CHAPTER I
INTRODUCTION

Nanotechnology has become a profound influence on the worldwide economy and society since early the twenty-first century, comparable to that of semiconductor technology, information technology, or cellular and molecular biology. Science and technology research in nanotechnology promises breakthroughs in a wide variety of areas such as materials and manufacturing, nanoelectrics, medicine and healthcare, biotechnology, energy, information technology, and national security. Consequently, the nanotechnology is likely to be the next industrial revolution (Bnushan, 2004).

The discovery of novel materials, processes, and phenomena at the nanoscale, as well as development of new experimental and theoretical techniques for research, provides new opportunities for the development of innovative nanosystems and nanostructured materials. Nanosystems are expected to find various unique applications. This field is expected to open new venues in science and technology. In addition to the fabrication of nanosystems, nanotechnology can also provides the continuous development of experimental and computational tools (Bhushan, 2004).

Nowadays, nanotechnology and nanoengineering stand to produce significant scientific and technological advances in diverse fields (Silva, 2004). Science and technology continue to move forward in making the fabrication of nanodevices and nanosystems, which are possible for a variety of industrial, consumer, and biomedical applications (Bhushan, 2004).

Nanomedicine is one of several fields of sciences and technology, which was developed from nanotechnology. It may be defined as the monitoring, repair, construction, and control of human biological systems at the molecular level, by using

engineered nanodevices and nanostructures. The nanomedicine can also be regarded as another implementation of nanotechnology in the field of medical sciences and diagnostics. The most important issue is the proper distribution of drugs and other therapeutic agents within the patient's body (Bogunia-Kubik and Sigisaka, 2002).

During the last two decade, considerable attention had been given to the development of novel drug delivery systems. The rationale is to alter the pharmacokinetics and pharmacodynamics of therapeutic agents in order to improve the therapeutic efficacy, while minimizing the toxicity. The major colloidal drug delivery systems investigated include liposomes, microparticles, and polymeric nanoparticles. These systems have been investigated for controlled drug delivery, and also the enhancement of dissolution rate and biovailability of poorly water-soluble drugs (Bodmeier and Maincent, 1996). The site-specific or targeting delivery of therapeutic agents to diseased lesions is one of the important aspects of the drug delivery system. Suitable carriers are needed to deliver a sufficient dose of drug to the target (Bogunia-Kubik and Sigisaka, 2002).

Liposomes have been used as potential drug carriers instead of conventional dosage forms because of their unique advantages, such as the ability to protect drugs from degradation, targeting delivery to the site of action, and reduction of toxicity and side effects. However, development of liposomes have been limited due to their inherent problem, such as low encapsulation efficiency, rapid leakage of drugs, and poor storage stability (Sharma *et al.*, 1997).

Microparticles have been investigated and used as potential drug carriers. However, due to the large size, it was impossible to direct delivery the therapeutic agents to target tissue via systemic circulation. Moreover, microparticles, cannot across the M cells in the Payer' patch and the mesentery on the surface of the gastrointestinal mucosa. Thus, it cannot deliver drug from peroral administration to systemic circulation (Jain, 2000).

Polymeric nanoparticles have been developed for drug delivery in recent years, since they offer suitable means of delivery small molecular weight drugs, as well as macromolecules such as proteins, peptides or genes by either localized or targeted delivery to the tissue of interest. These systems, in general, can be used to provide targeted (cellular or tissue) delivery of drugs, to improve oral bioavailability, to sustain therapeutic effect in target tissue, to solubilize drugs for intravascular delivery, and to improve the stability of therapeutic agents against enzymatic degradation (nucleases and proteases), especially of protein, peptide, and nucleic acid drugs. The nanometer size-ranges of these delivery systems offer certain distinct advantages for drug delivery over microparticles. Due to their sub-cellular and sub-micron size, nanoparticles can penetrate deep into tissues through fine capillaries, cross the fenestration present in the epithelial lining (e.g., liver), and are generally taken up efficiently by the cells. This allows efficient delivery of therapeutic agents to target sites in the body (Panyam *et al.*, 2003). Owing to their polymeric nature, nanoparticles may be more stable than liposomes in biological fluids and during storage (Pinto-Alphandary, *et al.*, 2000). In some cases, nanoparticles are more efficient drug carriers than liposomes due to their better stability and, thus, they possess more useful control release properties. These are the reason why many therapeutic agents, e.g. antibiotics and antiviral drugs, vitamins, protein and peptides, enzymes and hormones, etc., have been studied in association with nanoparticles. Therefore, nanoparticulate carriers may exert important potential applications for the administration of therapeutic agents (Bogunia-Kubik and Sigisaka, 2002). Firstly, the increasing attention of polymeric nanoparticles has been paid to injectable drug carriers, which would enable a long systemic circulation. Nowadays, the pharmaceutical application of polymeric nanoparticles has been extended to the field of non-parenteral deliveries of drugs via pulmonary, nasal or oral routes. In addition, the possibility of using polymeric nanoparticles as the new excipient for solid dosage forms has been explored. The results of many studies suggested that the extremely small size and large surface area of polymeric nanoparticles make it possible to

provide new function to the dosage forms. A number of different polymers, both synthetic polymers, e.g. poly (lactide-co-glycolide) copolymers (PLGA), polyacrylates, and polycaprolactones, and natural polymers, e.g. albumin, gelatin, alginate, collagen, and chitosan, have been utilized in formulating biodegradable nanoparticles. Among these polymers, poly (d, l-lactide-*co*-glycolide) (PLGA) has been the most extensively investigated for drug delivery because it is biodegradable and biocompatible (Murakami *et al.*, 1999).

Recently, natural occurring compounds tend to be more interesting as preventive and therapeutic agents than synthetic substances. Therefore, the use of phytochemicals, bioactive non-nutrients plant chemicals, which are presented varying in levels of different plants, e.g. flavonoids, isoflavones, phenolic compounds etc., becomes more interesting. Several fruits, vegetables, herbs, and plants, such as soybean, green tea, including turmeric, are the rich sources of phytochemicals (Bidlack *et al.*, 1998). In traditional medicine, usage of medicinal plants and their active principles in the prevention and treatment of diseases is based on the experience from different ethnic societies. In contrast, the use of medicinal plants in modern medicine has attracted the interest of scientists and been investigated for their biological activities and toxicities. One plant that has been widely investigated is *Curcuma longa* Linn. (Wang *et al.*, 1997).

Curcuma longa Linn. is a herbaceous plant in Zingiberaceae family. It originated in India and Southeast Asian countries and has been grown in many countries in this region, including Thailand. *Curcuma longa* Linn. has been used in the form of turmeric powder, which has yellowish orange color and pleasant aroma. Turmeric powder is widely used as a spice and natural coloring agent in several foods, such as curry, mustard and potato chip, as well as drugs and cosmetics. As cosmetics, it has been used as a skin caring agent for women. Medically, turmeric powder has been used as an anti-peptic ulcer agent, and used to treat a variety of inflammatory conditions and chronic diseases. Turmeric was found to be a rich source of

biologically active phenolic compounds, which are known as curcuminoids. In *Curcum longa* Linn., curcuminoids content presents about 2-9%, depending upon the geographic conditions and harvesting age (Jayaprakasha *et al.*, 2002).

Curcuminoids and individual constituents; curcumin, demethoxycurcumin, and bisdemethoxycurcumin, have exhibited various biological activities in many researches. The biological activities include anti-inflammatory (Brouet *et al.*,1995; Loe *et al.*, 1997; Chuang *et al.*, 2000; Jayadeep *et al.*, 2000; Literat *et al.*, 2001) antioxidant (Grinberg *et al.*, 1996; Bonté *et al.*, 1997; Das *et al.*, 2002), anti-carcinogenic (Limtrakul *et al.*, 1997; Huang *et al.*, 1997; Inano *et al.*, 2002), chemopreventive (Khafif *et al.*, 1998; Perkins *et al.*, 2002), hepatoprotective (Song *et al.*, 2001), antipsoriatic (Heng *et al.*, 2000), and anti-atherogenic activities (Ramirez-Tortosa *et al.*, 1999). In addition, other than the advantage of having several biological activities, curcuminoids were found to have no toxicity in the long-term administration (Sharma *el al.*, 2001).

Although curcuminoids have many advantages, they also possess some disadvantages, such as poor water solubility (THP I, 1995; Tønnesen *et al.*, 2002), rapid decomposition in neutral basic pH solution (Tønnesen *et al.*, 2002; Wang *et al.*, 1997), photodegradation (THP I, 1995; Tønnesen *et al.*, 2002), low biovailability (Sharma *et al.*, 2001; Perkins *et al.*, 2002), and short circulation half-life (Ireson *et al.*, 2001). These disadvantages result in the difficulties on their formulation and, in turn, curcuminoids cannot be effectively used as a preventive or therapeutic agent. Thus, in order to improve the potency of curcuminoids, an effective delivery device is required to overcome such problems.

In this study, the nanoparticulate carrier prepared from PLGA was selected for incorporating curcuminoids via the modified spontaneous emulsification solvent diffusion (modified-SESD) method. This method was developed and claimed to have more effectiveness and more safety than the classical SESD method by Murakami *et*

al. (1999). The nanoparticles were prepared with three formulation ingredients, which were PLGA copolymers, curcuminoids and stabilizers. Three PLGA copolymers were used, consisting of different ratios of PLA and PGA at 50:50, 75:25 and 85:15, respectively. The curcuminoids loaded in the formulation varied in three concentrations; 2, 6 and 10%. The stabilizer used in the preparation with varios concentrations were vitamine E TPGS (3, 5 and 7%), poloxamer 407 (9, 12 and 15%) and polyvinyl alcohol (3, 5 and 7%). The prepared formulations were characterized on five different responses, which were %recovery, particle size, size distribution (polydispersity index), curcuminoids content and %encapsulation efficiency. The different formulation ingredients with various concentrations were correlated with the responses to determine the optimal formulation to obtain the satisfactory formulation.

Basically, in order to prepare the nanoparticles with various types and concentrations of the ingredients described above for correlation study, total formulations would be 81, according to the three-factor, three-level (3^3) full factorial design. A three-factor, three-level Box-Behnken design was therefore used to reduce the number of formulations to 45. The three-factor, three-level Box-Behnken design can provide almost identical result compared to full factorial design, and hence, the method is more cost-effective (Smith, 1998).

The specific purposes of this study were as follows.

1) To prepare PLGA nanoparticles containing curcuminoids by varying formulation ingredients; poly (lactic acid) (PLA): poly (glycolic acid) (PGA) ratio in PLGA, PLGA: cucuminoids ratio, and type and/or concentration of emulsifying agents.

2) To characterize the %recovery, particle size, size distribution (polydispersity index), curcuminoids content and %encapsulation efficiency of the obtained PLGA nanoparticles containing curcuminoids.

3) To correlate the prepared PLGA nanoparticles containing curcuminoids with their characteristics to find the optimal formulation.

4) To evaluate the optimal formulation on %recovery, particle size, size distribution (polydispersity index), curcuminoids content, and %encapsulation efficiency, in order to see whether the optimization technique would work as expected, and investigate the particle morphology, and *in vitro* release profile of curcuminoids.

This thesis research would provide useful information about the preparation of nanoparticles containing curcuminoids. In addition, the obtained PLGA nanoparticles containing curcuminoids could serve as a controlled release raw material, which can be incorporated within formulations used via various routes of administration, such as parenteral, oral and topical administration. The effect of the formulation ingredients on the physicochemical characteristics of the nanoparticles inverstigated here would be helpful in understanding the formation of PLGA nanaparticles. Consequently, this study could be applied to develop PLGA nanoparticles containing other substances in the future.