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Thisirak Woraphatphadung

Warayuth Sajomsang

Theerasak Rojanarata

Tanasait Ngawhirunpat

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Thisirak Woraphatphadung[1], Warayuth Sajomsang[2], Theerasak Rojanarata[1], Tanasait Ngawhirunpat[1] and Praneet Opanasopit[1]

[1] *Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand*
[2] *National Nanotechnology Center, Thailand Science Park, Pathumthani, Thailand*

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INTRODUCTION

The poorly soluble of drug is one of the important obstacles for successful and effective therapy which frequently results in low bioavailability of the orally administered drug[1,2]. Polymeric micelles have been investigated intensively as oral drug delivery applications because it is more convenient and preferred by patient[3]. They are composted of amphiphilic block copolymers which consist of hydrophilic segment (stabilize micelles) and hydrophobic segment (contain lipophilic drugs). In this way, poorly soluble drugs can be successfully solubilized in aqueous media[4]. Several techniques have been offered for the preparation of drug-loaded micelles which the efficiency of drug loading depend on each technique such as chemical conjugates, physical entrapments, electrostatic technique, etc[5]. In this study, physical entrapment technique was selected because it was the simplest and most convenient technique and can be divided into dialysis, O/W emulsion, dropping and evaporation. Therefore, the aim of this study was to evaluate the effect of organic solvents and preparation methods on loading efficiency. Meloxicam (MX), a class of non-steroidal anti-inflammatory drug with poor aqueous solubility (BCS class II), was selected as a model drug. N-arylsuccinyl chitosan was used as synthesis pH-sensitive polymer.

MATERIALS AND METHODS

Materials Meloxicam (MX) was a gift from Siam pharmaceutical, Thailand. Chitosan with 96% deacetylation (MW 15 KDa) was purchased from OilZac Technologies Co., Ltd. (Bangkok, Thailand). 2-Naphthaldehyde 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 and used without further purification.

Synthesis of N-arylsuccinyl chitosan N-arylsuccinyl chitosan were synthesized by introducing hydrophobic and hydrophilic moieties by reductive arylation and succinylation, respectively [6].

Preparation of micelles with and without MX

Dialysis 5 mg of N-arylsuccinyl chitosan polymer and MX (0-40 % to polymer) was dissolved in 2 ml of dimethyl sulfoxide (DMSO) in a glass bottom. The mixture was stirred at room temperature until completely dissolved. The mixture was then placed in a dialysis bag and dialyzed against distilled water overnight.

O/W emulsion Micelles without MX were prepared as dialysis. For drug-loaded micelles, MX (5-40 % to polymer) was dissolved in dichloromethane (DCM) and then was injected under constant stirring to 2 ml of aqueous empty micellar solution. After that DCM was evaporated by overnight stirring at room temperature. The remaining aqueous solution was centrifuged at 1000 rpm for 2 min and the supernatant was collected.

Evaporation 5 mg of N-arylsuccinyl chitosan polymer and MX (0-40 % to polymer) was dissolved in N-dimethylformamide (DMF) in a glass bottom. The mixture was added acetone (1/3 of DMF) and stirred at room temperature under nitrogen gas flow until the solvent completely evaporated. 3ml of distilled water was added, and the solution was sonicated using a probe-type sonicator (model CV 244, Sonics Vibra Cell™, USA) in a cycle with a sonication time of 5 min and a standby time of 5 min at 80°C for 20 min. The obtained solution was centrifuged at 1000 rpm for 2 min and the supernatant was collected.

Dropping 5 mg of N-arylsuccinyl chitosan polymer and MX (0-40 % to polymer) was dissolved in DMSO or DMF 0.5 ml. The solution was slowly dropped into stirred water and the mixed solution was stirred overnight. Final ratio of DMSO or DMF : water was vary as 1:5 and 1:10. The mixture was then placed in a dialysis bag and dialyzed against distilled water overnight. After that the mixture solution was centrifuged at 1000 rpm for 2 min and the supernatant was collected.

Incorporation efficiency MX-loaded polymeric micelles were dissolved in a mixture solution of DMSO: H_2O (9:1). The amount of MX-loaded into polymeric micelles was determined by UV-vis

Spectrophotometer (Model 8453E, Agilent Technologies, USA) at 365 nm. The incorporation efficiency and loading capacity were calculated following equation(1) and (2), respectively.

$$Incorporation\ efficiency = \frac{amount\ of\ MX\ in\ micelles}{amount\ of\ MX\ used\ for\ micelles\ preparation} x\ 100 \quad (1)$$

$$Loading\ capacity = \frac{amount\ of\ MX\ in\ micelles}{amount\ of\ polymer} \quad (2)$$

Particle size The mean particle size of polymeric micelles with and without MX was determined in triplicate at 25°C by using the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The micelles samples were diluted with distilled water, which was passed through a 0.22 μm membrane filter prior to use.

In vitro release Release of MX from MX-loaded polymeric micelles was determined using a dialysis bag. 20 ml of 0.1N HCl (pH 1.2) or phosphate buffer solution pH 6.8 (PBS pH 6.8) was used as medium at 37°C under constant stirring. 1 ml of MX-loaded polymeric micelles was placed in a dialysis bag and immersed in the medium. At certain time intervals, 1 ml aliquots of the medium were withdrawn and the same volume of fresh medium was added. The sample solution was analyzed by UV-vis Spectrophotometer at 365 nm.

Statistical analysis All experimental measurements were collected in triplicate. The values are expressed as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Empty polymeric micelles A novel pH responsive polymeric self-assemblies were developed based on an introduction of hydrophobic and hydrophilic moieties into a chitosan backbone. The self-aggregates of N-arylsuccinyl chitosan can be formed in aqueous media. The critical micelle concentration (CMC) of the self-assembly micellar system of this polymer was determined by measuring the fluorescence intensity of the pyrene as a fluorescent probe[7]. The CMC value of was found to be 0.0749 mg/ml. The particle sizes and polydispersity index (PdI) of micelles prepared from different methods (dialysis, dropping and evaporation) were shown in the Table1. The results revealed that different methods influenced on the particle size of polymeric micelles. Micelles prepared by dropping technique showed the smallest particle sizes in both DMSO and DMF solvents. The rank of particle sizes of the micelles were dropping (84.31±0.88 nm) < dialysis (196.20±1.51nm) < evaporation (233.50±7.07 nm). The type and ratio of solvent to water was also the important factor which affected on the particle size of polymeric micelles prepared by dropping method. When using DMSO, the particle size of the micelles (84.31±0.88 nm) was smaller than DMF (98.35±0.46 nm) with the similar narrow size distribution. In addition, reduction in the ratio of organic solvent and water from 1:5 to 1:10 resulting in the increase in the particle size of the micelles from 84.31 to 97.97 nm for DMSO and from 98.35 to 107.40 nm for DMF.

Table1 The particle sizes and polydispersity index (PdI) of polymeric micelles prepared by different methods

Method	Organic solvent	Ratio (organic: water)	particle size ± S.D. (nm)	PdI
Dialysis	DMSO	-	196.20±1.51	0.061±0.031
	DMF		196.10±1.15	0.081±0.012
Dropping	DMSO	1:5	84.31±0.88	0.198±0.016
	DMF		98.35±0.46	0.198±0.015
	DMSO	1:10	97.97±0.77	0.237±0.001
	DMF		107.40±1.06	0.192±0.009
Evaporation	DMF:acetone (3:1)	-	233.50±7.07	0.384±0.006

Incorporation efficiency Polymeric micelles prepared by dropping method using DMSO as organic solvent showed a smaller particle size than DMF. Therefore, DMSO with the ratio of DMSO:water (1:5)

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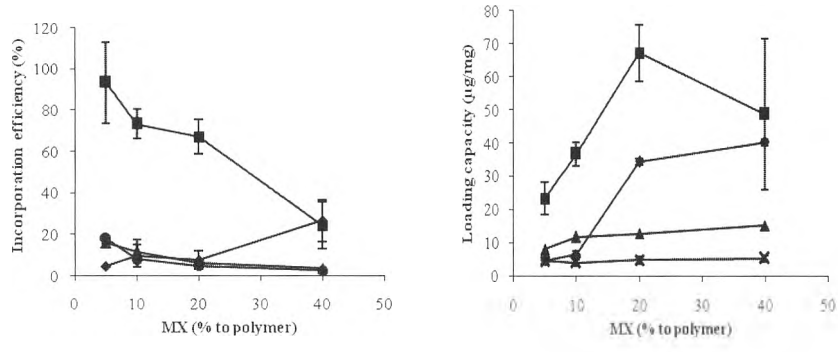
was selected to prepare MX-loaded polymeric micelles. For evaporation method, DMF was selected as appropriate solvent. The effect of entrapment methods on the incorporation efficiency and loading capacity of MX-loaded polymeric micelles was shown in the Fig.1. The X-axis represents the initial drug used in preparation (percentage of MX to polymer), ranging from 5% to 40%, and Y-axis represents the percentage of MX incorporated into the micelles (% CPT-loaded) (Fig 1a) and loading capacity (Fig.1b). The results revealed that evaporation method showed the highest incorporation efficiency (25-90 %) and loading capacity (20-65 μg/mg) when compared to other methods (less than 25 %). The loading capacity increased ranging from 20 to 65 μg/mg with an increase in the initial MX loading from 5% to 20%. Such a high content indicates successful incorporation of water-insoluble drug to polymeric micelles with good water solubility. Micelle formation and drug incorporation into micelle were expected to occur simultaneously. Interactions, mainly hydrophobic interactions, among the hydrophobic N-phthaloylchitosan chain, MX, and solvent may be an important key to control this incorporation process. This result was similar to the previous studies reported that the incorporation efficiency of dexamethasone-loaded into PEGylated poly-4-(vinylpyridine) polymeric micelles was also dependent on the incorporation methods[5]. Co-solvent evaporation method had percentage drug loading higher than direct dialysis and O/W emulsion. Thus, the incorporation method affected on drug loading efficiency. Table 2 shows the particle sizes of the polymeric micelles with and without MX. The MX-loaded polymeric micelles showed larger size (108 - 875 nm) than the empty micelles (84 - 233 nm). The particle sizes of the MX-loaded micelles had tendency to increase with an increase in the weight ratio of MX to polymer. The larger particle size of MX-loaded into polymeric micelles might be the increase of MX in the micelles and the aggregation of micelles[8].



(a) (b)

Figure1 Effect of incorporation technique and initial drug (5-40% to polymer) on (a) the incorporation efficiency and (b) loading capacity of MX-loaded polymeric micelles; (♦) dialysis method; (●) dropping method; (■) evaporation method; (▲) emulsion method.

Table 2 The particle sizes and polydispersity index (PdI) of polymeric micelles with and without MX

MX to polymer (%)	Dialysis		Dropping		Evaporation		O/W emulsion	
	Particle size (nm)	PdI	Particle size (nm)	PdI	Particle size (nm)	PdI	Particle size (nm)	PdI
0	196.20±1.51	0.061	84.31±0.88	0.198	233.50±7.07	0.384	-	-
5	275.03±6.12	0.169	108.77±4.03	0.217	291.77±6.35	0.383	192.93±0.85	0.070
10	364.50±8.35	0.250	113.87±1.32	0.156	382.17±12.02	0.523	194.83±3.02	0.100
20	654.07±23.91	0.444	127.07±0.29	0.153	312.17±12.00	0.381	192.13±2.54	0.076
40	875.23±28.60	0.510	142.73±0.57	0.174	293.80±6.84	0.310	195.37±2.17	0.120

Micelles drug release Polymeric micelle drug delivery systems are advantageous for their wide applicability in delivering hydrophobic drugs. pH-sensitive polymeric micelles are promising for oral drug delivery, especially for poorly water-soluble medicines. MX (20% to polymer)-loaded polymeric

micelles prepared by evaporation method showed the highest MX loaded. Therefore, this formulation was selected to investigate the release behavior. The in vitro release of 20% MX-loaded polymeric micelles was measured using simulated intestinal fluid (SIF) (PBS pH 6.8) and simulated gastric fluid (SIF) (0.1 N HCl pH 1.2) as release medium that mimic the conditions experienced in the body. The release profiles of MX-loaded polymeric micelles in both release mediums are shown in Fig. 2. In acidic medium (0.1 N HCl), MX released less than 50% of the MX. When the pH was changed to pH 6.8, the MX release increased due to ionization of succinic acid moiety into N-arylsuccinyl chitosan micelles. Therefore, this novel chitosan derivative would be the appropriate copolymer to protect poorly water-soluble drug in the gastric fluid and release at basic pH to absorb into intestinal.

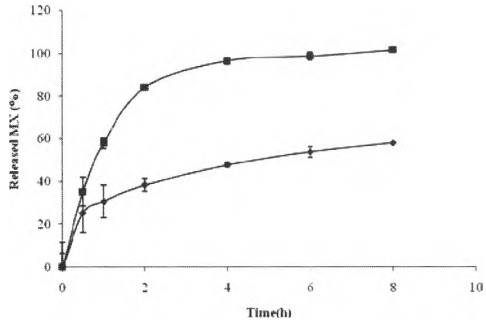


Figure 2 Release profile of 20% MX to polymer-loaded polymeric micelles in; (♦) 0.1 N HCl pH 1.2; (■) PBS pH 6.8

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CONCLUSION

The pH responsive N-arylsuccinyl chitosan was successfully synthesized. N-arylsuccinyl chitosan could be formed self-assembly in aqueous media and successfully incorporated poorly water-soluble drug (MX) in hydrophobic inner core by various physical entrapment methods. Among the methods, evaporation method and initial 20% MX to polymer showed the highest MX incorporation efficiency. The MX release behaviors could be adjusted by pH. Therefore, this N-arylsuccinyl chitosan polymeric micelle presents interest to develop MX carrier in oral drug delivery.

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REFERENCES

1. Aliabadi HM, Elhasi S, Mahmud A, Gulamhusein R, Mahdipoor P, Lavasanifar A. 2007. Encapsulation of hydrophobic drugs in polymeric micelles through co-solvent evaporation: The effect of solvent composition on micellar properties and drug loading. Int J Pharm. 329: 158–165.
2. Lu Y, Park K. 2013. Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. Int J Pharm. 453(1): 198-214.
3. Zhang Y, Chen J, Zhang G, Lu J, Yan H, Liu K. 2012. Sustained release of ibuprofen from polymeric micelles with a high loading capacity of ibuprofen in media simulating gastrointestinal tract fluids. React Funct Polym. 72: 359-364.
4. Yokoyama M. 2011. Clinical application of polymeric micelle carrier systems in chemotherapy and image diagnosis of solid tumors. J Exp Clin Med. 3(4): 151-158.
5. Miller T, Colen GV, Sander B, Golas MM, Uezguen S, Weigandt M et al. 2013. Drug loading of polymeric micelles. Pharm Res. 30: 584-595.
6. Sajomsang W, Tantayanon S, Tangpasuthadol V, Thatte M, Daly WH. 2008. Synthesis and characterization of N-aryl chitosan derivatives. Int J Biol Macromol. 43:79-87.
7. Zhao C, Wang Y, Winnik MA, Riess G, Croucher MD, 1990. Fluorescence probe technique used to study micelle formation inwater-soluble block co-polymer. Langmuir 6. 514–516.
8. Ngawhirunpat T, Wonglertnirant N, Opanasopit P, Ruktanonchai U, Yoksan R, Wasanasuk K et al. 2009. Incorporation methods for cholic acid chitosan-g-mPEG self-assembly micellar system containing camptothecin. Colloids Surf, B Biointerfaces. 74: 253-259.