CHAPTER 1
INTRODUCTION

1.1 Statement and significance of the research problem

Thailand is a great source of marine food industry. Therefore, a huge number of wastes and bio-wastes e.g. squid pens and shrimp shells and crab shells, etc., were regarded as a source of pollution. Nevertheless, the bio-wastes composed of biopolymers such as chitin, chitosan, protein and astaxanthin with high economical values. Currently, chitin and chitosan have been used in many other industries, such as agricultural, pharmaceutical, water treatment, food science and cosmetic applications [1]. However, the utilization of chitin has been restricted by its intractability and insolubility because it is highly hydrophobic and insoluble in water and most organic solvents. Chitin is soluble only in hexafluoroisopropanol, hexafluoroacetone, chloroalcohols in conjunction with aqueous solutions of mineral acids, thus chitosan is considerably more versatile than chitin [2].

Chitosan (2-amino-2-deoxy-(1-4)-β-D-glucopyranan) is a natural linear biopolyaminosaccharide. Its structure is very similar to cellulose and chitin, except that the amino group replaces the hydroxyl and acetyl amide group on the C-2 position of cellulose and chitin, respectively [3]. Chitosan is the N-deacetylated derivative of chitin by chemical or enzyme reaction [4]. Chitin is the second abundant polysaccharide next to cellulose in nature, which is the mainly component of exoskeleton of insects, crustaceans such as crabs, shrimps, lobsters, squid pens and cell walls of some fungi such as *Zygomicetes*, *Aspergillus* and *Mucor* [5]. In addition, the characteristics of chitosan especially, degree of N-deacetylation (40-98%) and molecular weight (50-2,000 kDa) are very important to their biological properties [6, 7]. Chitosan has a number of properties, such as biocompatibility, biodegradability and non-toxicity, which suitable for usage in biomedical and pharmaceutical formulations [8-11]. It is practically insoluble in water or aqueous solutions at pH above 6.5 because of the semi-crystalline structure which limits its applications [12-14]. Although chitosan is soluble in dilute organic acid, such as acetic acid, citric acid, malic acid and some inorganic acid, such as hydrochloric acid, due to the presence of free amino groups along the polymer [15], it may not desirable in many applications of chitosan e.g. food, cosmetics, biomedical. However, the amino functionality is a strong nucleophilic and reactive at higher pH values which could be suitably modified by various chemical reactions [16], resulting in N-substituted derivatives to enhance solubility and impart desired properties. Some N-substituted reactions include N-alkylation, N-acylation, N-Sulfation and N-hydroxyacylation [16-21].

N-acylation of chitosan is the most typical and extensively studied modification reaction. It can be obtained from acyl halide and anhydride [22]. Cyclic acid anhydrides e.g. succinic, maleic, glutaric, itaconic and phthalic anhydrides are also used for acylation of chitosan via ring-opening reaction giving N-carboxyacyl chitosans. This reaction is easy to prepare under mild condition [23, 24]. There are many reports about the development and physicochemical characterization of N-succinyl chitosan by the introduction of succinyl groups into amino groups of glucosamine units of chitosan in various solvent systems, such as acetic acid/ethanol, methanol, acetone, dimethylsulfoxide (DMSO) and dimethylformamide (DMF) [25-30]. N-succinyl chitosan displays good water soluble property at various pHs. It is initially developed as wound dressing materials. It is currently also applied as cosmetic material and drug carrier. Currently, only a few study of the use of other cyclic acid anhydrides such as phthalic anhydride has been reported. N-acylation of chitosan with various cyclic anhydride such as phthalic anhydride were reported [31]. The result showed that the N-phthaloyl chitosan with 34% degree of substitution showed the solubility even in the basic region above pH 7 and acidic region pH below 4. Although, it enhanced the solubility in basic region, the applications of colonic drug delivery are restricted due to the solubility in acidic region. Moreover, the physicochemical properties of N-phthaloyl chitosan such as FTIR, crystalline state and thermal property were not clarified sufficiently and there is no report about the toxicity of this derivative before. In addition, chitosan esters as chitosan succinate and chitosan phthalate have been used successfully as potential matrices for the colon-specific oral delivery.

Therefore, the aim of this study was to prepare N-phthaloyl chitosan as an enteric polymer by acylation at amino groups of chitosan via ring-opening reactions of phthalic anhydride. The suitable conditions for preparation and appropriate factors, i.e. temperature, stirring time, mole ratio of chitosan and phthalic anhydride, neutralization pH (step 3 of preparation process) and different molecular weights of CS (20 and 200 kDa) were studied. Physicochemical properties of N-phthaloyl chitosan, i.e. chemical structure, powder X-ray diffraction, thermal property and solubility in pH 1-10 media, were evaluated. The degree of substitution, cytotoxicity to Caco-2 cells, stability and film forming properties including mechanical properties, water vapor permeability, moisture content and pH solubility in SGF and SIF of N-phthaloyl chitosan were also investigated.

1.2 Objectives of this research

1. To study suitable conditions for preparation and appropriate factors for enteric polymer property of N-phthaloyl chitosan, i.e. temperature, stirring time, mole ratio between chitosan and phthalic anhydride and molecular weight of chitosan.
2. The physicochemical properties of N-phthaloyl chitosan, i.e. chemical structure, powder X-ray diffraction, thermal property, solubility and degree of substitution were studied.
3. To investigate cytotoxicity of N-phthaloyl chitosan in Caco-2 cells.
4. To evaluate the film forming properties of N-phthaloyl chitosan including tensile strength, water vapor permeability, moisture content and pH solubility.

1.3 Hypotheses of this research

1. N-phthaloyl chitosan can be prepared via ring-opening reactions of phthalic anhydride with amino groups of chitosan under the suitable condition.
2. N-phthaloyl chitosan with high degree of substitution can enhance the solubility of chitosan.

3. N-phthaloyl chitosan has low toxicity and cell compatibility.
4. The films of N-phthaloyl chitosan have good enteric film property.