Thai Journal of Pharmaceutical Sciences (TJPS)

Journal homepage: http://www.tjps.pharm.chula.ac.th

Development of silymarin-loaded polymeric micelles based on amphiphilic chitosan derivatives

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Keywords: Silymarin; Polymeric micelles; Chitosan; Physical entrapment methods

Objectives: The aim of this study was to incorporate silymarin into polymeric micelles which are amphiphilic chitosan derivatives to increase solubility of silymarin and to evaluate the properties of the micelles.
Methods: Three types of amphiphilic chitosan derivatives; N-benzyl-N,O-succinyl chitosan (BSCS), N-octyl-N-O-succinyl chitosan (OSCS) and N-naphthyl-N,O-succinyl chitosan (NSCS) were synthesized by reductive N-arylation and N,O-succinylation. Silymarin was incorporated into the micelles using various methods, including the dialysis method, the evaporation method, the dropping method and the cosolvent evaporation method. The entrapment efficiency, loading capacity, particle size and zeta potential were determined.
Results: Amphiphilic chitosan derivatives (BSCS, OSCS, NSCS) was synthesized and silymarin was incorporated into the inner core of polymeric micelles. The micelles prepared via the cosolvent evaporation method offered the highest percentage entrapment efficiency and loading capacity. The hydrophobic segment (benzyl, octyl and naphthyl) of polymers did not effect on the entrapment efficiency and loading capacity. We observed that increasing the initial amount of silymarin from 20% to 40% resulted in an increase in loading capacity from 194.1 to 334.7 µg/mg. This high amount reveals the successful encapsulation of the poor water soluble drugs into polymeric self-assemblies. The different methods also influenced the particle size of the micelles. The micelles ranged in size from 102 to 357 nm with negatively charge.
Conclusion: These amphiphilic chitosans was successfully synthesized and formed micelles in an aqueous solution by various methods. These micelles can be loaded with silymarin and demonstrate potential for increasing silymarin solubility. The micelles prepared from the cosolvent evaporation method demonstrated the highest entrapment efficiency and loading capacity. Therefore, these micelles have promising potential as silymarin delivery systems.

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Introduction

Polymeric micelles are colloidal carriers for poorly-water soluble drugs which are more stable than surfactant micelles.[1] Polymeric micelles are characterized by a core-shell structure.[2,3] Amphiphilic polymers with hydrophobic part and hydrophilic part can form polymeric micelles by self-aggregation[2,3] in aqueous media. Chitosan, a biocompatible polymer has been use in various drug delivery systems[4], including polymeric micelles. Amphiphilic chitosan derivatives, N-benzyl-N,O-succinyl chitosan (BSCS), N-octyl-N-O-succinyl chitosan (OSCS) and N-naphthyl-N,O-succinyl chitosan (NSCS) were synthesized by introducing hydrophobic (benzyl, octyl, naphthyl group) and hydrophilic moiety (succinyl group) into chitosan backbone[4,5,6], they have been reported for the successful preparation of polymeric micelles. Poorly soluble compounds can be incorporated into hydrophobic core to increase aqueous solubility by physical entrapment methods[7,9] such as dialysis method, dropping method, cosolvent evaporation method, solvent evaporation method, etc. In this study, silymarin was selected as a model phytomedicine to incorporate into polymeric micelles. Silymarin is a natural compound that can be extracted from milk thistle (*Silybum marianum*). It has been used as a hepatoprotective agent[10,11]. Mechanism of action[10] include: antioxidation activity, cell membrane stabilizer, anti-inflammation, liver regeneration and antifibrotic effect. The therapeutic effect of silymarin is limited by its low solubility. Therefore, these amphiphilic chitosan derivatives, BSCS, OSCS and NSCS were used to developed polymeric micelles as silymarin carriers.

Materials and methods

Materials:

Chitosan with 96% deacetylation (MW 15 KDa) was purchased from OilZac Technologies Co., Ltd. (Bangkok, Thailand). Silymarin, naphthaldehyde, octanaldehyde, benzaldehyde and succinic anhydride were purchased from Sigma Aldrich,

USA. Dialysis bag (CelluSep®, 6000–8000 MWCO) was purchased from Menbrane Filtration Products, USA. All other reagents and solvents were of analytical grade, high performance chromatography grade and used without further purification.

Synthesis of amphiphilic chitosan derivaties

BSCS, OSCS and NSCS were synthesized by introducing hydrophobic and hydrophilic moieties by reductive arylation and succinylation, respectively.[5]

Preparation of polymeric micelles with and without silymarin

Dialysis method: solutions of silymarin 20% wt to polymer in dimethyl sulfoxide (DMSO) were added to 5 mg of amphiphilic chitosan derivatives and diluted with DMSO to a final volume of 2 mL. The mixture was stirred at room temperature until completely dissolved. The mixture was then contained in a dialysis bag and dialyzed against distilled water for 24 h.

Evaporation method: solutions of silymarin 20% wt to polymer in dimethylformamide (DMF) were added to 5 mg of amphiphilic chitosan derivatives and diluted with acetone/DMF (1/3 ratio) to a final volume of 1 mL. The mixture was stirred at the room temperature under nitrogen gas flow. After the solvent completely evaporated, 3 mL of distilled water was added. A probe-type sonicator (CV 244, Sonics Vibracell™, Newtown, CT, USA) was used to sonicated the solution in 3 cycles (sonication time of 5 min and a stand by time of 5 min) at 80°C.Then the solution was centrifuged at 1000 rpm for 2 min and the supernatant was collected.

Dropping method: solutions of silymarin 20% wt to polymer in DMSO were added to 5 mg of amphiphilic chitosan derivatives and diluted with DMSO to a final volume of 0.5 mL. The solution was slowly dropped into 2.5 mL of stirred water in a glass bottle container. The mixture was stirred at the room temperature for 24 h then contained in a dialysis bag and dialyzed against distilled water for 24 h.

Cosolvent evaporation method: BSCS, OSCS, NSCS blank micelles were prepared as mentioned in the dialysis method without adding silymarin. Silymarin was incorporated into the blank micelles by dissolving silymarin in acetone and injecting into 2 mL of the blank micellar solution under regular stirring for 24 h to obtain the micelles with drug concentrations of 0.5, 0.75 and 1 mg/mL (20, 30 and 40% wt to polymer). Then, acetone was completely evaporated via stirring at 70°C for 15 min.

Characterization of micelles with and without silymarin

Particle size and zeta potential: the mean particle size, size distribution (polydispersity index; PDI) and zeta potential of the blank micelles and silymarin-loaded micelles were determined in triplicate at 25 °C using a Nano Zetasizer (Malvern, Worcestershire, UK).

Entrapment efficiency (%EE) and loading capacity: the content of silymarin in micelles prepared by each method was determined in triplicate by dissolving the silymarin-loaded micelles in a mixture of DMSO:H_2O at a 9:1 volume ratio then filtrated through a syringe filter membrane (0.45 µm pore size). The amount of silymarin was determined using high performance liquid chromatography (HPLC, Agilent 1100 series, USA) using a 5µ C18(2) HPLC column (phenomenex®, USA). Gradient mobile phases used were composed of solvent A (water:methanol:85% phosphoric acid, 80:20:0.5) and solvent B (water:methanol:85% phosphoric acid, 20:80:0.5), operating with the flow rate of 1.0 mL/min, injected volume of 10 µL and detection at 288 nm[12]. The average amount of silymarin was determined in triplicate. The %entrapment efficiency (%EE) and loading capacity (LC) were calculated from applying the results to the following equations:

$$\%EE = \frac{\text{The amount of determined silymarin in micelles}}{\text{Initial amount of silymarin used for the preparation}} \times 100 \quad (1)$$

$$LC = \frac{\text{The amount of determined silymarin in micelles}}{\text{Amount of graft copolymer used for the preparation}} \quad (2)$$

Results and discussion

Effect of entrapment method

The self-aggregation of amphiphilic chitosan derivatives can be formed in aqueous media. Polymeric micelles were prepared from amphiphilic chitosan derivative by physical entrapment method. The characteristics of micelles (entrapment efficiency, loading capacity, particle size, polydispersity index (PDI), zeta potential) that prepared from different methods with 20% silymarin were shown in Table 1. The 20% silymarin-loaded micelles prepared from different methods presented differences in size, entrapment efficiency and loading capacity. All polymeric micelles showed negative charges (-33 to -41). For dialysis and dropping method, silymarin could not be incorporated into micelles. It may result from the large amount of distilled water that had been used during dialysis. The solubility of silymarin in water is 0.04 mg/mL[7], when added a lot of water, silymarin dissolved and released from dialysis bag to the water outside. Silymarin-loaded polymeric micelles were successfully prepared by the evaporation and the cosolvent evaporation method. This indicates that silymarin-loaded micelles are occurred through hydrophobic interactions[9] between silymarin and inner core of polymer. Cosolvent evaporation method show highest silymarin entrapment efficiency and loading capacity compared to the other methods. The silymarin-loaded micelles prepared via the cosolvent evaporation method exhibited the small particles. Therefore, this method was selected to prepared silymarin-loaded polymeric micelles with different initial drug concentrations.

Table 1. The entrapment efficiency (%EE), loading capacity (LC), particle size, polydispersity index (PDI), zeta potential of polymeric micelles prepared by different methods

Sample	%EE	LC	Particle size (nm)	PDI	Zeta potential (mV)
Dialysis	0.00	0.00	148.3 ± 5.7	0.207	-35.5 ± 5.0
Evaporation	56.85 ± 1.36	113.70 ± 2.71	357.7 ± 97.5	0.463	-40.5 ± 4.3
Dropping	0.00	0.00	129.8 ± 12.9	0.271	-35.2 ± 4.7
Cosolvent evaporation	80.31 ± 4.71	160.62 ± 9.43	157.3± 10.4	0.211	-33.7± 2.5

Effect of polymers and initial amount of drug

The effect of hydrophobic moiety of polymer and initial amount of silymarin on particle size, polydispersity index (PDI) and zeta potential of silymarin-loaded micelles prepared via the cosolvent evaporation method was shown in Table 2. The silymarin-loaded micelles prepared from different type of polymers and different initial amount of silymarin presented differences in size. The overall sizes of the micelles ranged from 102 to 196 nm. Both blank micelles and silymarin-loaded micelles showed negative charges because of succinyl moiety in the structure of polymers.

Table 2. The particle size, polydispersity index (PDI), zeta potential of polymeric micelles prepared by different initial amount of silymarin by Cosolvent evaporation method.

Amphiphilic chitosan derivatives	Silymarin (%to polymer)	Particle size (nm)	PDI	Zeta potential (mV)
BSCS	0	154.8 ± 3.3	0.282	-38.8 ± 1.6
	20	173.5 ± 3.1	0.249	-32.6 ± 6.8
	30	187.6 ± 3.9	0.347	-31.7 ± 5.7
	40	177.3 ± 14.3	0.256	-28.4 ± 1.3
OSCS	0	128.3 ± 12.0	0.318	-28.7 ± 0.1
	20	148.8 ± 12.8	0.290	-25.8 ± 3.9
	30	156.5 ± 9.1	0.294	-27.5 ± 1.3
	40	161.5 ± 1.7	0.343	-27.6 ± 5.3
NSCS	0	102.5 ± 16.1	0.612	-30.5 ± 1.0
	20	140.1 ± 13.8	0.367	-26.0 ± 3.5
	30	164.8 ± 16.4	0.395	-26.8 ± 4.8
	40	130.7 ± 16.1	0.263	-32.7 ± 2.0

Silymarin was loaded into the micelles during micelle formation.The effect of hydrophobic moiety of polymer and initial amount of silymarin on entrapment efficiency and loading capacity of silymarin-loaded micelles prepared via the cosolvent evaporation method was illustrated in Figure 1. We observed in all polymers that increasing the initial amount of silymarin from 20% to 40% resulted in a decrease in % entrapment efficiency. On the other hand, the loading capacity continued to increase when the initial amount of silymarin was increased. The loading capacity rose from 194.1 to 334.7 µg/mg, with an increase in the initial silymarin loading from 20% to 40%. This high amount reveals the successful encapsulation of the poor water soluble drugs into polymeric self-assemblies and demonstrates the presence of hydrophobic interactions between the loaded drugs and the hydrophobic moieties of the polymeric micelles[13]. The hydrophobic interactions among the hydrophobic chain, silymarin, and solvent may be principal factors that control this entrapment process.

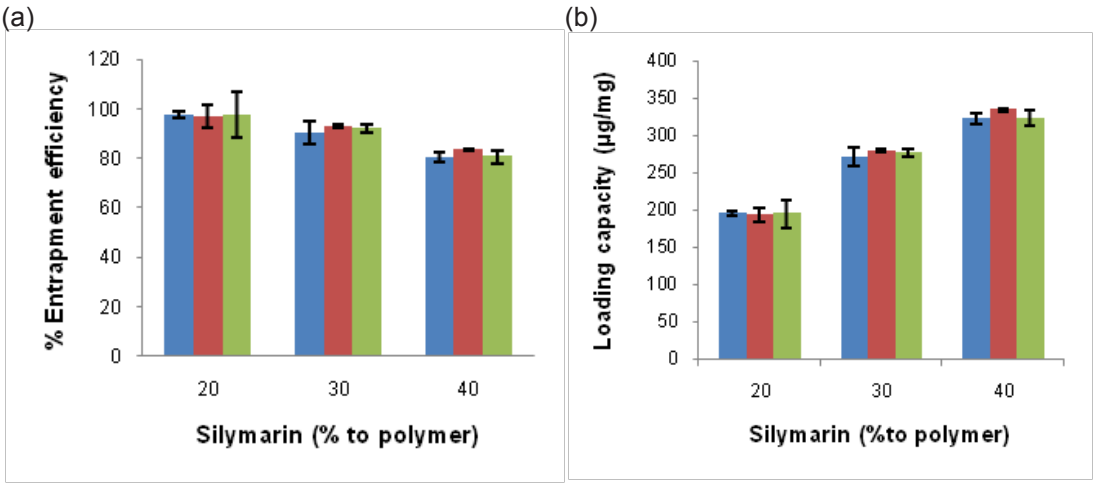

Figure 1. Effect of hydrophobic core of polymers and initial amount of silymarin (20-40% to polymer) on (a) the entrapment efficiency and (b) loading capacity of silymarin-loaded polymeric micelles prepared via the cosolvent evaporation method; (■) BSCS;(■) OSCS; (■) NSCS polymer.

Conclusion

In this present study, amphiphilic chitosan derivatives (BSCS, OSCS and NSCS), were successfully synthesized and formed micelles in an aqueous solution by various methods. These micelles can be loaded with poorly water soluble phytomedicine (silymarin). The micelles prepared from the cosolvent evaporation method demonstrated the highest entrapment efficiency and loading capacity, and they also presented the nano-size range. These polymeric micelles may be an alternative for potential as silymarin carrier.

Acknowledgements

The authors would like to acknowledge the Thailand Toray Science Foundation (TTSF) in 2015, Bangkok Lab and Cosmetic Company Limited, the Pharmaceutical Development of Green Innovations Group (PDGIG) the Faculty of Pharmacy, Silpakorn University and the National Nanotechnology Center (NANOTEC), Thailand.

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