CHAPTER I
INTRODUCTION

1.1 Introduction

Doxorubicin (DOX) is a commercial and widely used as anticancer drug [1]. Nevertheless, this drug has certain severe side effects, such as cytotoxicity in normal tissue, inherent multidrug resistance effect and acute cardiotoxicity [2]. To reduce the side effects and improve therapeutic efficiency of doxorubicin, various drug delivery systems based on polymer conjugates [3], polyethyleneglycol modified liposomes [4], stearic acid-grafted chitosan oligosaccharide micelles [5], and polymer-nanoparticles [6] have been intensively explored.

Nowaday, mucoadhesive polymers become more interesting for transmucosal routes such as nasal, pulmonary, oral and parenteral routes due to several advantages, for example, increasing the localization at target site, a prolonged residence time at the site of drug absorption and intensified contact with the mucosa increasing the drug concentration gradient. Numerous polymers adhere to mucosal tissues, for instance, chitosan [7], chitosan-poly (lactide-co-glycolide) [8], 4-carboxybenzenesulfonamide-chitosan conjugate [9] and poly lactic acid [10].

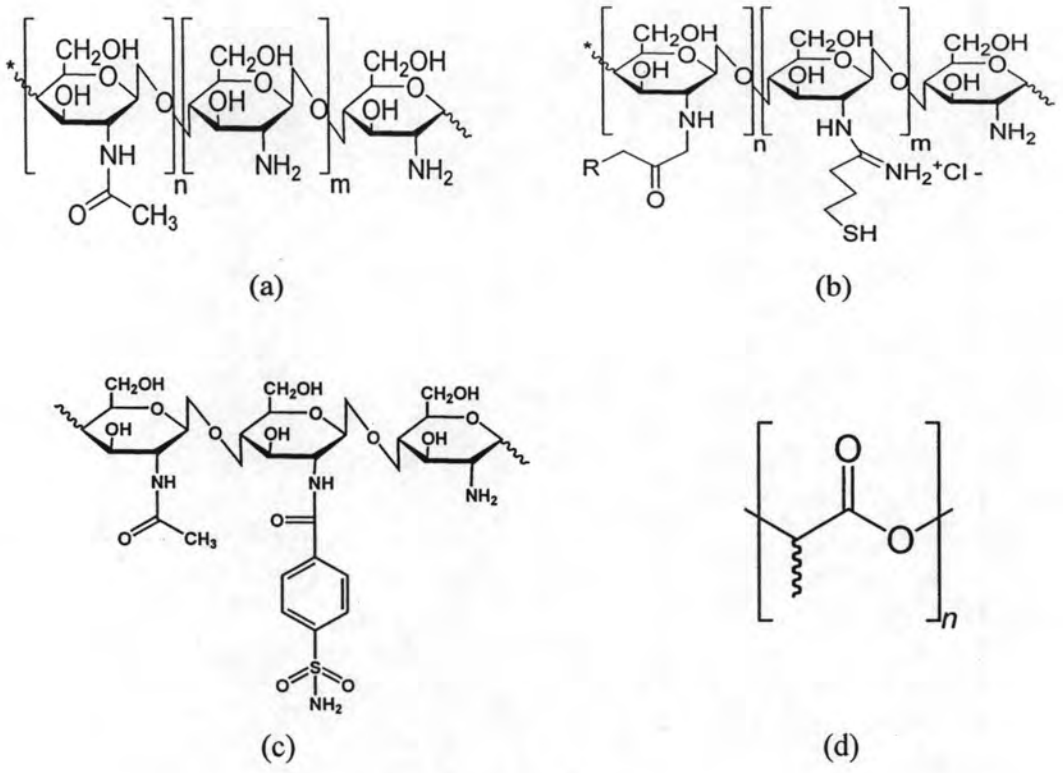

Figure 1.1 Chemical structures of a) chitosan b) chitosan-poly (lactide-co-glycolide) c) 4-carboxybenzenesulfonamide-chitosan conjugate d) polylactic acid

Chitosan (CS) is a natural polysaccharide derived from chitin by alkaline deacetylation, consists mainly of the repeating unit of 2-amino- and 2- acetamido-2-deoxy-β-D-glucopyranose and is soluble in dilute aqueous acidic solution (pH<6.5) [11]. It has gained increasing attention in pharmaceutical field due to its favorable biological properties such as non-toxic, biocompatible, biodegradable, mucoadhesive properties etc. It was widely accepted as material of drug delivery carrier.

4-Carboxybenzenesulfonamide-chitosan (4-CBS-CS) is modified chitosan by sulfonamide group which the carboxylic group of 4-carboxybenzenesulfonamide will react with amines group of chitosan to give the desired amide group of 4-carboxybenzenesulfonamide-chitosan. The mucoadhesive properties of 4-CBS-CS and its usefulness in drug delivery system design have been well documented. Strong

electrostatic interaction of positively charged 4-CBS-CS with the negatively charged mucosal surface is the underlying mechanism for its mucoadhesive properties. The 4-CBS-CS also possesses bioadhesive properties that can be used to enhance the therapeutic efficacy of the dosage forms by increasing the contact time at the site of action and intensified contact with the mucosa increasing the drug concentration gradient. Furthermore, it is non-toxic to Vero cell and inactive against to anticancer cell lines of KB cell line (epidermoid carcinoma of oral cavity), MCF-7 cell line (breast adenocarcinoma) and NCL-H187 (small cell lung carcinoma), which can be implied that the 4-CBS-CS is biocompatible to the human body.

Poly (lactic acid) (PLA) is a kind of biodegradable materials with low toxicity, excellent biocompatibility and bioabsorbability in vivo [12]. It has been widely used in several biomedical applications, such as controlled release of drug delivery systems, implants for orthopedic devices and absorbable fibers. However, the low hydrophilicity and high crystallinity of PLA reduce its degradation rate, which results in poorer soft tissue compatibility. Since poly (lactic acid) is well known to undergo scission to monomer units of lactic acid which is a natural intermediate in carbohydrate metabolism [13]. These characteristics make this polymer suitable for further uses such as resorbable sutures, implants for orthopedic surgery or blood vessels, which finally can be replaced by the body's tissues [14]. As to the sustained release, PLA has been used for delivery of antimycobacterial drugs [15], quinolones [16], antimalarial and antiinflammatory drugs [17], hormones [18] and antitumor agents [19].

Polymer-based delivery system, especially in micro/nanospheres, has been demonstrated that it could reduce such side effects and extend the release time [6]. Because of their size, drug carrying micro/nanoparticles can be internalized by cells thereby allowing for easy transport of the bioactive agent across the cell membrane [20]. Several preparation methods have been employed for the fabrication of polymer micro/nanoparticles. For example, chitosan nanoparticles can be prepared from, such as solvent evaporation and emulsification crosslinking method [21], nanoprecipitation method [22], ionic gelation technique [23] etc. These methods generally employed

chemical cross-linkers and high speed stirring for long durations of time. The chemical crosslinking agents can be the source of undesirable toxic effects if left unremoved. Glutaraldehyde which is commonly used to act as a crosslinking agent in order to strengthened in preparation of chitosan micro/nanoparticles. It has been found to cause irritation to mucosal membranes due to its toxicity [20], [24]. Therefore, it causes impeding the biocompatibility of microspheres and limiting their applications in the biomedical field [24]. For this reason, tripolyphosphate (TPP), non-toxic solvent, biocompatible crossliking agent with improved mechanical and transport properties, was used to prepare of chitosan micro/nanoparticles by ionic interaction between positively charged amino groups and negatively charged counterions of TPP [11]. This work focus on a new technique for preparation of doxorubicin loaded chitosan (DOX-CS) nanoparticles by using electrospray ionization.

Various methods for preparation of chitosan micro/nanoparticles have been reported such as ionotropic gelation [25] and solvent emulsification/internal gelation [26], but the droplet easily agglomerate and coaglulate, moreover they gave a wide size distribution. One of the novel techniques for preparation nanoparticles is known as electrohydrodynamic atomization or electrospray ionization.

Electrospray ionization has been used to generate nanoparticles and quantum dots [27], which is a slightly modified form of the electrospinning process that is widely used for making micro/nanofibers [28]. The principles of electrospraying is applied high voltage to polymeric solution to forces the polymer to come out of the syringe in form of micro/nanoparticles [20]. Electrospray ionization can give uniform particle size, narrow size distribution, simplicity, fast preparation and unique one-step technique [29]. Moreover, the droplet produced by electrospraying are highly charged, that prevents their coaglulation and agglomeration [30]. Electrospray technique has many applications such as fabrication of inorganic nanoparticles, thin films, and fibers, production of pharmaceutical particles, deposition of nanoparticles and generation of micro/nanoencapsulation [31].

Therefore, the purpose of the present work was to prepare controlled release nanoparticles of doxorubicin, using the matrix polymer consisting of chitosan, modified-chitosan and poly (lactic acid) as a retarding material, by applying electrospray ionization technique. Various process and formulation parameters such as working distance, needle gauge, flow rate, stirring rate, electrospraying voltage, and drug ratio were optimized. These nanoparticles were evaluated for drug encapsution efficiency and in vitro release. The physical characteristics were evaluated by scanning electron microscopy (SEM), particle size analyzer, Fourier Transform Infrared Spectroscopy (FT-IR), Differential scanning calorimetry (DSC), Thermogravimetric analysis (TGA). Moreover, we emphasis on encapsulation efficiency, *in vitro* drug release behavior and also performed *in vitro* studies to demonstrate the feasibility of this delivery system on controlling Topoisomerase II inhibitory activity using gel electrophoresis.

1.2 The objectives of research

1.21. To Prepare CS, 4-CBS-CS, CS/PLA and 4-CBS-CS/PLA nanoparticles for doxorubicin delivery system by electrospray technique

1.22. To study the release behaviors of doxorubicin form polymer nanoparticles and evaluate Topoisomerase II inhibitory activity

1.3 The scope of research

The scope of this research was carried out by stepwise methodology as follow:

1.3.1 Literature review of related works

1.3.2 Preparation of polymer nanoparticles and doxorubicin-loaded polymer nanoparticles by varying electrospray parameters including voltage, flow rate, needle gauge and working distance.

1.3.3 Characterization of the obtained nanoparticles in terms of morphology, size and size distribution, zeta potential, chemical analysis and thermal behavior.

1.3.4 Evaluation of drug content and drug encapsulation efficiency as a function of electrospary preparation parameters; weight ratio of polymer to drug.

1.3.5 Study the *In vitro* release behavior of doxorubicin from polymer nanoparticles at various weight ratios of polymer to drug in SIF (phosphate buffer pH 7.4).

1.3.6 Evaluation of Topoisomerase II inhibitory activity.

1.3.7 Report, Discussion and Writing up thesis.