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INTRODUCTION

Lawsone methyl ether (LME, 2-methoxy-1,4-napthoquinone), isolated from *Impatiens balsamina* Linn. (Balsaminaceae), exhibited potential antifungal and antibacterial activities [1]. Both minimal inhibitory and minimal fungicidal concentrations of LME against *Candida* were 1.25μg/ml [2]. In addition, Sritrairat et al. (2011) suggested that LME might be used as alternative mouthwash in the prophylaxis of oral candidiasis (OC) for denture wearers or among HIV-infected individuals or other immune-compromised patients [3]. However, the limitation of conventional mouthwashes is their short retention time leading to low therapeutic efficacy [4]. To overcome this problem, mucoadhesive formulations involving micro- and nanoparticles have been developed [5]. In this study, chitosan-dextran sulfate nanoparticles (CDNPs) were examined. Chitosan is a naturally biopolymer derived from the shells of crustaceans such as crabs, shrimps and lobsters. It is biodegradable, biocompatible and nontoxic [6]. Dextran sulfate is a biodegradable polyanionic polymer that is widely used for pharmaceutical applications [7]. CDNPs are formed due to the electrostatic interaction between chitosan and dextran sulfate. The surface charge and mean particle size are adjustable by varying the concentration ratio of both polymers and pH sensitive swelling has been reported [7, 8]. Based on these considerations, the purpose of this study was to develop and characterize the LME loaded CDNPs. In addition, the amount of LME released was determined using *in vitro* release study.

MATERIALS AND METHODS

Materials Chitosan from shrimp (C, MW 30kDa with 95% deacetylation) was obtained from Aquapremier, Inc. (Bangkok, Thailand). Dextran sulfate (D, MW 500kDa), α-Amylase from *Aspergillus oryzae* and mucin from porcine stomach, Type II were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals and solvents were of analytical grade. De-ionized (DI) water was used in the preparation of solutions and dispersion of CDNPs.

Preparation of LME loaded CDNPs Lawsone methyl ether was semisynthesized by methylation of lawsone. Then, the LME loaded CDNPs were prepared by polyelectrolyte complexation as reported earlier [5], with minor modification. LME in dimethylsulfoxide (DMSO) solution (10 mg/ml) and 0.5 ml of 1%Tween 20 were added to 1 ml of the dextran sulfate aqueous solution. The resulting mixture was continuously stirred at 500 rpm for 30 min. Then, the mixture of 1.67 ml of chitosan aqueous solution (pH 4) and DI water (total volume: 5 ml) was added drop-wise to the dextran sulfate mixture using homogenization at 10,000 rpm for 15 min. Subsequently, 0.025 ml of PEG-400 was added with mixing over a period of 15 min. CDNPs were prepared with different processing parameters to study the effect of a number of variables on their physicochemical properties. Process parameters were varied as follows: the polymer concentration was varied from 0.1 to 0.2%; the mass ratio of chitosan to dextran sulfate was varied from 1:0.4 to 1:1. The ranges of these variable values were selected based on preliminary experiments. All samples were prepared in triplicate.

Physicochemical characterization of the CDNPs: Mean particle size The mean particle size and size distribution were measured by dynamic light scattering (DSL) using ZetaPAL/90plus (Brookhaven Instrument, Holtsville, NY). This instrument was equipped with 35 mV HeNe laser diode operating at 632.8 nm (JDS Uniphase, San Jose, CA, USA) and a BI-200SM Goniometer with an EMI-9863 photomultiplier tube connected to a BI-9000AT digital correlator. An aliquot of nanoparticle was diluted in DI water. The particle size of each sample was measured at 25°C at a detection angle of 90° for 10 repeated measurements. The mean particle size and polydispersity index were obtained from the cumulative measurements [6].

Zeta potential The zeta potential was determined using phase analysis light scattering with ZetaPAL/90plus. Measurements were carried out at 25°C at 14.8° to the incident light. Samples were prepared by redispersing the CDNPs in DI water. The zeta potential was calculated using the electrophoretic mobility based on the Smoluchowski approximation.

Determination of entrapment efficiency (EE) LME loaded CDNPs were centrifuged at 18,000 rpm for 30 min. After discarding the supernatant, LME was extracted from CDNPs using the mixture of 2% aqueous acetic acid and methanol at a ratio of 60:40 (v/v) and applied to the HPLC system. The HPLC system (Shimadzu, Japan) consisted of a LC20-AT pump connected to a SIL-10ADVP auto-injector, a SPD-6AV system controller, SPD-20A UV-visible detector. LME was separated using a Gemini 5u C18 110A (5µm, 150x4.6 mm).

In viro release studies *In vitro* release profile of LME loaded CDNPs was determined by the shake-flask method [5]. LME exhibits poor water soluble, thus, artificial saliva (pH 7.4) containing 0.6% (v/v) Tween 80 was used as a dissolution medium to provide sink condition. A known amount of the LME loaded CDNPs was mixed into 5 ml of dissolution medium and shaken at 100 rpm with an orbital shaker at 37±0.5°C. An aliquot (0.3 ml) was taken at predetermined time intervals of 5, 15, 30, 60,120 and 180 min in triplicate. The samples were centrifuged at 18,000 rpm for 30 min and the supernatant was analyzed for the amount of LME released using HPLC method as described above.

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RESULTS

Physicochemical characterization of the CDNPs: Mean particle size and size distribution The effect of the polymer concentration (0.1, 0.125, 0.15 and 0.2% chitosan and dextran sulfate) was studied by maintaining the polymer ratio at 1:0.6. The mean particle size was increased from 301 to 411 upon an increase of polymer concentration (Table 1). When the concentration of polymer was less than 0.1%, the system became translucent suggesting that only a small amount of nanoparticle was obtained. In contrast, when the concentration of polymer was more than 0.15%, particle precipitation occurred. In addition, the effect of the mass ratio of chitosan and dextran sulfate at 1:0.4, 1:0.6, 1:0.8 and 1:1 was studied by maintaining the polymer concentration at 0.125%. The mean particle size was increased from 317 to 428 upon an increase of mass ratio of chitosan and dextran sulfate (Table 1). When the mass ratio of chitosan and dextran sulfate was 1:1, particle precipitation occurred.

Zeta potential Regardless of the processing parameter, the zeta potential value of all formulations exhibited a positive charge with the same zeta potential of approximately 20 mV (Table 1).

Determination of entrapment efficiency (EE) The entrapment efficacy of LME loaded CDNPs showed low entrapment efficacy within the range of 7-17%.

In vitro release studies The effect of polymer concentration and mass ratio of chitosan and dextran sulfate on the drug release profile was evaluated. For all formulation tested, the LME loaded CDNPs showed biphasic drug release pattern with a burst release, approximately 60%, within 5 min, followed by a sustained release up to 3 h. (Figure 1 and 2). In addition, LME solution was used as a control. As expected, approximately 90% of LME rapidly dissolved within 5 min.

DISCUSSION

To overcome the limitation of conventional mouthwash, polymeric nanoparticles are proposed as a promising delivery system for topical oral delivery as they possess a long retention time and prolong released characteristic. Thus, in this study, LME loaded mucoadhesive CDNPs were developed. CDNPs were formed rapidly by phase separation induced by electrostatic interaction between the positively charge chitosan and negatively charge dextran sulfate. In this preliminary study, the influence of polymer concentration and mass ratio of chitosan and dextran sulfate on physicochemical properties was evaluated. These experimental conditions resulted in LME loaded CDNPs with a mean particle size in a range of 300-450 nm and polydispersity index of ~0.2-0.3 indicating a narrow distribution. The mean particle size of LME loaded CDNPs was slightly larger compared with blank CDNPs, ~ 200 nm (data not shown). The mean particle size was found to be dependent upon the polymer concentration and polymer ratio. The mean particle size tended to increase when increasing the polymer concentration. Larger complex nuclei may be formed when formulated with higher polymer concentration. Similarly, mean particle size tended to increase when increasing the mass ratio of polymer concentration. In addition,

precipitation was observed when the mass of dextran sulfate was equal and/or higher than chitosan. However, there were no significant differences in the resulting zeta potentials when varying the processing parameters. The zeta potential of all formulations was in a range of +16 to +21 mV, suggesting CDPNs should be adhered to the negatively charged oral cavity. Unfortunately, CDNPs showed low LME entrapment efficacy. It might be the effect of Tween 20 that was used as an emulsifier to disperse LME in dextran sulfate solution. Tween 20 is a non-ionic surfactant that has HLB value of 16.7 which may help solubilize LME in aqueous medium, leading to low entrapment efficacy. Nevertheless, formulation without Tween 20 showed LME precipitation in dextran sulfate solution resulting in no entrapment of LME. Therefore, various types of surfactants and concentrations should be examined to overcome this problem, and the surfactants with low HLB might be candidate.

In addition, *in vitro* release profile studies showed burst release of LME from nanoparticles (60% within 5 min), suggesting that LME was likely adsorbed onto the particle surface, and some were entrapped in particles. Moreover, the mechanism of LME release from the CDNPs was analyzed using three models as follows: zero order, first order and Higuchi equation. The correlation coefficients for the release kinetics were calculated from the graph. All formulations were best fitted in the Higuchi equation, indicating diffusion to be the predominant mechanism of LME release from particles (Figures 1B and 2B).

Table 1 The mean particle size, zeta potential and entrapment efficiency of LME loaded CDNPs

Formulation	Mean size (nm±SD)	PI±SD	Zeta potential (mV±SD)	EE (%±SD)
C-D 0.1%	301±12	0.270±0.035	19±2	17±4
C-D 0.125%	346±16	0.251±0.036	19±2	9±1
C-D 0.15%	411±10	0.211±0.015	21±1	11±0
C-D 0.2%	P	–	–	–
C:D 1:0.4	317±10	0.309±0.033	19±0	7±1
C:D 1:0.6	346±16	0.251±0.036	19±2	9±1
C:D 1:0.8	428±17	0.271±0.052	16±2	17±1
C-D 1:1	P	–	–	–

EE = entrapment efficiency

P = precipitation which could not be measured by ZetaPAL/90plus.

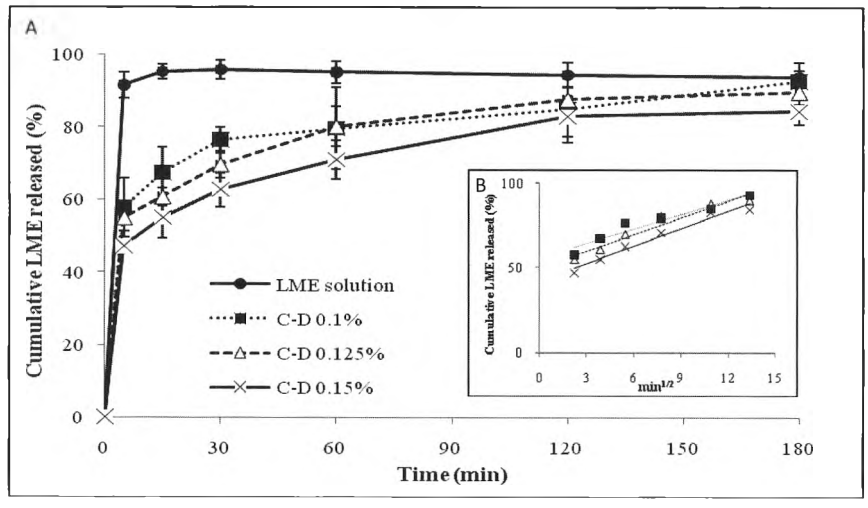

Figure 1 (A) *In vitro* release profiles of LME loaded CDNPs prepared with a mass ratio of chitosan to dextran sulfate of 1:0.6 and polymer concentration of (■) 0.1%, (Δ) 0.125%, and (×) 0.15% chitosan and dextran sulfate aqueous solution compared with (●) LME solution. (B) Higuchi release model of LME loaded CDNPs.

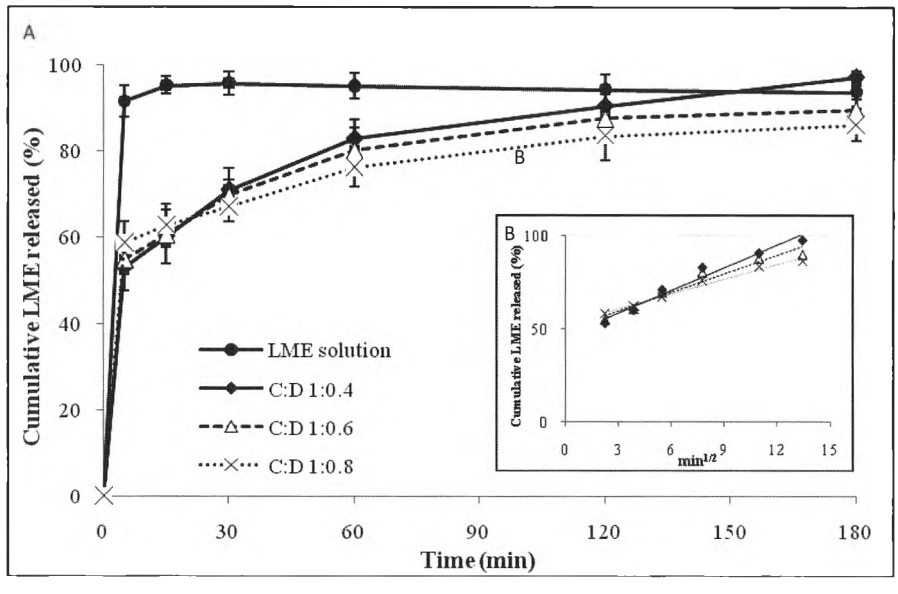

Figure 2 (A) *In vitro* release profiles of LME loaded CDNPs prepared with a polymer concentration of 0.125% and a mass ratio of chitosan and dextran sulfate of (♦) 1:0.4, (Δ) 1:0.6, and (×) 1:0.8 compared with (●) LME solution. (B) Higuchi release model of LME loaded CDNPs.

CONCLUSION

LME loaded CDNPs prepared by electrostatic interaction between the positively and negatively charge polymers could be successfully obtained. The prepared nanoparticles showed mean particle size of 300-450 nm and are positively charged (+20mV), suggesting its mucoadhesive properties. In addition, the *in vitro* release profile showed their ability to provide a sustained release, hence, CDNPs can reasonably be considered as a promising oral cavity delivery system. Various type of surfactant should be further investigated to enhance entrapment efficacy of LME incorporated.

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