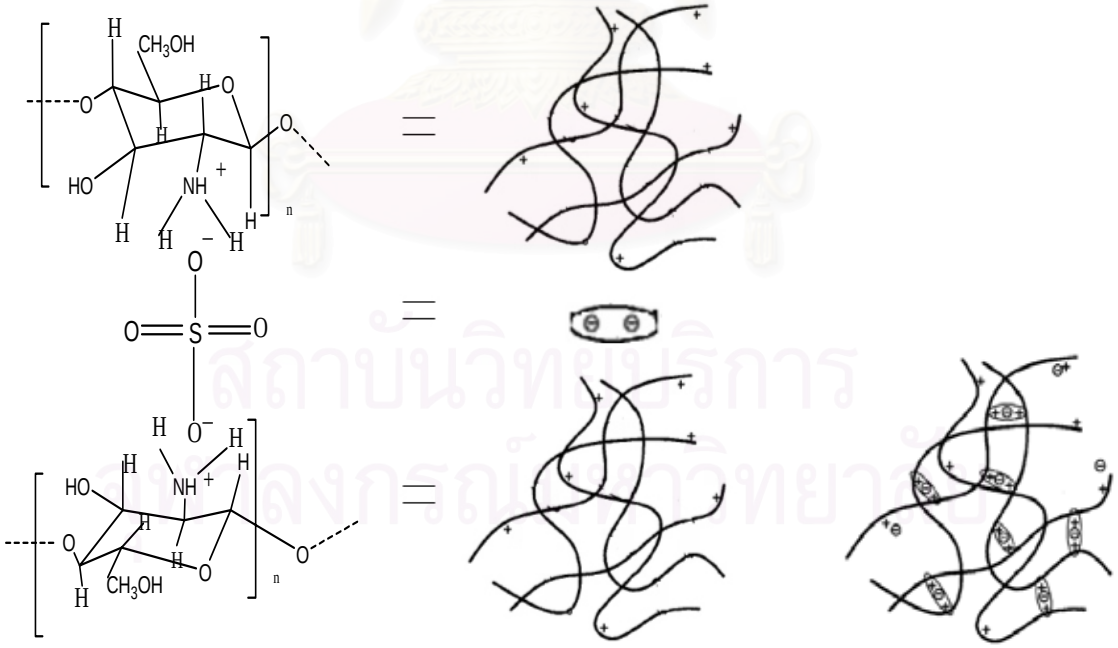


Figure 3.8 Illustrative drawing of coulombic interaction between sulfate anion and chitosan chain in acidic media.

The spherical particles observed from light microscopic and SEM image are probably formed by the coulombic interactions which form bridges between the positively charged chitosan polymeric chains and negatively charged sodium sulfate as represented in Figure 3.8. This network is inside micelle form of anionic surfactant (AOT) in order to form particles with sphere like shape after that the surfactant was removed.

3.3.3 Factors affecting particle sizes prepared from chitosan/AOT/polyanion system

Three types of polyanions *i.e.* sulfate, citrate and tripolyphosphate were each used in the preparation system. The average particle size determined from laser light scattering showed that the system using sulfate anion gave the smallest particle size in comparison to the system using citrate and tripolyphosphate (Figure 3.9). As a dianion, a sulfate ion can interact with two cationic ammonium groups on chitosan chain while citrate and tripolyphosphate, which are trianions, can simultaneously interact with three cationic ammonium groups. The crosslinking caused by citrate and tripolyphosphate anions are thus likely to involve more chitosan chains in a particle resulting in the greater particle sizes.

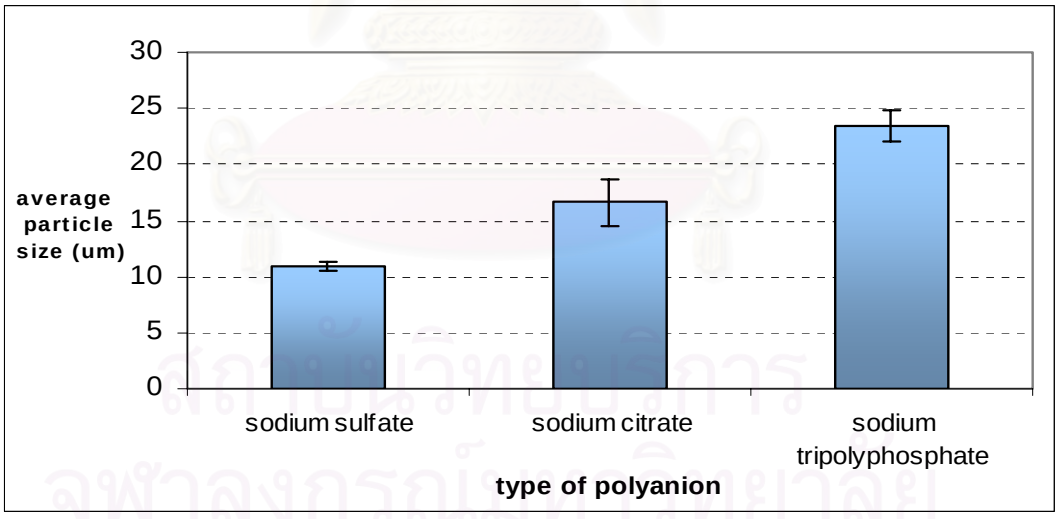


Figure 3.9 The average particle size of particles prepared from chitosan/AOT/polyanion system using three different polyanions. [AOT] = 50 mM, [chitosan] = 5 mM, [polyanion] 0.6 M

When the chitosan concentration in the preparation system increased, the average particle size also increased (Figure 3.10). The results may be explained in terms of the

increasing of the numbers of chitosan chains coacervated in one particle at higher concentration of chitosan under constant concentration of the surfactant, AOT.

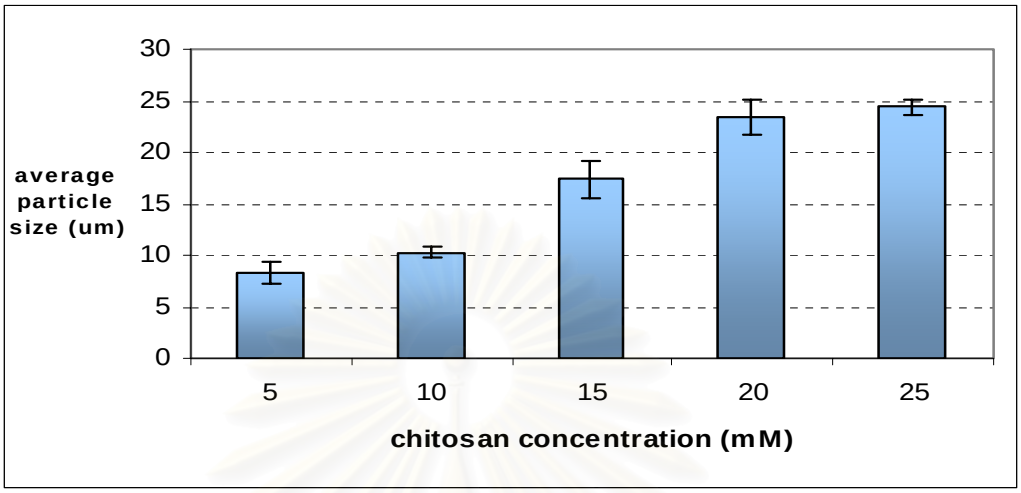


Figure 3.10 The average particle size of chitosan/AOT/sodium sulfate particles with chitosan concentration. [AOT] = 50 mM, [sodium sulfate] = 0.6 M

3.4 Drug loading

3.4.1 Loading efficiency

Drug loading of lidocaine hydrochloride and diclofenac sodium were studied at varied initial drug concentration in the range of 0.1-0.5 mg/mL. Interestingly, both cationic and anionic drugs can be loaded into the chitosan/AOT/sodium sulfate particles. The loading efficiency of the cationic lidocaine hydrochloride was higher than that of the anionic diclofenac sodium. The increase of the initial drug concentration led to higher loading amount of drug into the chitosan particles but lower loading efficiency (Figure 3.11). For example, the chitosan particles contained lidocaine hydrochloride with loading efficiency of 66% when the loading was conducted at 0.1 mg/mL and the loading increased with lower loading efficiency of 50% when the loading was conducted at 0.5 mg/mL.

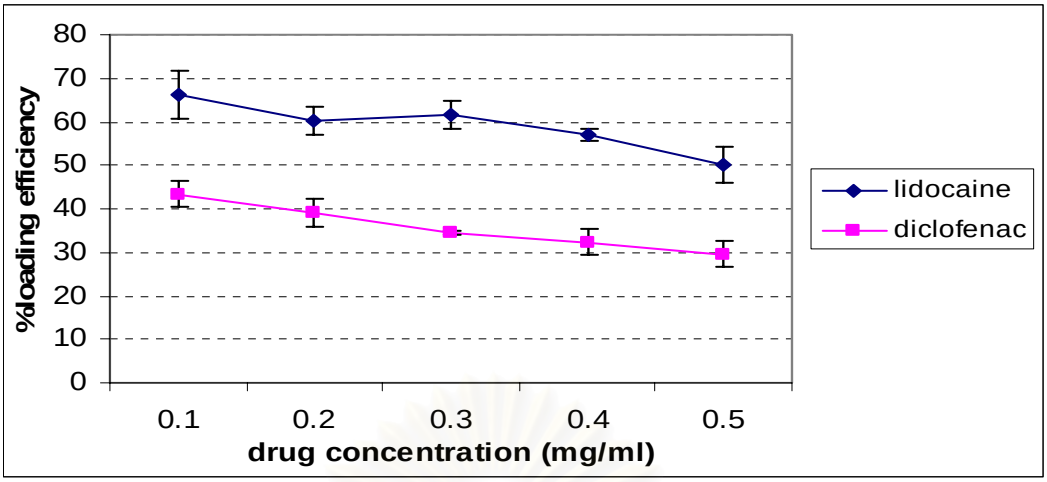


Figure 3.11 The drug loading efficiency of lidocaine hydrochloride and diclofenac sodium to chitosan/AOT/sodium sulfate particles by incubation method. [AOT] = 50 mM, [chitosan] = 5mM and [sodium sulfate] = 0.6 M.

The relationship between the chitosan concentration used during the particle preparation and the drug loading efficiency was investigated. When the chitosan concentration used during the particle preparation increased the loading efficiency significantly reduced (Figure 3.12). The results may be explained by the information described in section 3.3.3 that the particle size increased with the chitosan concentration. Therefore, the formation of the larger particle lower the surface area to adsorption the drug.

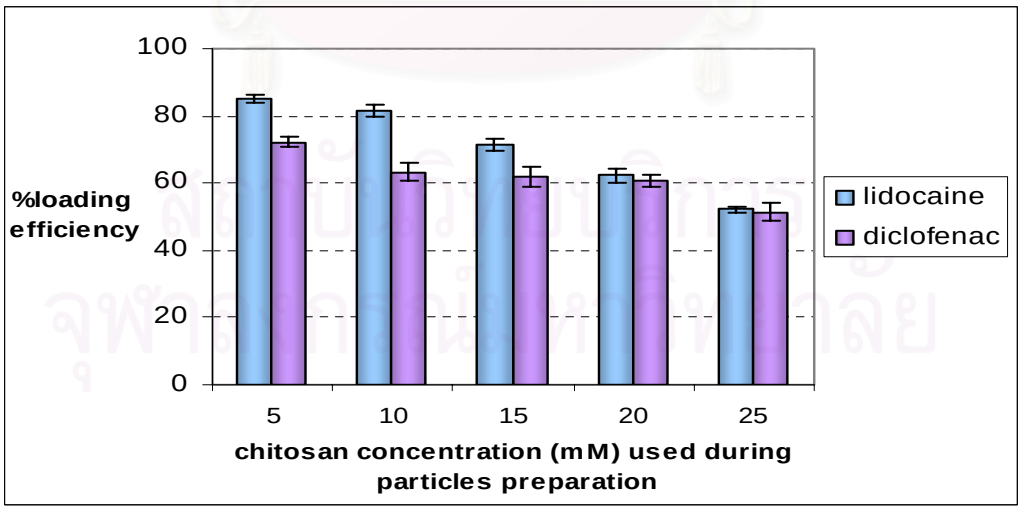


Figure 3.12 The drug loading efficiency of chitosan/AOT/sodium sulfate particles in relation to the chitosan concentration used during the particle preparation. [AOT] = 50 mM, [sodium sulfate] = 0.6 M, [drug] = 0.4 mg/ml

The drug loading efficiency from influence of different loading method found that adding the drug after the formation of the particles (incubation) can load drug more than adding the drug while the formation of the particles (incorporation) as shown in Figure 3.13. The maximum loading efficiency of incorporation was to be only 61% and 44% for lidocaine and diclofenac , respectively. On the other hand, the loading efficiency increased to maximum of 85% when the drug loading by incubation.

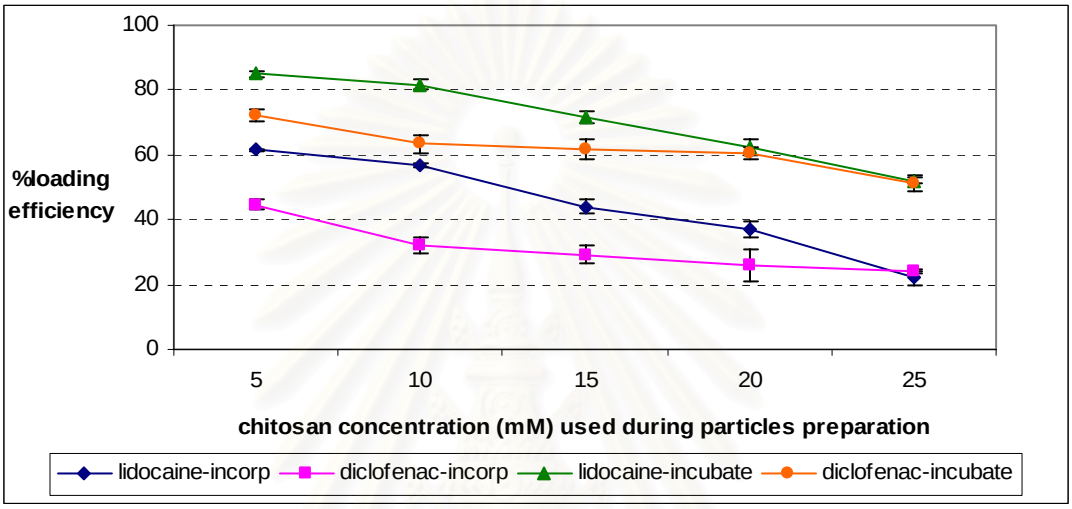


Figure 3.13 The drug loading efficiency of chitosan/AOT/sodium sulfate particles using different loading methods, incubation and incorporation. [AOT] = 50 mM, [sodium sulfate] = 0.6 M, [drug] = 0.4 mg/ml

3.4.2 Change of particle size upon drug loading

When the particle size was monitored by laser light scattering before and after drug loading, the average particle size appeared to be larger upon the drug loading and remained indifferent when the drug concentration used during loading increased from 0.1 to 0.5 mg/ml (Figure 3.14). The initial increase in the size of the particles indicated the increase of void volume inside the particles. However this void volume did not increase further when more drug was absorbed suggesting the inclusion of the drug molecules into the same voided space.

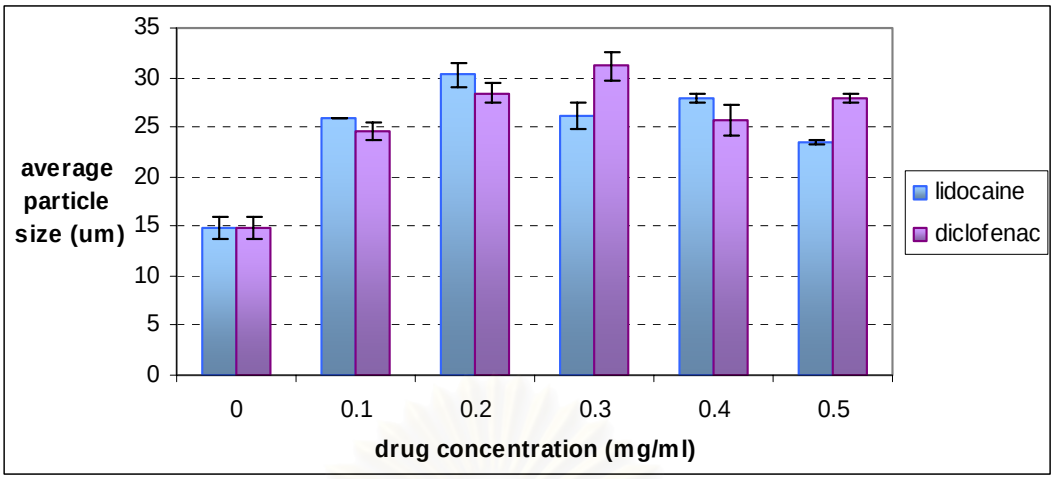


Figure 3.14 The particle size of lidocaine and diclofenac loaded chitosan/AOT particles as a function of the drug concentration. [AOT] = 50 mM, [chitosan] = 5 mM, [sodium sulfate] = 0.6 M, [drug] = 0.4 mg/ml

3.5 In vitro drug release

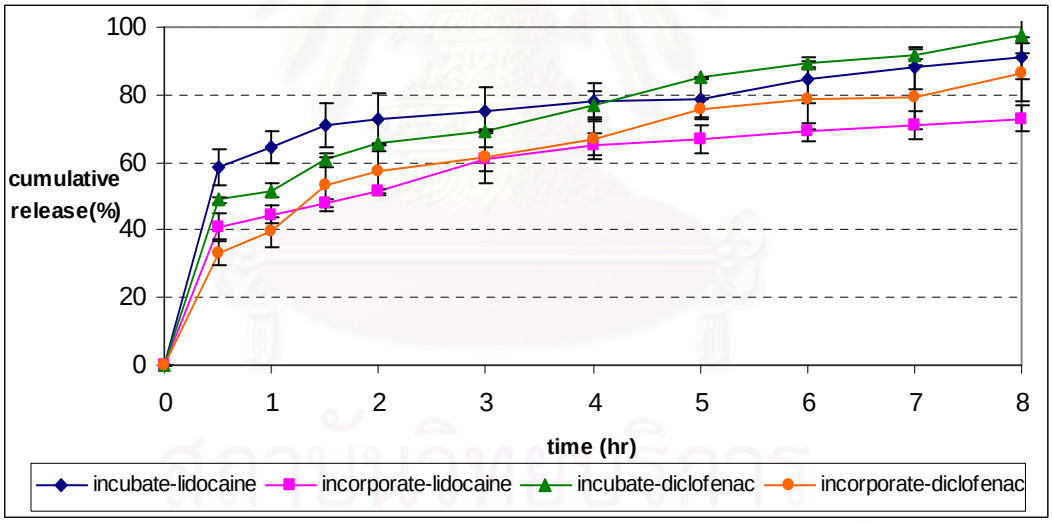


Figure 3.15 Drug release profile of chitosan/AOT/sodium sulfate particles loaded with lidocaine hydrochloride and diclofenac sodium using different methods of loading, incubation and incorporation ([AOT] = 50 mM, [chitosan] = 5 mM, [sodium sulfate] = 0.6 M, [drug] = 0.4 mg/ml). The release is conducted in phosphate buffer saline pH 7.4.

In vitro drug release was performed in pH 7.4 phosphate buffer saline solution for 8 hours. All the drug loaded particles showed similar release profile that is about 40-60% of the drug was released quickly within half an hour and the rest of the drug released slowly

over a period of 8 hours (Figure 3.15). The profiles fit the control release model containing a fast initial surface release and a slow diffusion release of the drug from inside of the particles. It is also interesting to note that the release rates from the drug-loaded particles prepared from the incubation method are slightly faster than those prepared from the incorporation suggesting that the drugs loaded into the chitosan particles are closer to the particle surface in the incubation method. The release profiles found in this work are quite suitable to provide an instant drug action and maintain the drug concentration over the effective threshold for about 8 hours.

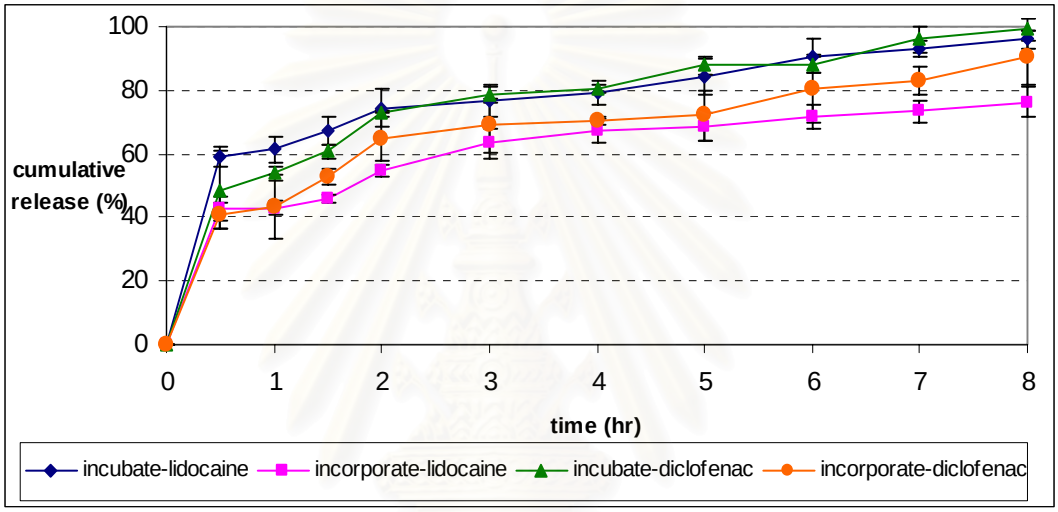


Figure 3.16 Drug release profile of chitosan/AOT/sodium sulfate particles loaded with lidocaine hydrochloride and diclofenac sodium using different methods of loading, incubation and incorporation ([AOT] = 50 mM, [chitosan] = 5 mM, [sodium sulfate] = 0.6 M, [drug] = 0.4 mg/ml). The release is conducted in phosphate buffer pH 5.5.

The drug release profiles from the drug-loaded particles in pH 5.5 phosphate buffer solution showed very similar pattern as those observed in pH 7.4 solution (Figure 3.16). This finding suggested that the chitosan/AOT/sodium sulfate particles may be used as a controlled release of lidocaine and diclofenac for specific local application because the skin pH is mildly acidic range (5.5-7).

CHAPTER IV

CONCLUSION

4.1 Conclusion

The chitosan nanoparticles could be prepared from the chitosan/AOT system. However the particles were not stable and tended to aggregate upon surfactant removal. With an addition of sodium sulfate, the chitosan/AOT/sodium sulfate microparticles were produced. These particles were stable after the removal of AOT by aqueous washing. The particles could be loaded with lidocaine and diclofenac with loading efficiencies up to 80% and 70%, respectively by incubation loading. The controlled release study revealed that the drug loaded particles could sustain the drug release up to 8 hours in mildly acidic pH that might find real specific local application.

4.2 Suggestion for further work

Further study for fine tuning of this research work should be studied to bring about real application by in vivo test e.g. applying the particles on skin of panelists to test drug efficiency and drug release comparing to the commercial drugs.

REFERENCES

[1] O. Felt, P. Furrer, J.M. Mayer, B. Plazonnet, P. Buri, R. Grny. Topical use of chitosan in ophthalmology : tolerance assessment and evaluation of precorneal retention. International Journal Pharmacy 180 (1999) : 185-193.

[2] S. Patashnik, L. Rabinovich, G. Golomb. Preparation and evaluation of chitosan microspheres containing biphosphates. Journal Drug Target 4 (1997) : 371-380.

[3] J.S. Song, C.H. Such, Y.B. Park, S.H. Lee, N.C. Yoo, J.D. Lee, K.H. Kim, S.K. Lee. A phase, I/IIa study on intra-articular injection of holmium-166-chitosan complex for the treatment of knee synovitis of rheumatoid arthritis. European Journal Nuclear Medical 28 (2001) : 489-497.

[4] R.A.A. Muzzarelli. Human enzymatic activities related to the therapeutic administration of chitin derivatives. Cell Molecular Life Science 53 (1997) : 131-140.

[5] D. Koga. Chitin enzymology-chitinase, In : R.Chen and H.C. Chen, (Eds.). Advance Chitin Science 3 (1998) : 16-23.

[6] V.R. Sinha, A.K. Singla, S. Wadhawan, R. Kaushik, R. Kumria, K.Bansal, S. Dhawan. Review chitosan microspheres as a potential carrier for drugs. International Journal of Pharmaceutics 274 (2004) : 1-33.

[7] O.Felt,P.Buri,R.Gurny. Chitosan : a unique polysaccharide for drug delivery. Drug Dev. Ind. Pharm. 24 (11) (1998) : 979-993.

[8] Chandy, T., Sharma, C.P. Chitosan- as a biomaterial. Biomater. Artif. Cells Artif. Organs 18 (1990) : 1-24.

[9] X.Z.Shu, K.J.Zhu. Controlled drug release properties of ionically cross-linked chitosan beads : the influence of anion structure. International Journal of pharmaceutics 233 (2002) : 217-225.

[10] O.Skaugrud. Chitosan-new biopolymer for cometics and drug. Drug Cosmetic Ind. 148 (1991) : 24-29.

[11] VJ Mohanraj and Y Chen. Nanoparticles – A review. Tropical Journal of Pharmaceutical Research 5(1) (2006) : 561-573.

[12] S.A.Agnihotri, N.N.Mallikarjuna, T.M.Aminabhavi. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. Journal of Controlled Release 100 (2004) : 5-28.

[13] I.Orienti, K.Aiedeh, E.Gianasi, V.Bertasi. Indomethacin loaded chitosna microspheres. Correlation between the erosion process and release kinetics. Journal Microencapsulation 13 (1996) : 463-472.

[14] Y.Xu, Y.Du. Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. International Journal of Pharmaceutics 250 (2003) : 215-226.

[15] Z.Ma, H.H. Yeoh, L.Y.Lim. Formulation pH modulates the interaction of insulin with chitosan nanoparticles. Journal of Controlled Release 37 (2000) : 124-132.

[16] R. Fernandez-Urrusuno, P. Calve, C.Remunan-Lopez, J.L. vila-Jato, M.J. Alonso. Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharmaceutical Research 16 (1999) : 1576-1581.

[17] Q.Gan, T.Wang. Chitosan nanoparticle as protein delivery carrier – Systematic examination of fabrication conditions for efficient loading and release. Colloids and Surfaces B : Biointerfaces (2007).

[18] S.Ozbax-Turan, J.Akbuga, C.Aral. Controlled release of interleukin-2 from chitosan microspheres. Journal Pharmaceutical Science 91 (2002) : 1245-1251.

[19] M.N.V. Ravi Kumar. A review of chitin and chitosan applications. Reactive & functional polymers 46 (2000) : 1-27.

[20] Y.Xia, E.Chen, D.L.Tibbits, T.E.Reilley, T.D.McSweeney. Comparison of effects of lidocaine hydrochloride, buffer lidocaine, diphenhydramine, and normal saline after intradermal hydrochloride gels. European Journal Pharmaceutical Science 17 (2002) : 161-167.

[21] H.Piao, N.Kamiya, J.Watanabe, H.Yokoyama. Oral delivery of diclofenac sodium using a novel solid-in-soil suspension. International Journal of Pharmaceutics 313 (2006) : 159-162.

[22] A.Berthold, K.Cremer, J. Kreuter. Preparation and characterization of chitosan microspheres as drug carrier for prednisolone sodium phosphate as model for anti-inflammatory drugs. Journal of controlled release 39 (1996) : 17-25.

[23] I.M. van der Lubben, J.C. Verhoef, A.C. van Aelst, G.Borchard, H.E. Junginger. Chitosan microparticles for oral vaccination : preparation, characterization and preliminary in vivo uptake studies in murine Peyer's patches. Biomaterials 22 (2001) : 687-694.

[24] H.-Q. Mao, K.Roy,V.L.Troung-Le, K.A.Janes, K.Y.Lin, Y.Wang, J.T.August, K.W.Leong. Chitosan-DNA nanoparticles as gene carriers : synthesis, characterization and transfection efficiency. Journal of Controlled Release 70 (2001) : 399-421.

[25] J.A. Ko, H.J. Park , S.J. Hwang, J.B. Park, J.S Lee. Preparation and characterization of chitosan microparticles intended for controlled drug delivery. International Journal of Pharmaceutics 249 (2002) : 165-174.

[26] R.Hejazi, M.Amiji. Stomach-specific anti-H.pylori therapy. I : preparation and characterization of tetracycline-loaded chitosan microspheres. International Journal of Pharmaceutics 235 (2002) : 87-94.

[27] I.M. van der Lubben, G.Kersten, M.M. Fretz, C.Beuvery, J.C.Verhoef, H.E. Junginger. Chitosan microparticles for mucosal vaccination against diphtheria : oral and nasal efficacy studies in mice. Vaccine 21 (2003) : 1400-1408.

[28] T.Dastan, K.Turan. In vitro characterization and delivery of chitosan –DNA micropaticles into mammalian cells. Journal Pharmaceutical Science 7 (2) (2004) : 205-214.

[29] C. Saratid, "Effect of surfactants on chitosan nanoparticles size. Program of Petrochemistry and Polymer Science, Faculty of Science, Master Degree Thesis Chulalongkorn University (2005) : 1-79.

[30] M.H. Lameiro, A. Lopes, L.O. martins, P.M. Alves, E. Melo. Incorporation of a model protein into chitosan-bile salt microparticles. International Journal of Pharmaceutics 312 (2006) : 119-130.

[31] O.Celik, J.Akbuga. Preparation of superoxide dismutase loaded chitosan microspheres :Characterization and release studies. European Journal of Pharmaceutics and Biopharmaceutics 66 (2007) : 42-47.

[32] S.H.Chiou, W.T. Wu, Y.Y. Huang, T.W.Chung. Effects of the characteristics of chitosan on controlling drug release of chitosan coated PLLA microspheres. Journal of Microencapsulation 18 (2001) : 613-625.

[33] S.G. Kumbar, A.R. Kulkarni, M.Aminabhavi. Crosslinked chitosan microspheres for encapsulation of diclofenac sodium: effect of crosslinking agent" Journal Microencapsulation 19 (2002) : 173-180.

[34] M.L.Gonzalez-Rodriguez, M.A.Holgado, C.Sanchez-Lafuente, A.M.Rabasco, A.Fini. Alginate/chitosan particulate systems for sodium diclofenac release." International Journal Pharmaceutics 232 (2002) 225-234.

[35] N.Praphairaksit, K.Klywong. The controlled release of diclofenac sodium from chitosan-diclofenac bead. 31st Congress on science and technology of Thailand at Suranaree university of technology (2007) : 18-20.

REFERENCES

[1] O. Felt, P. Furrer, J.M. Mayer, B. Plazonnet, P. Buri, R. Grny. Topical use of chitosan in ophthalmology : tolerance assessment and evaluation of precorneal retention. International Journal Pharmacy 180 (1999) : 185-193.

[2] S. Patashnik, L. Rabinovich, G. Golomb. Preparation and evaluation of chitosan microspheres containing biphosphates. Journal Drug Target 4 (1997) : 371-380.

[3] J.S. Song, C.H. Such, Y.B. Park, S.H. Lee, N.C. Yoo, J.D. Lee, K.H. Kim, S.K. Lee. A phase, I/IIa study on intra-articular injection of holmium-166-chitosan complex for the treatment of knee synovitis of rheumatoid arthritis. European Journal Nuclear Medical 28 (2001) : 489-497.

[4] R.A.A. Muzzarelli. Human enzymatic activities related to the therapeutic administration of chitin derivatives. Cell Molecular Life Science 53 (1997) : 131-140.

[5] D. Koga. Chitin enzymology-chitinase, In : R.Chen and H.C. Chen, (Eds.). Advance Chitin Science 3 (1998) : 16-23.

[6] V.R. Sinha, A.K. Singla, S. Wadhawan, R. Kaushik, R. Kumria, K.Bansal, S. Dhawan. Review chitosan microspheres as a potential carrier for drugs. International Journal of pharmaceutics 274 (2004) : 1-33.

[7] O.Felt,P.Buri,R.Gurny. Chitosan : a unique polysaccharide for drug delivery. Drug Dev. Ind. Pharm. 24 (11) (1998) : 979-993.

[8] Chandy, T., Sharma, C.P. Chitosan- as a biomaterial. Biomater. Artif. Cells Artif. Organs 18 (1990) : 1-24.

[9] X.Z.Shu, K.J.Zhu. Controlled drug release properties of ionically cross-linked chitosan beads : the influence of anion structure. International journal of pharmaceutics 233 (2002) : 217-225.

[10] O.Skaugrud. Chitosan-new biopolymer for cometics and drug. Drug Cosmetic Ind. 148 (1991) : 24-29.

[11] VJ Mohanraj and Y Chen. Nanoparticles – A review. Tropical journal of pharmaceutical research 5(1) (2006) : 561-573.

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[15] Z.Ma, H.H. Yeoh, L.Y.Lim. Formulation pH modulates the interaction of insulin with chitosan nanoparticles. Journal Control Release 37 (2000) : 124-132.

[16] R. Fernandez-Urrusuno, P. Calve, C.Remunan-Lopez, J.L. vila-Jato, M.J. Alonso. Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharm. Res. 16 (1999) : 1576-1581.

[17] Q.Gan, T.Wang. Chitosan nanoparticle as protein delivery carrier – Systematic examination of fabrication conditions for efficient loading and release. Colloids and Surfaces B : Biointerfaces (2007).

[18] S.Ozbax-Turan, J.Akbuga, C.Aral. Controlled release of interleukin-2 from chitosan microspheres. Journal Pharmaceutical Science 91 (2002) : 1245-1251.

[19] M.N.V. Ravi Kumar. A review of chitin and chitosan applications. Reactive & functional polymers 46 (2000) : 1-27.

[20] Y.Xia, E.Chen, D.L.Tibbits, T.E.Reilley, T.D.McSweeney. Comparison of effects of lidocaine hydrochloride, buffer lidocaine, diphenhydramine, and normal saline after intradermal hydrochloride gels. European Journal Pharmaceutical Science 17 (2002) : 161-167.

[21] H.Piao, N.Kamiya, J.Watanabe, H.Yokoyama. Oral delivery of diclofenac sodium using a novel solid-in-soil suspension. International Journal of Pharmaceutics 313 (2006) : 159-162.

[22] A.Berthold, K.Cremer, J. Kreuter. Preparation and characterization of chitosan microspheres as drug carrier for prednisolone sodium phosphate as model for anti-inflammatory drugs. Journal of controlled release 39 (1996) : 17-25.

[23] I.M. van der Lubben, J.C. Verhoef, A.C. van Aelst, G.Borchard, H.E. Junginger. Chitosan microparticles for oral vaccination : preparation, characterization and preliminary in vivo uptake studies in murine Peyer's patches. Biomaterials 22 (2001) : 687-694.

[24] H.-Q. Mao, K.Roy,V.L.Troung-Le, K.A.Janes, K.Y.Lin, Y.Wang, J.T.August, K.W.Leong. Chitosan-DNA nanoparticles as gene carriers : synthesis, characterization and transfection efficiency. Journal of controlled release 70 (2001) : 399-421.

[25] J.A. Ko, H.J. Park , S.J. Hwang, J.B. Park, J.S Lee. Preparation and characterization of chitosan microparticles intended for controlled drug delivery. International journal of pharmaceutics 249 (2002) : 165-174.

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[27] I.M. van der Lubben, G.Kersten, M.M. Fretz, C.Beuvery, J.C.Verhoef, H.E. Junginger. Chitosan microparticles for mucosal vaccination against diphtheria : oral and nasal efficacy studies in mice. Vaccine 21 (2003) : 1400-1408.

[28] T.Dastan, K.Turan. In vitro characterization and delivery of chitosan –DNA micropaticles into mammalian cells. Journal pharm. pharmaceutical science 7 (2) (2004) : 205-214.

[29] C. Saratid, “Effect of surfactants on chitosan nanoparticles size. Program of Petrochemistry and Polymer Science, Faculty of Science, Master Degree Thesis Chulalongkorn University (2005) : 1-79.

[30] M.H. Lameiro, A. Lopes, L.O. martins, P.M. Alves, E. Melo. Incorporation of a model protein into chitosan-bile salt microparticles. International Journal of Pharmaceutics 312 (2006) : 119-130.

[31] O.Celik, J.Akbuga. Preparation of superoxide dismutase loaded chitosan microspheres : Characterization and release studies. European Journal of Pharmaceutics and Biopharmaceutics 66 (2007) : 42-47.

[32] S.H.Chiou, W.T. Wu, Y.Y. Huang, T.W.Chung. Effects of the characteristics of chitosan on controlling drug release of chitosan coated PLLA microspheres. Journal of Microencapsulation 18 (2001) : 613-625.

[33] S.G. Kumbar, A.R. Kulkarni, M.Aminabhavi. Crosslinked chitosan microspheres for encapsulation of diclofenac sodium: effect of crosslinking agent” Journal Microencapsulation 19 (2002) : 173-180.

[34] M.L.Gonzalez-Rodriguez, M.A.Holgado, C.Sanchez-Lafuente, A.M.Rabasco, A.Fini. Alginate/chitosan particulate systems for sodium diclofenac release.” International Journal Pharmaceutics 232 (2002) 225-234.

[35] N.Praphairaksit, K.Klywong. The controlled release of diclofenac sodium from chitosan-diclofenac bead. 31st Congress on science and technology of Thailand at Suranaree university of technology (2007) : 18-20.

APPENDICES

APPENDIX A

Preparation of calibration curve of drug

Two calibration curve lines for lidocaine hydrochloride and diclofenac sodium were obtained from aqueous solution to give linear calibration lines. The UV-Vis absorbance of lidocaine hydrochloride and diclofenac sodium was measured at 262 nm and 276 nm, respectively.

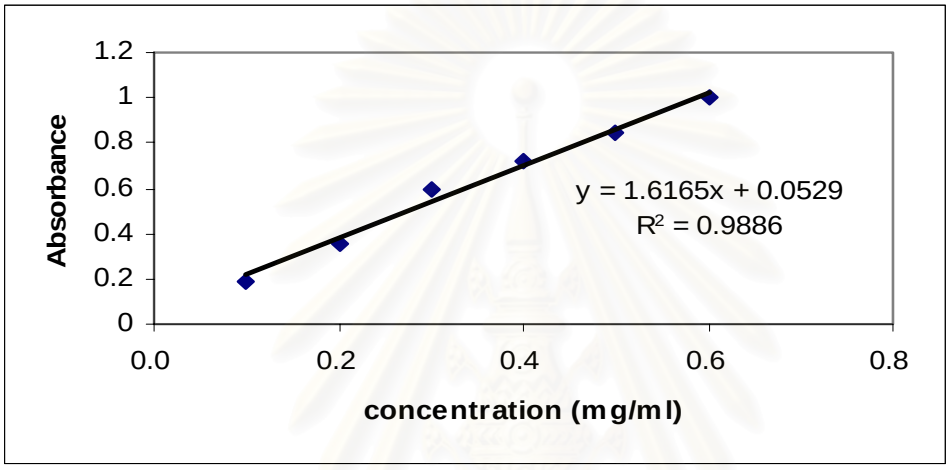


Figure A1 Calibration curve of lidocaine hydrochloride in water

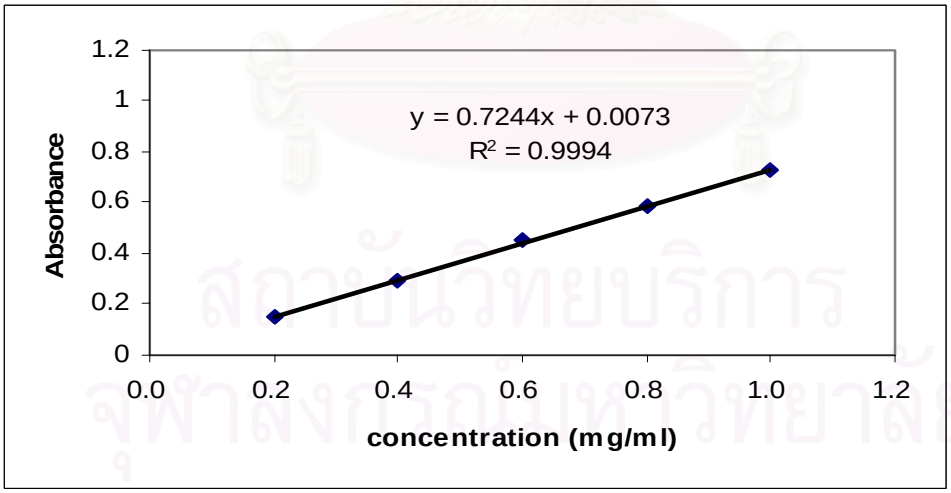


Figure A2 Calibration curve of diclofenac sodium in water

APPENDIX B

Table B1 Time of chitosan solution traveling through the Ubbelohde Viscometer.

C (g/l)	Time				η_{rel}	η_{sp}	η_{sp}/C
	t1	t2	t3	t			
0.000	88.59	88.65	88.64	88.63	1.000	0.000	-
3.064	135.58	135.8	135.77	135.72	1.531	0.531	173
2.662	127.44	127.17	127.19	127.27	1.436	0.436	164
2.321	121.27	121.51	121.54	121.44	1.370	0.370	159
1.675	108.99	108.59	108.41	108.66	1.226	0.226	135
1.485	105.89	105.97	105.44	105.77	1.193	0.193	130
1.269	102.79	102.21	102.52	102.51	1.157	0.157	123

Table B2 Molecular weight of chitosan calculated from $[\eta] = K M_v^a$ ($K = 1.8 \times 10^{-3}$, $a = 0.93$)

$[\eta]$	$\log M_v$	M_v
102.5	5.113	129,752

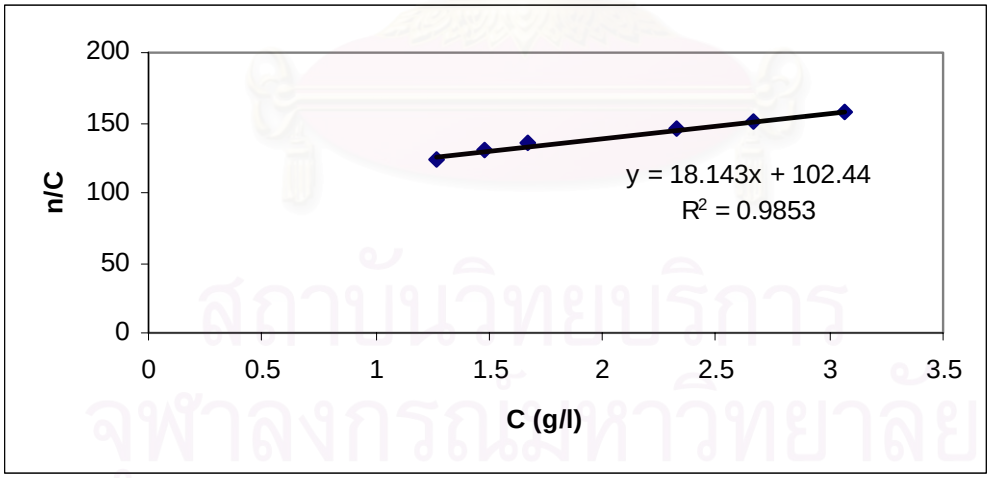


Figure B1 Plot of η_{sp}/C against C of chitosan

Table B3 Volume of the PVSK solution for blank and CPC titration

Time	PVSK (ml)	
	CPC Titration	Blank Titration
1	3.5	0.1
2	3.5	0.1
3	3.45	0.1
Avg.	3.48	0.1

Table B4 Volume of the PVSK solution for titration of chitosan

Time	PVSK (ml)
1	3.5
2	3.4
3	3.4
Avg	3.43

APPENDIX C

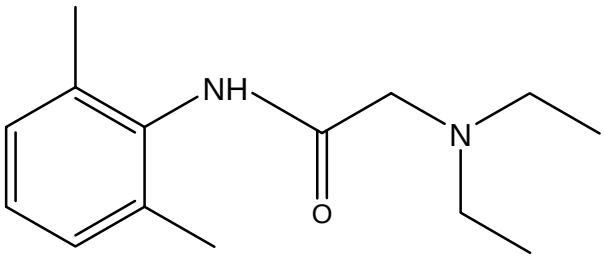


Figure C1 Structure of lidocaine hydrochloride , MW. = 234.34 g/mol

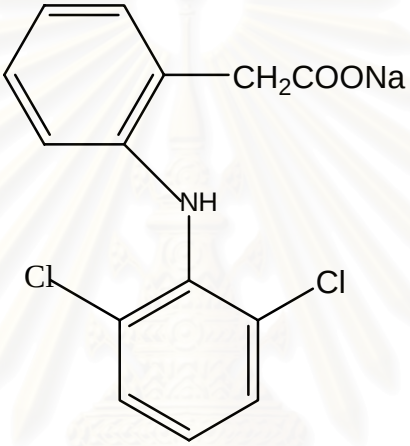


Figure C2 Structure of diclofenac sodium , MW. = 296.14 g/mol

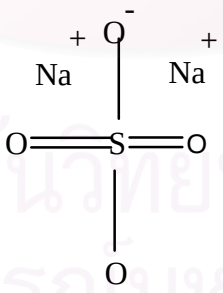


Figure C3 Structure of sodium sulfate , MW. = 142.04 g/mol

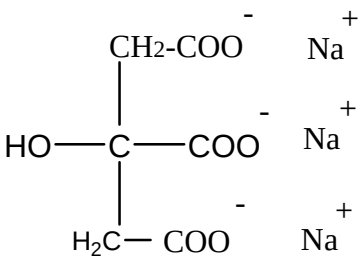


Figure C4 Structure of sodium citrate , MW. = 258.07 g/mol

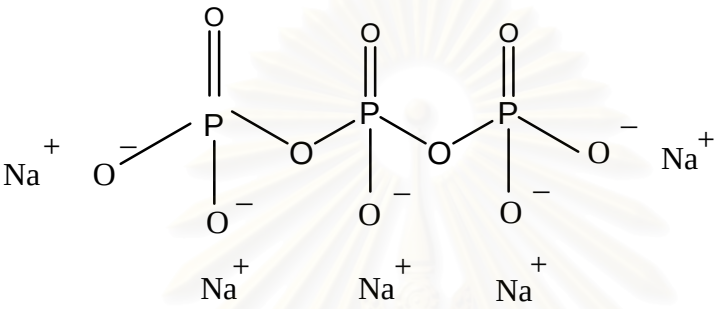


Figure C5 Structure of sodium tripolyphosphate , MW. = 367.86 g/mol

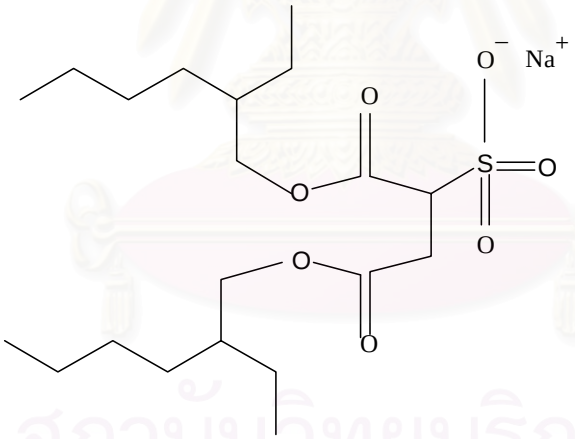
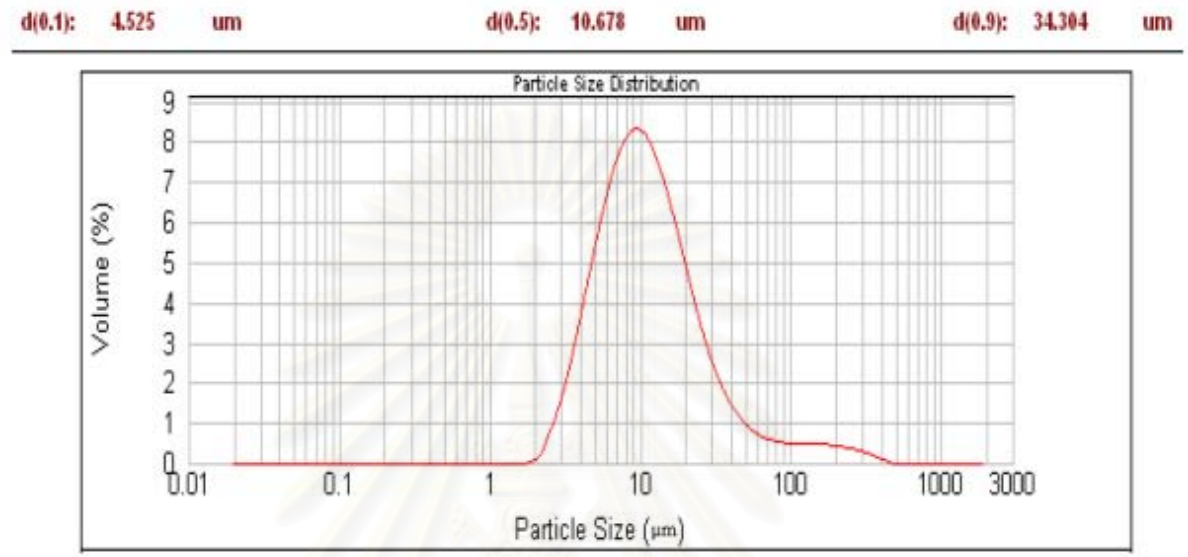


Figure C6 Structure of sodium bis (ethylhexyl) sulfosuccinate , MW = 444.56 g/mol

APPENDIX D



Size (nm)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

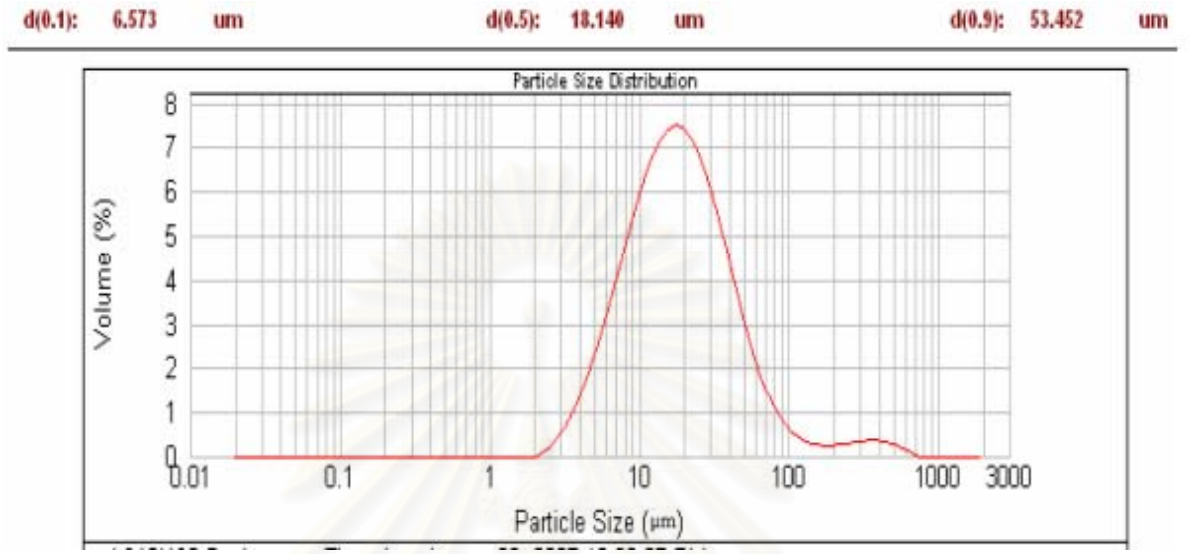
Size (nm)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.10
2.188	0.43
2.512	0.96
2.884	1.63
3.311	2.46
3.802	3.39
4.365	4.37
5.012	5.31
5.754	6.17
6.607	6.85
7.586	7.31
8.710	7.50
10.000	7.40
11.482	

Size (nm)	Volume in %
11.482	7.05
13.183	6.47
15.136	5.74
17.378	4.92
19.953	4.10
22.909	3.32
26.303	2.63
30.200	2.05
34.674	1.57
39.811	1.21
45.709	0.94
52.481	0.74
60.256	0.60
69.183	0.52
79.433	0.47
91.201	0.44
104.713	0.43
120.226	

Size (nm)	Volume in %
120.226	0.43
138.038	0.43
158.489	0.41
181.970	0.39
208.930	0.36
239.883	0.32
275.423	0.26
316.228	0.20
363.078	0.11
416.869	0.03
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (nm)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D1 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by using 0.6 M sodium sulfate.



Size (nm)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (nm)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.11
2.512	0.27
2.884	0.54
3.311	0.86
3.802	1.28
4.365	1.77
5.012	2.33
5.754	2.96
6.607	3.63
7.586	4.31
8.710	4.97
10.000	5.59
11.482	

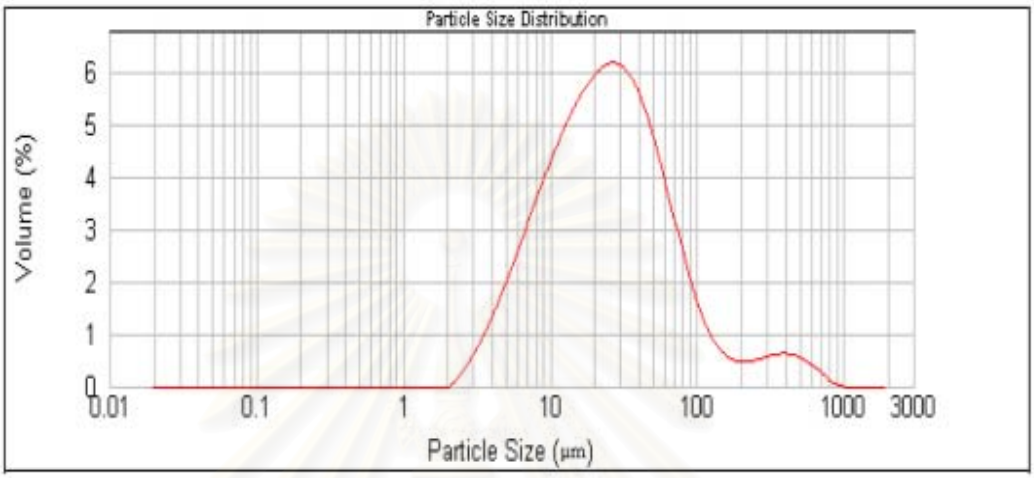
Size (nm)	Volume in %
11.482	6.10
13.183	6.48
15.136	6.70
17.378	6.75
19.953	6.60
22.909	6.27
26.303	5.79
30.200	5.17
34.674	4.46
39.811	3.72
45.709	2.99
52.481	2.31
60.256	1.72
69.183	1.24
79.433	0.86
91.201	0.60
104.713	0.42
120.226	

Size (nm)	Volume in %
120.226	0.31
138.038	0.25
158.489	0.22
181.970	0.22
208.930	0.24
239.883	0.27
275.423	0.30
316.228	0.32
363.078	0.32
416.869	0.29
478.630	0.24
549.541	0.14
630.957	0.06
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (nm)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D2 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by using 0.6 M sodium citrate.

d(0.1): 6.908 um d(0.5): 24.418 um d(0.9): 86.995 um



Size (nm)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (nm)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.17
2.512	0.35
2.884	0.60
3.311	0.88
3.802	1.21
4.365	1.58
5.012	1.97
5.754	2.38
6.607	2.80
7.586	3.22
8.710	3.62
10.000	4.01
11.482	

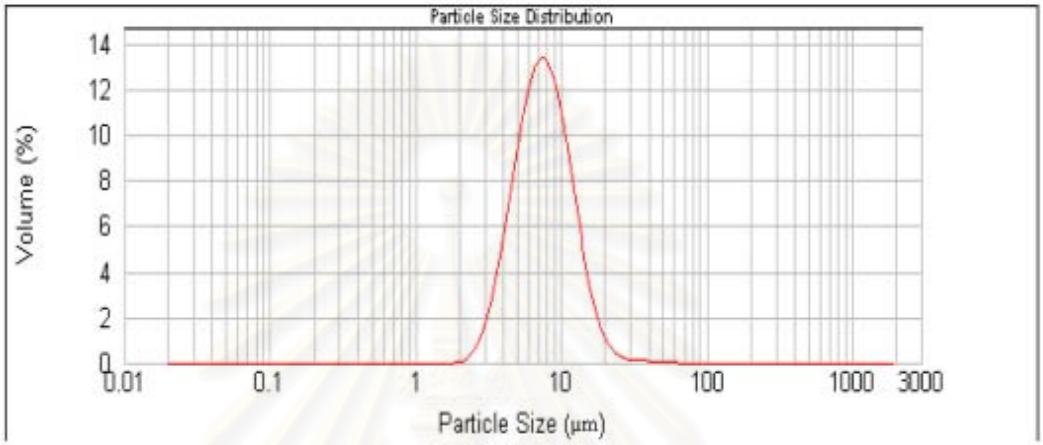
Size (nm)	Volume in %
11.482	4.36
13.183	4.69
15.136	4.96
17.378	5.23
19.953	5.41
22.909	5.53
26.303	5.55
30.200	5.47
34.674	5.27
39.811	4.94
45.709	4.48
52.481	3.92
60.256	3.30
69.183	2.66
79.433	2.06
91.201	1.54
104.713	1.11
120.226	

Size (nm)	Volume in %
120.226	0.80
138.038	0.60
158.489	0.48
181.970	0.43
208.930	0.43
239.883	0.46
275.423	0.50
316.228	0.55
363.078	0.57
416.869	0.55
478.630	0.49
549.541	0.40
630.957	0.29
724.436	0.14
831.764	0.04
954.993	0.00
1096.478	0.00
1258.925	

Size (nm)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D3 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by using 0.6 M sodium tripolyphosphate.

d(0.1): 4.272 um d(0.5): 7.523 um d(0.9): 13.448 um



Size (nm)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

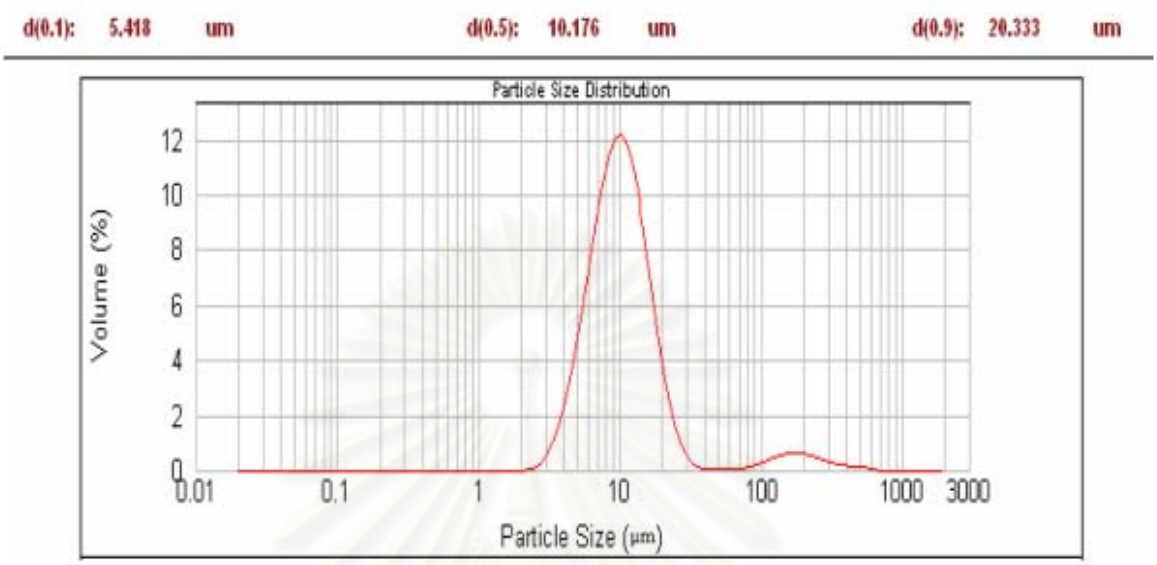
Size (nm)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.02
2.188	0.21
2.512	0.73
2.884	1.69
3.311	3.17
3.802	5.11
4.365	7.30
5.012	9.41
5.754	11.10
6.607	11.98
7.586	11.87
8.710	10.81
10.000	8.96
11.482	

Size (nm)	Volume in %
11.482	6.82
13.183	4.66
15.136	2.88
17.378	1.58
19.953	0.80
22.909	0.36
26.303	0.17
30.200	0.11
34.674	0.09
39.811	0.07
45.709	0.06
52.481	0.03
60.256	0.00
69.183	0.00
79.433	0.00
91.201	0.00
104.713	0.00
120.226	

Size (nm)	Volume in %
120.226	0.00
138.038	0.00
158.489	0.00
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.00
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (nm)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D4 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by using 5 mM chitosan concentration.



Size (µm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (µm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

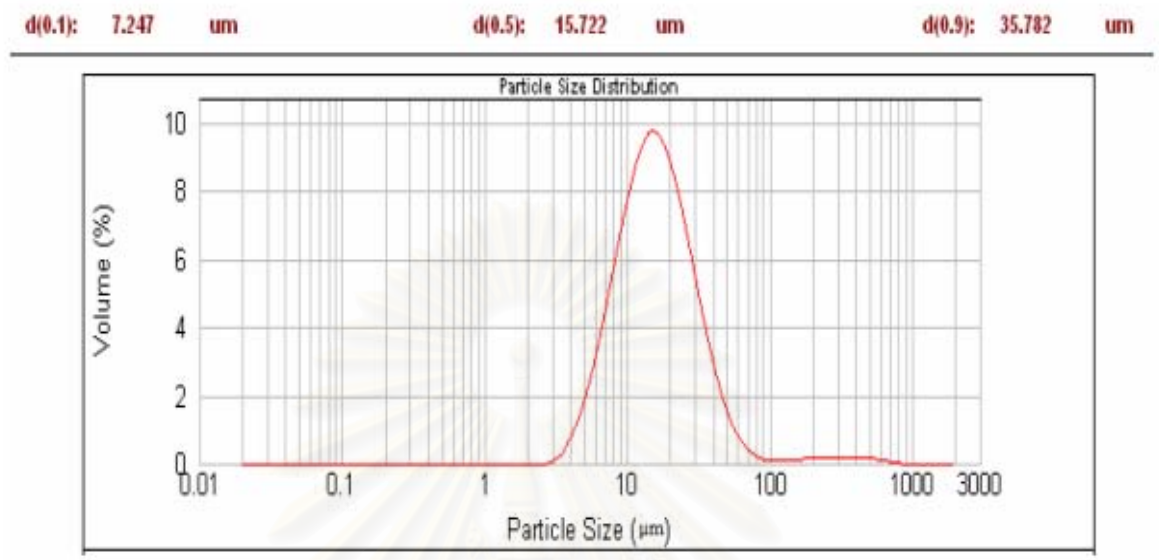
Size (µm)	Volume In %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.02
2.512	0.16
2.884	0.52
3.311	1.15
3.802	2.11
4.365	3.42
5.012	5.02
5.754	6.81
6.607	8.56
7.586	10.00
8.710	10.83
10.000	10.88
11.482	10.68

Size (µm)	Volume In %
11.482	10.68
13.183	8.57
15.136	6.67
17.378	4.69
19.953	2.95
22.909	1.63
26.303	0.77
30.200	0.29
34.674	0.08
39.811	0.04
45.709	0.02
52.481	0.01
60.256	0.01
69.183	0.05
79.433	0.12
91.201	0.22
104.713	0.33
120.226	

Size (µm)	Volume In %
120.226	0.44
138.038	0.52
158.489	0.57
181.970	0.56
208.930	0.50
239.883	0.41
275.423	0.31
316.228	0.22
363.078	0.17
416.869	0.13
478.630	0.10
549.541	0.06
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (µm)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D5 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by using 10 mM chitosan concentration.



Size (?m)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (?m)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

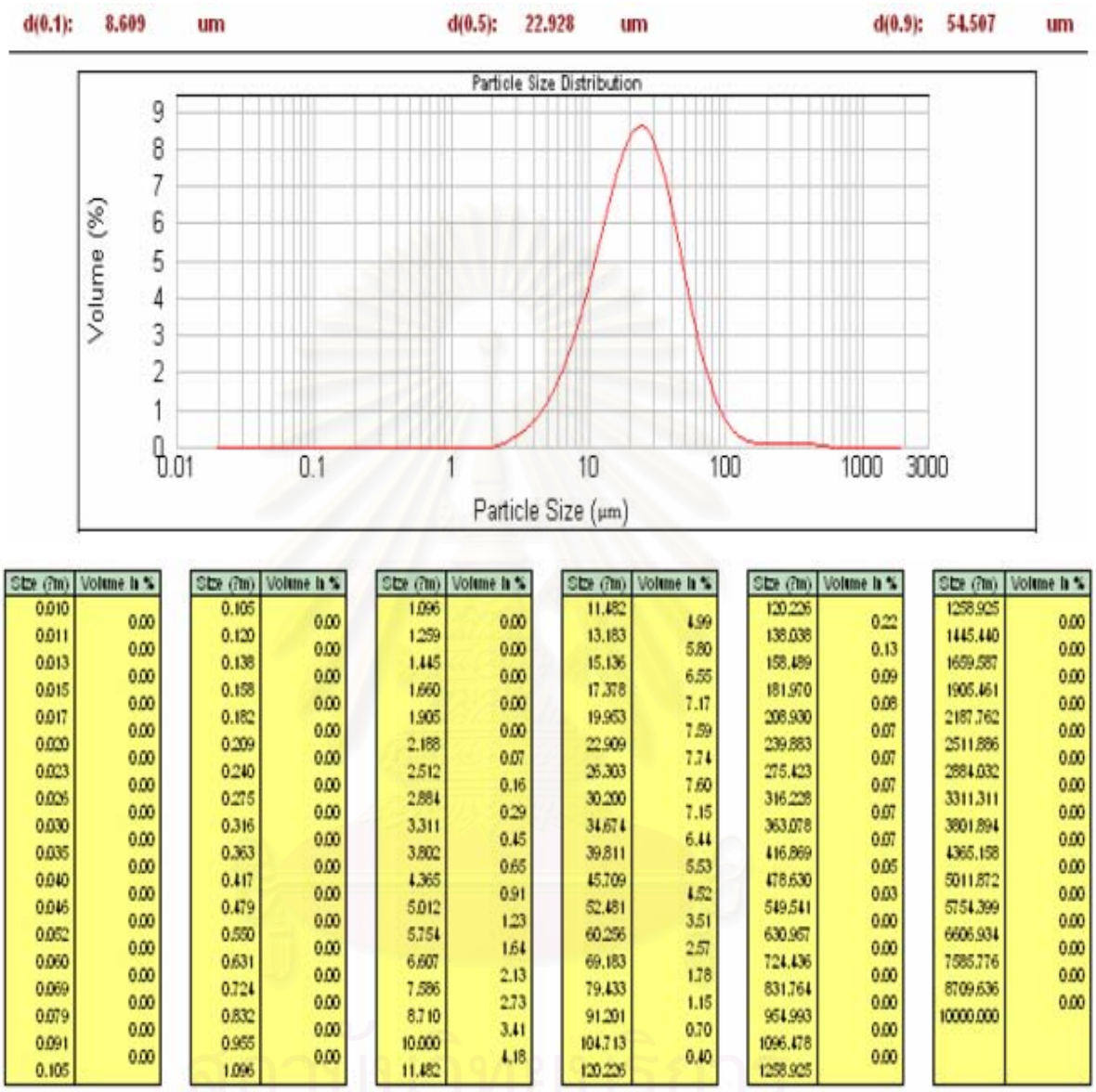
Size (?m)	Volume In %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.00
2.512	0.00
2.884	0.08
3.311	0.28
3.802	0.71
4.365	1.27
5.012	2.04
5.754	3.00
6.607	4.10
7.586	5.28
8.710	6.43
10.000	7.46
11.482	

Size (?m)	Volume In %
11.482	8.24
13.183	8.69
15.136	8.75
17.378	8.40
19.953	7.70
22.909	6.72
26.303	5.59
30.200	4.41
34.674	3.30
39.811	2.33
45.709	1.54
52.481	0.95
60.256	0.55
69.183	0.29
79.433	0.15
91.201	0.09
104.713	0.07
120.226	

Size (?m)	Volume In %
120.226	0.08
138.038	0.10
158.489	0.12
181.970	0.13
208.930	0.13
239.883	0.14
275.423	0.14
316.228	0.14
363.078	0.14
416.869	0.14
478.630	0.14
549.541	0.10
630.957	0.06
724.436	0.02
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (?m)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D6 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by using 15 mM chitosan concentration.



Size (?m)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (?m)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

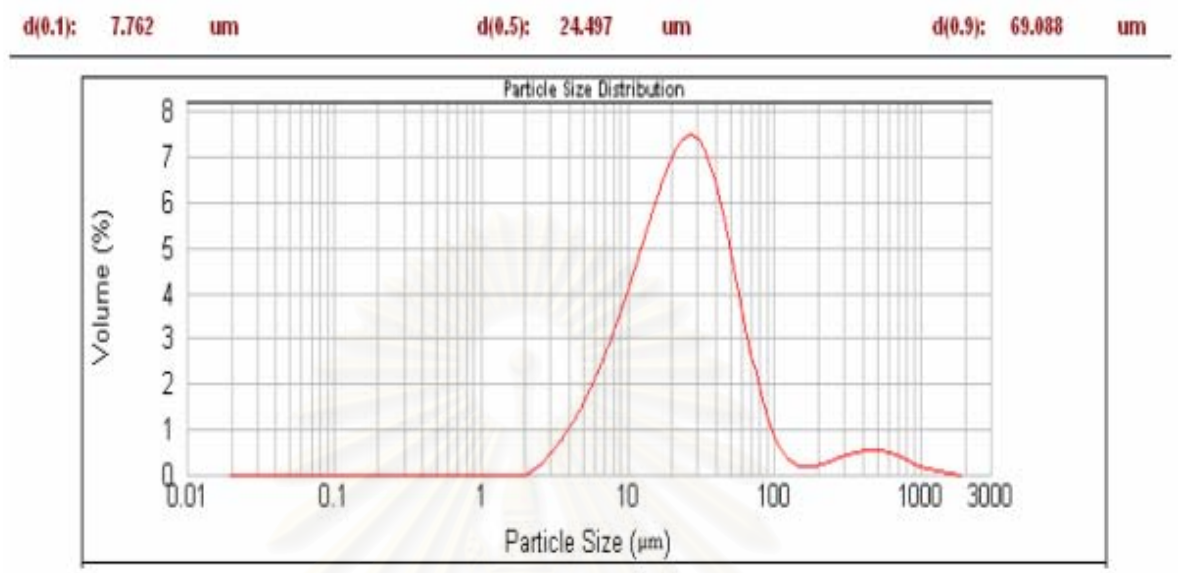
Size (?m)	Volume In %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.07
2.512	0.16
2.884	0.29
3.311	0.45
3.802	0.65
4.365	0.91
5.012	1.23
5.754	1.64
6.607	2.13
7.586	2.73
8.710	3.41
10.000	4.18
11.482	4.99

Size (?m)	Volume In %
11.482	4.99
13.183	5.80
15.136	6.55
17.378	7.17
19.953	7.59
22.909	7.74
26.303	7.60
30.200	7.15
34.674	6.44
39.811	5.53
45.709	4.52
52.481	3.51
60.256	2.57
69.183	1.78
79.433	1.15
91.201	0.70
104.713	0.40
120.226	0.22

Size (?m)	Volume In %
120.226	0.22
138.038	0.13
158.489	0.09
181.970	0.08
208.930	0.07
239.883	0.07
275.423	0.07
316.228	0.07
363.078	0.07
416.869	0.07
478.630	0.05
549.541	0.03
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	0.00

Size (?m)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D7 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by using 20 mM chitosan concentration.



Size (?m)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (?m)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

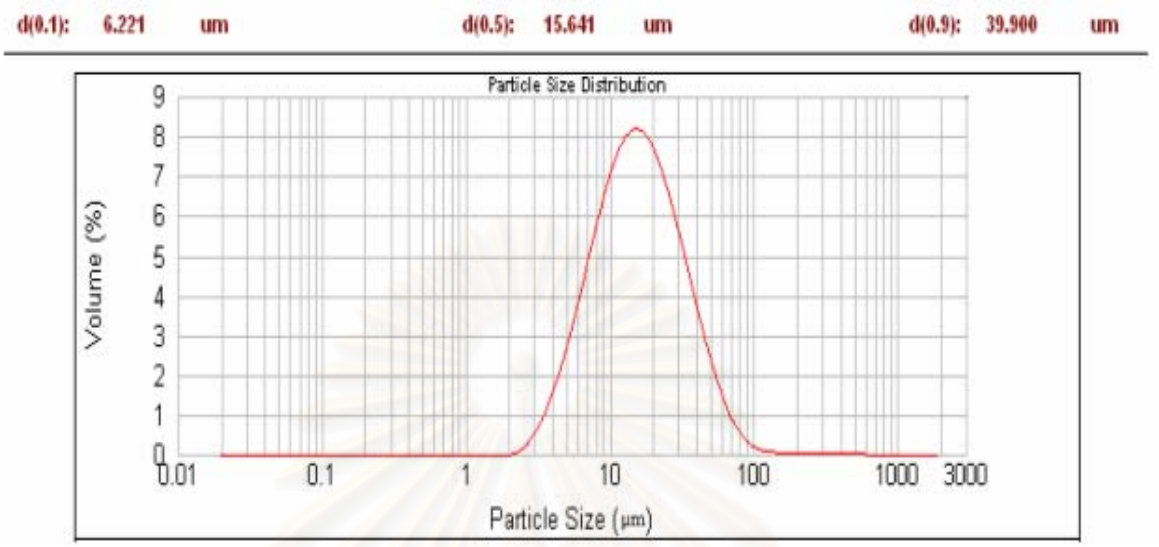
Size (?m)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.12
2.512	0.26
2.884	0.46
3.311	0.68
3.802	0.94
4.365	1.24
5.012	1.57
5.754	1.95
6.607	2.36
7.586	2.82
8.710	3.31
10.000	3.84
11.482	

Size (?m)	Volume in %
11.482	4.39
13.183	4.96
15.136	5.50
17.378	6.00
19.953	6.40
22.909	6.66
26.303	6.73
30.200	6.57
34.674	6.17
39.811	5.55
45.709	4.76
52.481	3.86
60.256	2.94
69.183	2.09
79.433	1.38
91.201	0.85
104.713	0.48
120.226	

Size (?m)	Volume in %
120.226	0.27
138.038	0.17
158.489	0.15
181.970	0.17
208.930	0.23
239.883	0.29
275.423	0.36
316.228	0.41
363.078	0.45
416.869	0.48
478.630	0.47
549.541	0.45
630.957	0.39
724.436	0.32
831.764	0.23
954.993	0.14
1096.478	0.10
1258.925	

Size (?m)	Volume in %
1258.925	0.07
1445.440	0.03
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D8 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by using 25 mM chitosan concentration.



Size (?m)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (?m)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (?m)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.08
2.512	0.25
2.884	0.55
3.311	0.93
3.802	1.44
4.365	2.06
5.012	2.78
5.754	3.57
6.607	4.39
7.586	5.21
8.710	5.96
10.000	6.60
11.482	

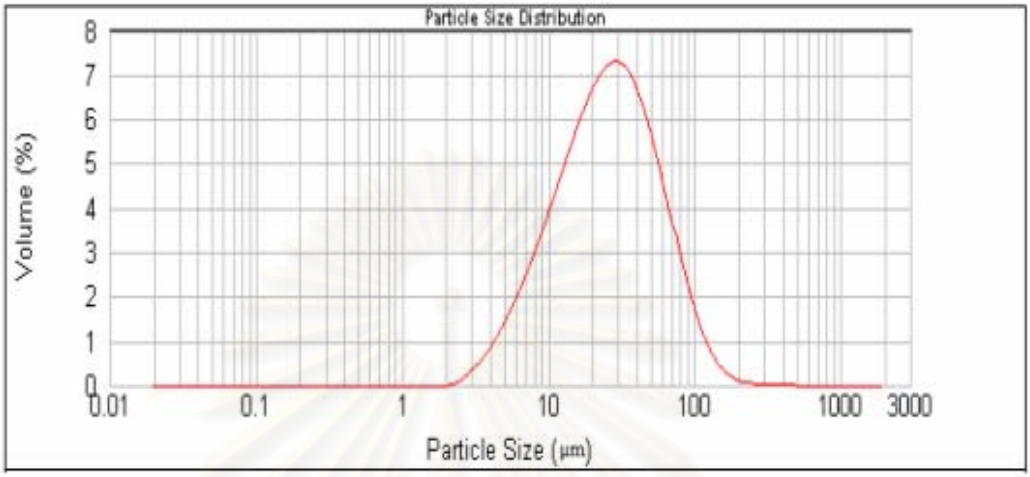
Size (?m)	Volume in %
11.482	7.07
13.183	7.34
15.136	7.38
17.378	7.19
19.953	6.79
22.909	6.21
26.303	5.50
30.200	4.72
34.674	3.91
39.811	3.12
45.709	2.38
52.481	1.74
60.256	1.17
69.183	0.68
79.433	0.37
91.201	0.19
104.713	0.10
120.226	

Size (?m)	Volume in %
120.226	0.06
138.038	0.04
158.489	0.03
181.970	0.02
208.930	0.02
239.883	0.02
275.423	0.02
316.228	0.03
363.078	0.03
416.869	0.02
478.630	0.02
549.541	0.01
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (?m)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D9 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by no loaded drug.

d(0.1): 8.167 um d(0.5): 25.737 um d(0.9): 68.692 um



Size (?m)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (?m)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

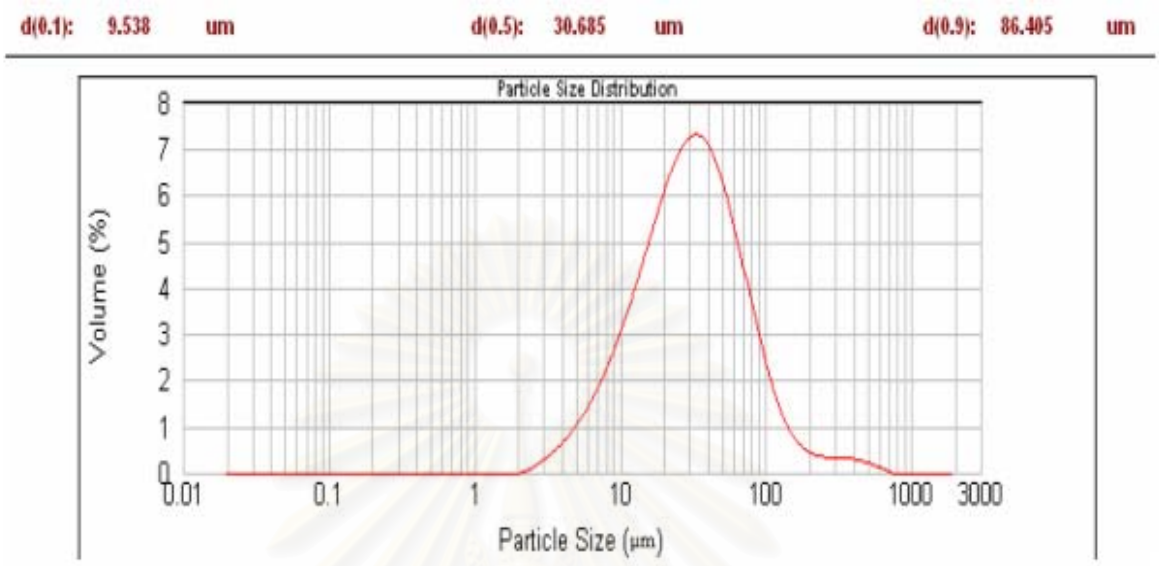
Size (?m)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.07
2.512	0.18
2.884	0.36
3.311	0.56
3.802	0.81
4.365	1.11
5.012	1.45
5.754	1.83
6.607	2.25
7.586	2.72
8.710	3.21
10.000	3.73
11.482	

Size (?m)	Volume in %
11.482	4.26
13.183	4.80
15.136	5.31
17.378	5.78
19.953	6.17
22.909	6.45
26.303	6.58
30.200	6.54
34.674	6.32
39.811	5.90
45.709	5.33
52.481	4.63
60.256	3.85
69.183	3.07
79.433	2.32
91.201	1.66
104.713	1.11
120.226	

Size (?m)	Volume in %
120.226	0.70
138.038	0.40
158.489	0.22
181.970	0.11
208.930	0.06
239.883	0.04
275.423	0.04
316.228	0.03
363.078	0.03
416.869	0.02
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (?m)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D10 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading lidocaine hydrochloride 0.1 mg/ml.



Size (nm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

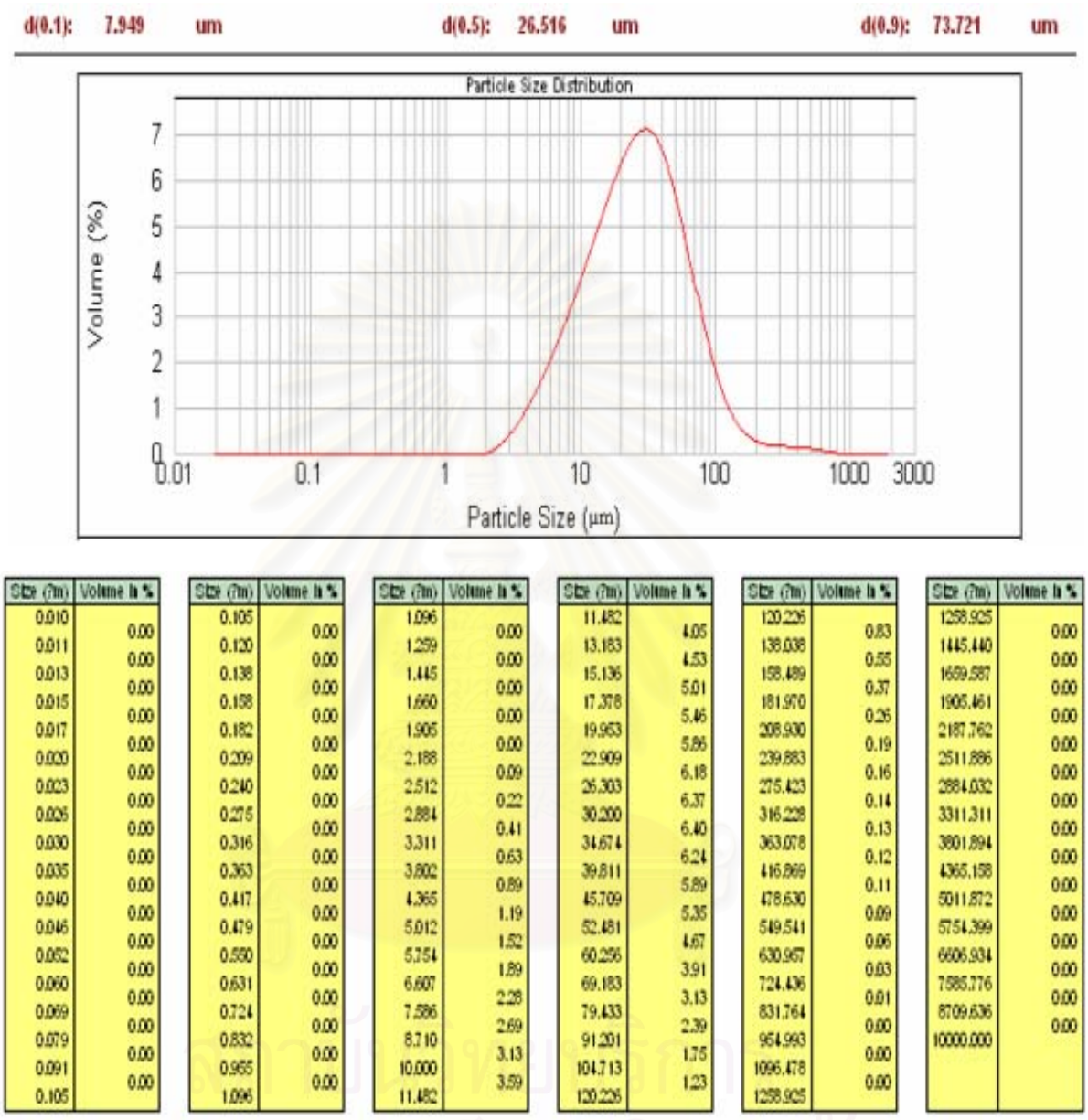
Size (nm)	Volume In %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.06
2.512	0.16
2.884	0.29
3.311	0.43
3.802	0.61
4.365	0.81
5.012	1.05
5.754	1.33
6.607	1.66
7.586	2.03
8.710	2.46
10.000	2.94
11.482	

Size (nm)	Volume In %
11.482	3.45
13.183	4.01
15.136	4.58
17.378	5.15
19.953	5.67
22.909	6.11
26.303	6.43
30.200	6.59
34.674	6.55
39.811	6.31
45.709	5.87
52.481	5.26
60.256	4.53
69.183	3.75
79.433	2.97
91.201	2.25
104.713	1.64
120.226	

Size (nm)	Volume In %
120.226	1.15
138.038	0.80
158.489	0.56
181.970	0.42
208.930	0.34
239.883	0.31
275.423	0.30
316.228	0.29
363.078	0.27
416.869	0.24
478.630	0.20
549.541	0.12
630.957	0.05
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (nm)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D11 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading lidocaine hydrochloride 0.2 mg/ml.



Size (?m)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (?m)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

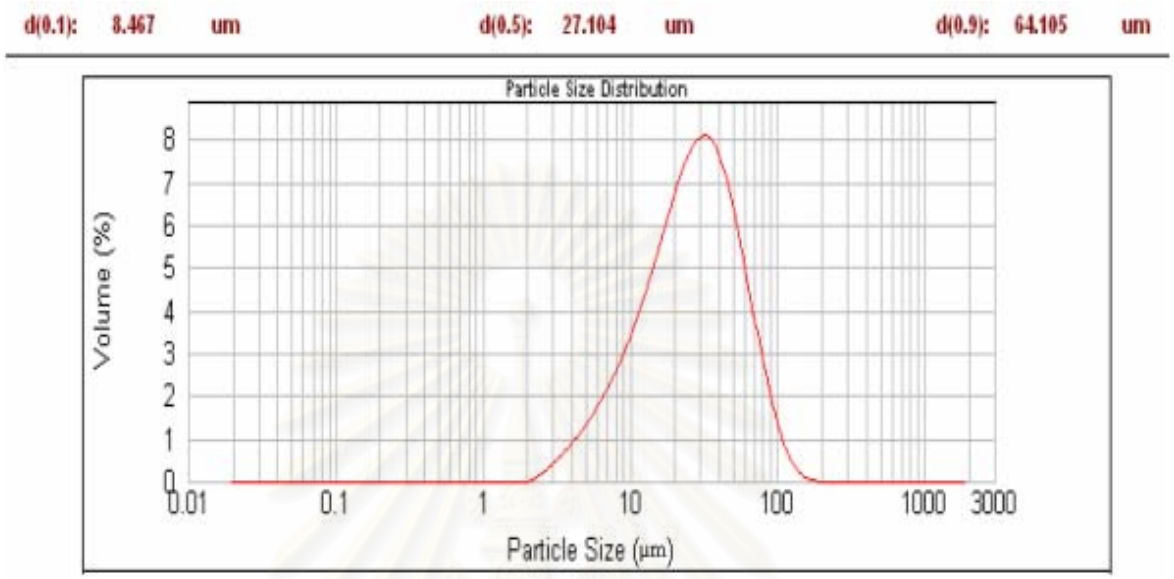
Size (?m)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.09
2.512	0.22
2.884	0.41
3.311	0.63
3.802	0.89
4.365	1.19
5.012	1.52
5.754	1.89
6.607	2.28
7.586	2.69
8.710	3.13
10.000	3.59
11.482	

Size (?m)	Volume in %
11.482	4.05
13.183	4.53
15.136	5.01
17.378	5.46
19.953	5.86
22.909	6.18
26.303	6.37
30.200	6.40
34.674	6.24
39.811	5.89
45.709	5.35
52.481	4.67
60.256	3.91
69.183	3.13
79.433	2.39
91.201	1.75
104.713	1.23
120.226	

Size (?m)	Volume in %
120.226	0.83
138.038	0.55
158.489	0.37
181.970	0.25
208.930	0.19
239.883	0.16
275.423	0.14
316.228	0.13
363.078	0.12
416.869	0.11
478.630	0.09
549.541	0.06
630.957	0.03
724.436	0.01
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (?m)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D12 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading lidocaine hydrochloride 0.3 mg/ml.



Size (nm)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

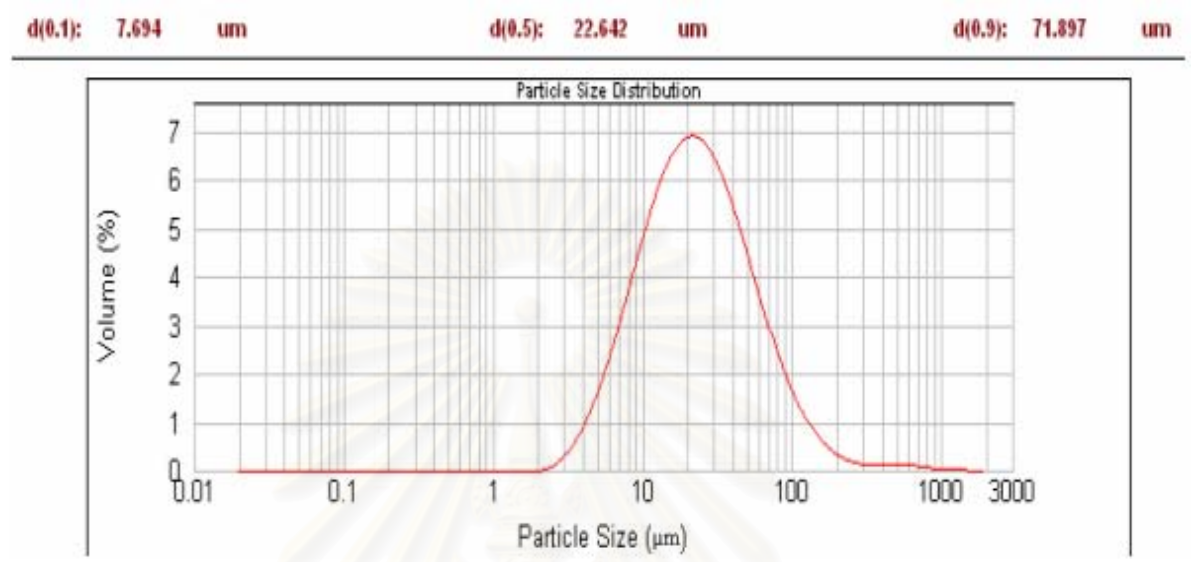
Size (nm)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.11
2.512	0.23
2.884	0.41
3.311	0.60
3.802	0.82
4.365	1.06
5.012	1.33
5.754	1.63
6.607	1.97
7.586	2.34
8.710	2.77
10.000	3.25
11.482	

Size (nm)	Volume in %
11.482	3.79
13.183	4.39
15.136	5.02
17.378	5.67
19.953	6.28
22.909	6.80
26.303	7.16
30.200	7.30
34.674	7.16
39.811	6.74
45.709	6.05
52.481	5.15
60.256	4.13
69.183	3.09
79.433	2.14
91.201	1.36
104.713	0.75
120.226	

Size (nm)	Volume in %
120.226	0.36
138.038	0.12
158.489	0.04
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.00
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (nm)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D13 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading lidocaine hydrochloride 0.4 mg/ml.



Size (nm)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

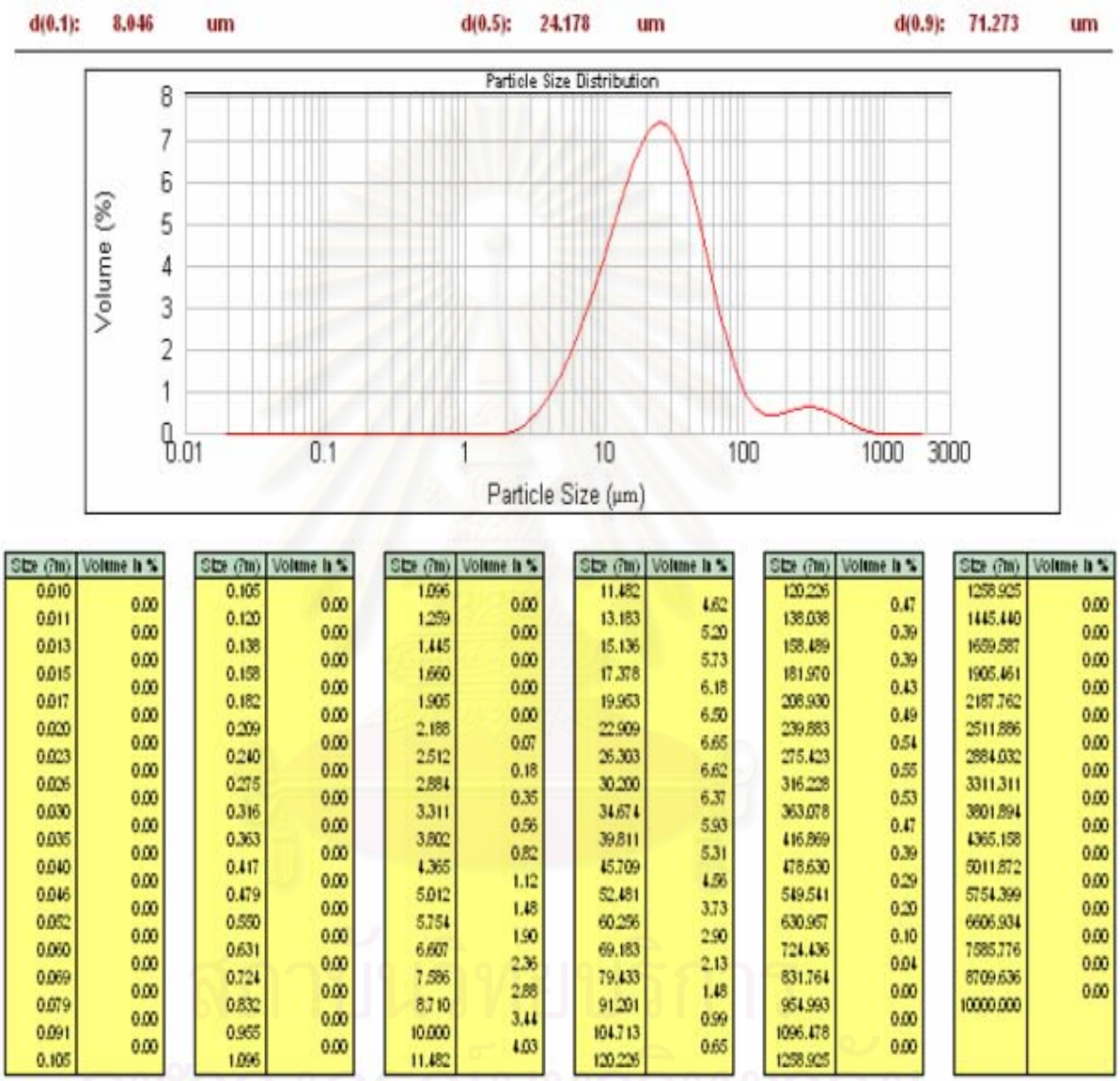
Size (nm)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.04
2.512	0.14
2.884	0.31
3.311	0.54
3.802	0.84
4.365	1.22
5.012	1.66
5.754	2.18
6.607	2.75
7.586	3.37
8.710	3.98
10.000	4.57
11.482	

Size (nm)	Volume in %
11.482	5.11
13.183	5.56
15.136	5.91
17.378	6.13
19.953	6.22
22.909	6.17
26.303	5.99
30.200	5.68
34.674	5.26
39.811	4.77
45.709	4.21
52.481	3.62
60.256	3.03
69.183	2.48
79.433	1.98
91.201	1.55
104.713	1.18
120.226	

Size (nm)	Volume in %
120.226	0.88
138.038	0.64
158.489	0.46
181.970	0.32
208.930	0.22
239.883	0.15
275.423	0.12
316.228	0.10
363.078	0.10
416.869	0.11
478.630	0.11
549.541	0.10
630.957	0.09
724.436	0.06
831.764	0.03
954.993	0.03
1096.478	0.02
1258.925	

Size (nm)	Volume in %
1258.925	0.01
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D14 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading lidocaine hydrochloride 0.5 mg/ml.



Size (?m)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (?m)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

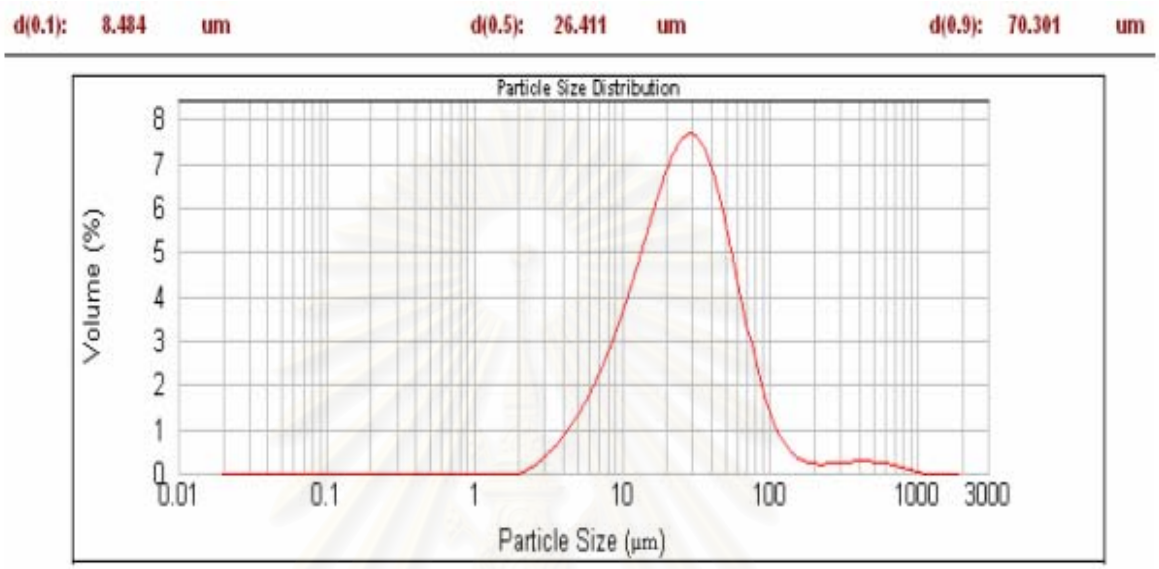
Size (?m)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.07
2.512	0.18
2.884	0.35
3.311	0.56
3.802	0.82
4.365	1.12
5.012	1.48
5.754	1.90
6.607	2.36
7.586	2.88
8.710	3.44
10.000	4.03
11.482	

Size (?m)	Volume in %
11.482	4.62
13.183	5.20
15.136	5.73
17.378	6.18
19.953	6.50
22.909	6.65
26.303	6.62
30.200	6.37
34.674	5.93
39.811	5.31
45.709	4.56
52.481	3.73
60.256	2.90
69.183	2.13
79.433	1.48
91.201	0.99
104.713	0.65
120.226	

Size (?m)	Volume in %
120.226	0.47
138.038	0.39
158.489	0.39
181.970	0.43
208.930	0.49
239.883	0.54
275.423	0.55
316.228	0.53
363.078	0.47
416.869	0.39
478.630	0.29
549.541	0.20
630.957	0.10
724.436	0.04
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (?m)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D15 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading diclofenac sodium 0.1 mg/ml.



Size (nm)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

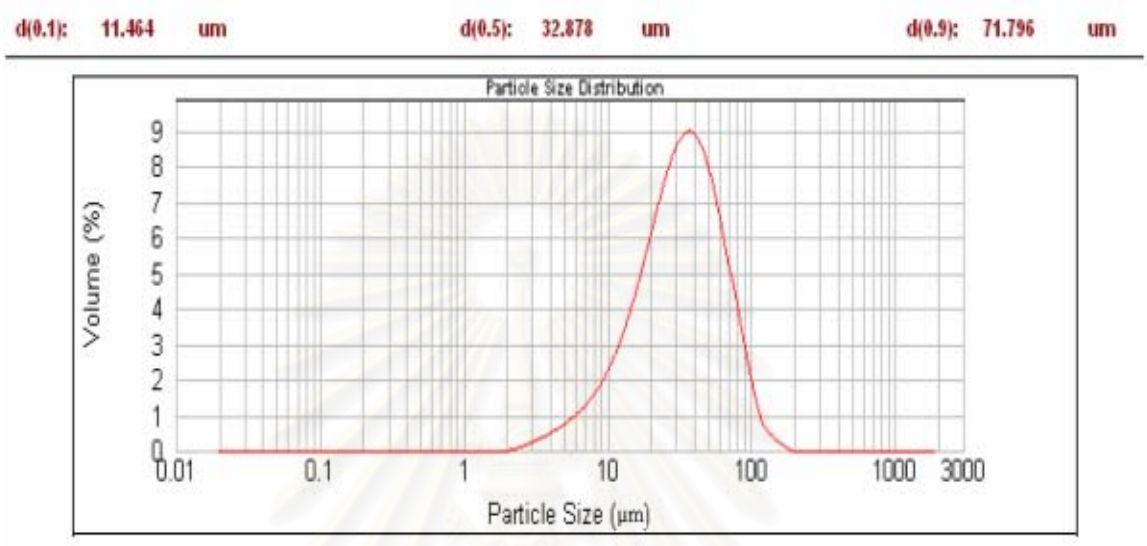
Size (nm)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.10
2.512	0.21
2.884	0.38
3.311	0.56
3.802	0.78
4.365	1.03
5.012	1.32
5.754	1.65
6.607	2.03
7.586	2.46
8.710	2.94
10.000	3.47
11.482	

Size (nm)	Volume in %
11.482	4.05
13.183	4.66
15.136	5.26
17.378	5.85
19.953	6.35
22.909	6.72
26.303	6.91
30.200	6.87
34.674	6.58
39.811	6.06
45.709	5.34
52.481	4.49
60.256	3.59
69.183	2.73
79.433	1.96
91.201	1.34
104.713	0.87
120.226	

Size (nm)	Volume in %
120.226	0.55
138.038	0.35
158.489	0.25
181.970	0.20
208.930	0.18
239.883	0.19
275.423	0.21
316.228	0.23
363.078	0.24
416.869	0.24
478.630	0.23
549.541	0.21
630.957	0.17
724.436	0.12
831.764	0.07
954.993	0.02
1096.478	0.00
1258.925	

Size (nm)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D16 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading diclofenac sodium 0.2 mg/ml.



Size (?m)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (?m)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

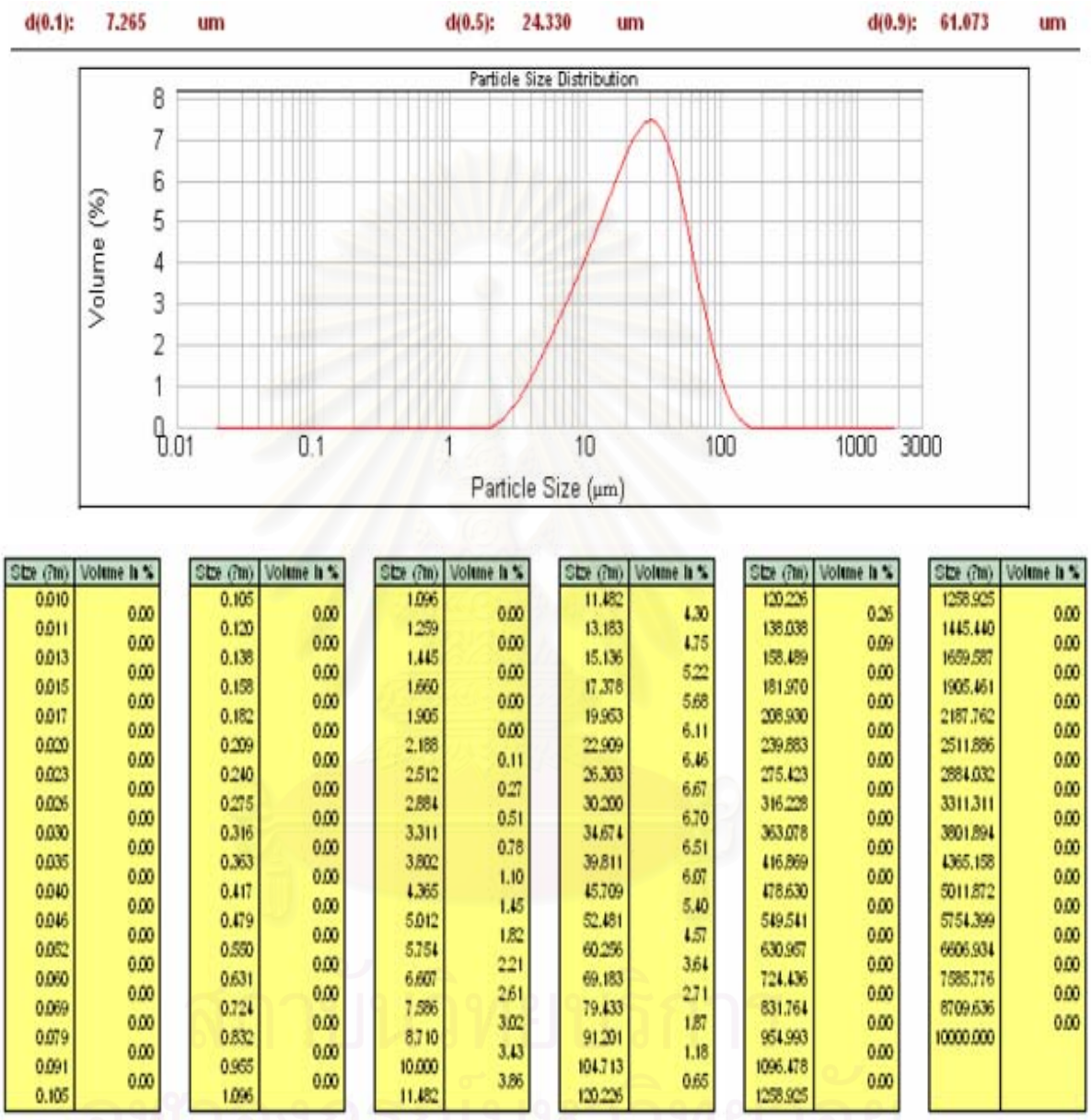
Size (?m)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.05
2.512	0.13
2.884	0.23
3.311	0.34
3.802	0.46
4.365	0.59
5.012	0.75
5.754	0.93
6.607	1.15
7.586	1.43
8.710	1.77
10.000	2.21
11.482	

Size (?m)	Volume in %
11.482	2.74
13.183	3.39
15.136	4.14
17.378	4.97
19.953	5.84
22.909	6.68
26.303	7.40
30.200	7.90
34.674	8.11
39.811	7.96
45.709	7.45
52.481	6.60
60.256	5.51
69.183	4.31
79.433	3.11
91.201	1.97
104.713	1.01
120.226	

Size (?m)	Volume in %
120.226	0.50
138.038	0.27
158.489	0.11
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.00
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (?m)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D17 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading diclofenac sodium 0.3 mg/ml.



Size (nm)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

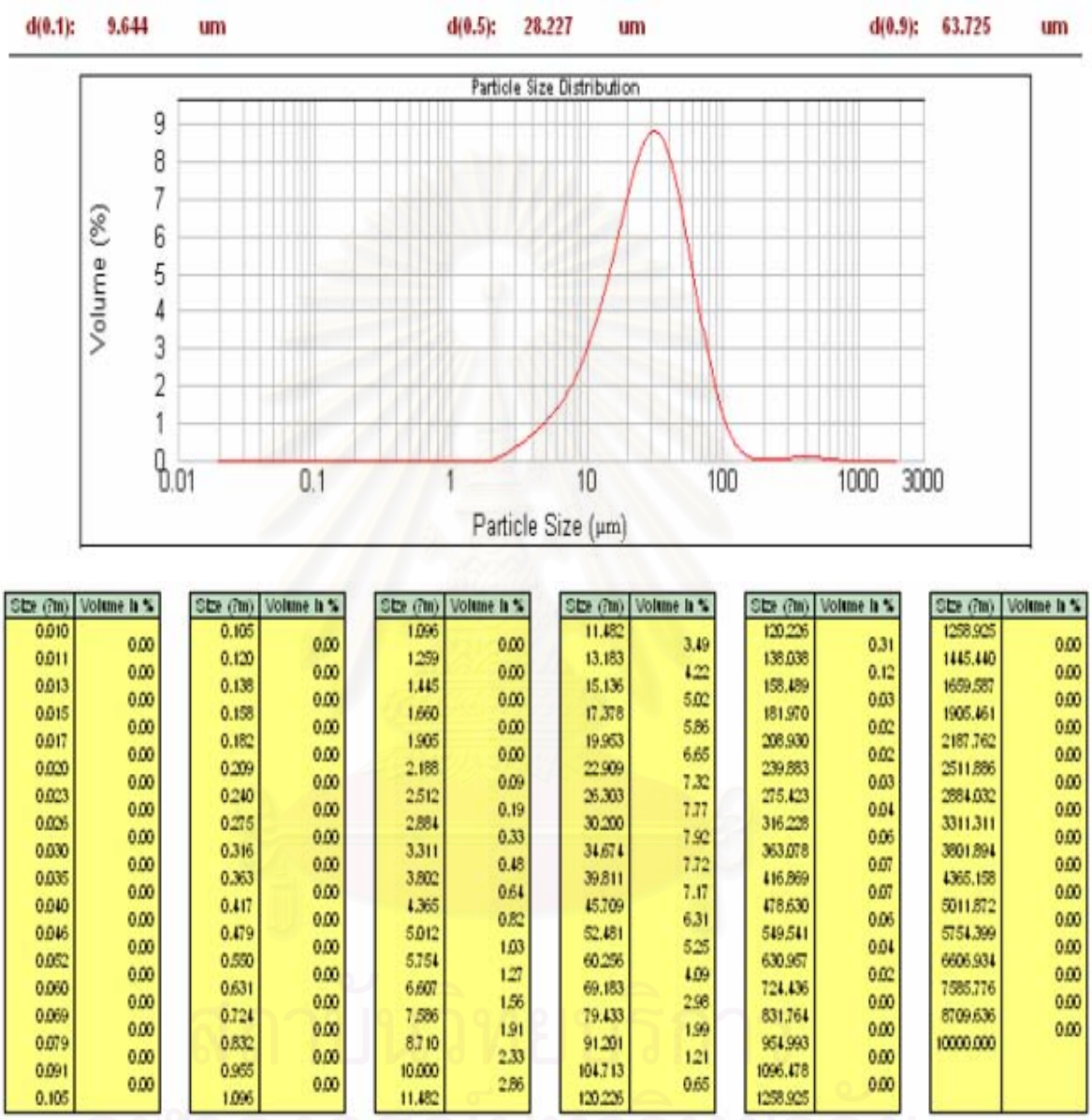
Size (nm)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.11
2.512	0.27
2.884	0.51
3.311	0.78
3.802	1.10
4.365	1.45
5.012	1.82
5.754	2.21
6.607	2.61
7.586	3.02
8.710	3.43
10.000	3.86
11.482	

Size (nm)	Volume in %
11.482	4.30
13.183	4.75
15.136	5.22
17.378	5.68
19.953	6.11
22.909	6.46
26.303	6.67
30.200	6.70
34.674	6.51
39.811	6.07
45.709	5.40
52.481	4.57
60.256	3.64
69.183	2.71
79.433	1.87
91.201	1.18
104.713	0.65
120.226	

Size (nm)	Volume in %
120.226	0.26
138.038	0.09
158.489	0.00
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.00
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (nm)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D18 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading diclofenac sodium 0.4 mg/ml.



Size (nm)	Volume in %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (nm)	Volume in %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (nm)	Volume in %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.09
2.512	0.19
2.884	0.33
3.311	0.48
3.802	0.64
4.365	0.82
5.012	1.03
5.754	1.27
6.607	1.56
7.586	1.91
8.710	2.33
10.000	2.86
11.482	

Size (nm)	Volume in %
11.482	3.49
13.183	4.22
15.136	5.02
17.378	5.86
19.953	6.65
22.909	7.32
26.303	7.77
30.200	7.92
34.674	7.72
39.811	7.17
45.709	6.31
52.481	5.25
60.256	4.09
69.183	2.98
79.433	1.99
91.201	1.21
104.713	0.65
120.226	

Size (nm)	Volume in %
120.226	0.31
138.038	0.12
158.489	0.03
181.970	0.02
208.930	0.02
239.883	0.03
275.423	0.04
316.228	0.06
363.078	0.07
416.869	0.07
478.630	0.06
549.541	0.04
630.957	0.02
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (nm)	Volume in %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Figure D19 Chromatogram and table of the chitosan/AOT/sodium sulfate particle size distribution by loading diclofenac sodium 0.5 mg/ml.

APPENDIX E

Formulation of buffer

Phosphate Buffer Saline (pH 7.4)

1. NaCl 8 g
2. KCl 0.2 g
3. NaH_2PO_4 1.44 g
4. KH_2PO_4 0.24 g

In 800 ml of distilled water. Adjust the pH to 7.4 with HCl. Add water to 1 litre.

Phosphate Buffer (pH 5.5)

1. 0.06 M NaH_2PO_4 50 ml
2. 0.06 M KH_2PO_4 950 ml

CURRICULUM VITAE

Miss Rattana Miphokasap was born in Bangkok, Thailand. In 1998, she graduated from Chulalongkorn University, Faculty of Science, Department of Genetics. In 2004, she was admitted as a Master degree student at Chulalongkorn University , Faculty of Science in Petrochemistry and Polymer Science Program. While the studying , she also worked as research and development supervisor with Unilever Thai Tradings Co.,Ltd. She graduated from this program in 2007.