Original Article

การเตรียมพอลิเมอริกไมเซลล์บรรจุเคอร์คูมินสำหรับนำส่งยาสู่ลำไส้ใหญ่ทางการรับประทาน

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บทคัดย่อ

บทนำ: เคอร์คูมินมีประสิทธิภาพในการต้านมะเร็งโดยเฉพาะใช้เพื่อป้องกันและรักษามะเร็งลำไส้ใหญ่ แต่เคอร์คูมินมีปัญหาด้านการละลายน้ำน้อย พอลิเมอริกไมเซลล์ได้รับการออกแบบให้บรรจุยาที่ละลายน้ำน้อยเพื่อเพิ่มการละลายของยา ดังนั้นการศึกษานี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพการบรรจุเคอร์คูมินในพอลิเมอริกไมเซลล์ ที่เตรียมจาก *N*-octyl-*N, O*-succinyl chitosan (OSCS) คุณลักษณะ และการควบคุมการปลดปล่อยยาบริเวณเป้าหมายที่ลำไส้ใหญ่เมื่อให้รูปแบบรับประทาน **วิธีดำเนินการวิจัย:** เตรียมพอลิเมอริกไมเซลล์จาก OSCS เพื่อบรรจุเคอร์คูมินด้วยวิธีแยกสารผ่านเยื่อและวิธีการระเหย การประเมินประสิทธิภาพการบรรจุยา ขนาดอนุภาค ความเป็นพิษต่อเซลล์ และการปลดปล่อยยาแบบภายนอกร่างกาย **ผลการวิจัย**: สามารถเตรียมพอลิเมอริกไมเซลล์จาก OSCS เพื่อบรรจุเคอร์คูมินไว้ภายในแกนด้านในที่ไม่ชอบน้ำด้วยวิธีแยกสารผ่านเยื่อและวิธีการระเหย โดยวิธีแยกสารผ่านเยื่อมีประสิทธิภาพการบรรจุเคอร์คูมินร้อยละ 22.75±5.54 และความสามารถในการบรรจุเท่ากับ 78.42±5.94 ไมโครกรัม/มิลลิกรัม มากกว่าวิธีการระเหย ซึ่งมีประสิทธิภาพการบรรจุยาร้อยละ 7.80±1.46 และความสามารถในการบรรจุ เท่ากับ 15.59±2.91 ไมโครกรัม/มิลลิกรัม ขนาดอนุภาคเฉลี่ยของไมเซลล์ที่เตรียมด้วยวิธีแยกสารผ่านเยื่ออยู่ในช่วง 193-260 นาโนเมตร ซึ่งมีขนาดเล็กกว่าวิธีการระเหย (310-354 นาโนเมตร) พอลิเมอริกไมเซลล์ มีความเป็นพิษต่อเซลล์ Caco-2 ต่ำ โดยมีค่า IC_{50} เท่ากับ 2.95±0.06 มิลลิกรัม/มิลลิลิตร การปลดปล่อยปริมาณเคอร์คูมินสะสมในระบบจำลองกระเพาะที่มีพีเอช 1.2 ประมาณร้อยละ 20 และเมื่อปรับเป็นระบบลำไส้เล็กพีเอช 6.8 และลำไส้ใหญ่ 7.4 พบว่าการปลดปล่อยเคอร์คูมินจากไมเซลล์เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ โดยลำไส้เล็กจำลองร้อยละ 50-55 และลำไส้ใหญ่จำลองร้อยละ 60-70 เมื่อเทียบกับยาเดี่ยวร้อยละ 20 ($p<0.05$) **สรุปผลการวิจัย**: การศึกษานี้ชี้ให้เห็นว่าไมเซลล์ที่เตรียมจาก OSCS อาจช่วยเพิ่มการละลายของยาและควบคุมการปลดปล่อยยาละลายน้ำน้อยไปบริเวณเป้าหมายที่ลำไส้ใหญ่เมื่อให้รูปแบบรับประทาน

คำสำคัญ: พอลิเมอริกไมเซลล์, เคอร์คูมิน, เป้าหมายที่ลำไส้ใหญ่, รับประทาน

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Preparation of Curcumin-loaded Polymeric micelles for Oral Colon-Targeted Drug Delivery

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Abstract

Introduction: Curcumin is an anticancer agent to use in chemoprevention and treatment colorectal cancer. However, one problem of curcumin is its low solubility. Polymeric micelles has been designed for entrapment of hydrophobic drugs to improve drug solubility. Thus, this study aimed to investigate entrapment efficiency of curcumin-loaded *N*-octyl-*N,O*-succinyl chitosan (OSCS) polymeric micelles, characterization and control drug release at colon targeted site by oral route. **Methods:** The OSCS micelles were prepared to load curcumin. The physical entrapment methods (dialysis and evaporation) were applied. Curcumin-loaded OSCS micelles were determined loading efficiency, loading capacity, particle size, *in vitro* cytotoxicity and *in vitro* drug release. **Results:** The OSCS micelles were able to load curcumin in the hydrophobic inner core by dialysis and evaporation methods. The curcumin-loaded OSCS micelles by dialysis method showed loading efficiency (22.75±5.54%) and loading capacity (78.42±5.94 µg/mg) higher than evaporation method (loading efficiency 7.80±1.46%; loading capacity 15.59±2.91 µg/mg, respectively). The mean particle sizes of micelles by dialysis method were in the range of 193-260 nm which were smaller than that by evaporation method (310-354 nm). The cytotoxicity of OSCS micelles on Caco-2 cells depended on the concentration of OSCS with the IC_{50} value of 2.95±0.06 mg/mL. The cumulative release of curcumin from OSCS micelles in simulated gastric fluid (SGF) was about 20%. When pH medium was changed to pH 6.8 (simulated intestinal fluid; SIF) and pH 7.4 (simulated colonic fluid; SCF), the curcumin release was significantly increased (SIF; 50-55%) and (SCF; 60-70%) when compared to curcumin free drug (20%) ($p<0.05$). **Conclusion:** These finding supports the potential of these pH-sensitive OSCS polymeric micelles and it might be improved solubility of hydrophobic drugs and control drug release at colon targeted site by oral administration.

Keywords: polymeric micelles, curcumin, colon-targeted, oral delivery

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Introduction

Curcumin is a hydrophobic polyphenolic compound from the rhizome of turmeric *Curcuma longa* Linn (Zingiberaceae). It is widely used due to low cost and pharmacological safety. The curcumin has been extensively investigated for its potential to use in chemoprevention and treatment of a wide variety of tumors (Akl et al., 2016; Mehanny et al., 2016). Several studies have shown that curcumin inhibited colorectal cancer formation in the initiation and progression stage of carcinogenesis in *in vivo* study (Kawamori et al., 1999; Shemesh and Arber, 2014). For example, curcumin exhibits potent inhibitory activity in human colorectal cancer cell lines, SW480, HT-29, and HTC116 (Cen et al., 2009). It inhibits proliferation and induces apoptosis of human colorectal cells by activating the mitochondria apoptotic pathway (Guo et al., 2013). However, anti-cancer activity of curcumin is hindered by its low aqueous solubility (11 ng/mL in aqueous buffer at pH 5) (Akl et al., 2016; Mehanny et al., 2016). The low solubility is the one parameter of the crucial obstacles with effect absorbed in the gastrointestinal (GI) tract leading to low bioavailability (Li et al., 2009; Lu and Park, 2013). Therefore, novel carriers were generated for raising higher level treatment such as polymeric micelles, microemulsions and nanoparticles (Sharma et al., 2009).

Polymeric micelles are formed through self-assembly of amphiphilic copolymer in aqueous solution when the concentration of polymer is above the critical micelle concentration (CMC). The inner core is the hydrophobic segment, which entraps hydrophobic drugs while the outer hydrophilic shell stabilizes interface between the hydrophobic core and aqueous solution, protects the hydrophobic drugs from the environmental stimuli (e.g. gastric pH, enzyme) and decreases side effects of drugs on healthy cells and tissues (Ghaemy, Ziaei, and Alizadeh, 2014). The pH sensitive of polymeric micelles has been designed to control or enhance drug release from carriers (Bromberg, 2008). Previously, in our study, we found that pH sensitive polymeric micelles prepared from chitosan derivatives could be designed to improve the stability of meloxicam-loaded micelles in the stomach and could achieve a controlled release when it was tested in the intestine (Woraphatphadung et al., 2015). Therefore, the aim of this study was to entrap curcumin into pH sensitive polymeric micelles from amphiphilic chitosan based (*N*-octyl-*N,O*-succinyl chitosan; OSCS) and control drug release at colon targeted site by oral route. The loading efficiency, loading capacity, particle size, *in vitro* cytotoxicity of the polymeric micelles on Caco-2 cells and *in vitro* of curcumin-loaded OSCS micelles were investigated.

Materials and Methods

Chemicals and Materials

N-octyl-N,O-succinyl chitosan (OSCS) was synthesized by introducing hydrophobic and hydrophilic moieties via reductive amination and succinylation as previously reported (Woraphatphadung et al., 2016). Curcumin was purchased from Sigma Aldrich, USA. Dialysis bag (CelluSep®, 3500 MWCO) was purchased from Membrane Filtration Products, USA. The human colon adenocarcinoma (Caco-2) cell line was

obtained from American Type Culture Collection (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Trypsin—EDTA, and penicillin-streptomycin were purchased from Gibco BRL Rockville, MD, USA. All other chemicals and solvents were of analytical grade and used without further purification.

Preparation of curcumin-loaded OSCS micelles

Dialysis method: 5 mg of OSCS and curcumin (10 and 20% to polymer) were dissolved in 2 mL of dimethyl sulfoxide (DMSO) in a glass bottom container. Then, the mixture was stirred at room temperature until completely dissolved and transferred to dialysis bag. The deionized water was replaced every 4 h for 24 h. The solution was centrifuged at 1000 rpm for 5 min. Then, the supernatant was filtered through a 0.45-μm membrane filter and collected. Moreover the polymeric micelles without drugs (blank micelles) were also prepared in the same method.

Evaporation method: 5 mg of OSCS and curcumin were dissolved in dimethylformamide (DMF) in a glass bottom container. The solution was mixed with acetone (1/3 of DMF) and stirred at room temperature under nitrogen gas flow until the solvent completely evaporated. Next, 3 mL of deionized water was added, and the solution was sonicated using a probe-type sonicator (CV 244, Sonics VibraCellTM, Newtown, CT, 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 solution was centrifuged at 1000 rpm for 5 min. Then, the supernatant was filtered through a 0.45-μm membrane filter and collected.

In vitro cytotoxicity

The *in vitro* cytotoxicity of the OSCS was evaluated by MTT assay (Mosmann, 1983). Briefly, the Caco-2 cells were cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 1% nonessential amino acid solution and 0.1% penicillin-streptomycin solution at pH 7.4 in a humidified atmosphere (5% CO_2, 95% air, 37°C). Then, the Caco-2 cells were seeded into each well of 96-well plates and pre-incubated for 24 h at a seeding density of 10000 cells/well. After that, the cells were treated with the blank micelles at various concentrations (0.01–5 mg/mL) and further incubated for 24 h. After the treatment, the cell viability was assessed using MTT assay and calculated based on the absorbance measurements at 550 nm using a microplate reader (Universal Microplate Analyzer, Model AOPUS01 and AI53601, Packard BioScience, CT, USA). The viability of non-treated control cells was arbitrarily defined as 100%.

Characterization

Entrapment efficiency

The drug loading efficiency and drug loading capacity of the curcumin-loaded OSCS micelles were determined by HPLC analysis (Agilent 1100 Series HPLC System, Agilent Technologies, USA). The separation was performed using an Eclipse XDB-C18 column (particle size 5 μm; column dimension 4.6 mm × 150 mm). A mobile phase was used acetonitrile:1% v/v acetic acid (43:57, v/v), and the flow rate was 1 mL/min. The injection volume was 20 μL, and the wavelength was set at 428 nm (Sajomsang et al., 2014). The micelle samples were dissolved in a mixture solution of DMSO:H_2O (9:1) and filtered through a 0.45-μm membrane filter prior to injection. The loading efficiency and loading capacity were calculated according to equation (1)

and (2), respectively.

$$\text{Loading efficiency (\%)} = (C1/C2)\times 100 \quad (1)$$

where C1 is the amount of curcumin in OSCS micelles and C2 is the initial of amount curcumin used for preparation

$$\text{Loading capacity (mg/mg)} = (L1/L2) \quad (2)$$

where L1 is the amount of curcumin in micelles and L2 is the amount of OSCS copolymer used for preparation.

Particle size

The mean particle size and the size distribution of the polymeric micelles were determined in triplicate at 25°C by dynamic light scattering (DLS) (Malvern, Worcestershire, UK). The micelle samples were diluted with deionized water and were passed through a 0.45-μm membrane filter prior to use.

In vitro release

The release of curcumin from the curcumin-loaded OSCS micelles was performed using the dialysis technique. The medium was supplemented for three different stages with 0.1 N HCl (pH 1.2) for 2 h, then the pH of the medium was changed to 6.8 with potassium dihydrogen phosphate (KH_2PO_4) and 5.0 M NaOH for 3 h and then pH was changed to 7.4 until for 8 h (Sajomsang et al., 2014). One milliliter of curcumin-loaded OSCS micelles in water was placed in a dialysis bag and immersed in the medium containing 30% (v/v) methanol and 1% (v/v) Tween 20 under constant stirring with sink conditions at 37±0.5°C. At the time intervals of 0.5, 1, 2, 4, 6 and 8 h, 1 mL aliquots of the medium was withdrawn, and replaced with the same volume of fresh medium. The content of curcumin was analyzed by HPLC system equipped with UV spectrophotometer, and the cumulative release of curcumin from the micelles was calculated. All experiments were done in triplicate.

Statistical analysis

All data were expressed as the mean ± standard deviation (SD) of triplicate experiments. The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by an LSD post hoc test. The significance level was set at $p < 0.05$.

Results

pH-sensitive self-assembly micelles could improve the solubility of poorly water soluble drugs by entrapping the drugs into hydrophobic core. In this study, OSCS was selected as a micelle forming polymer because it has shown to exhibit the highest MX loading efficacy, excellent stability and controlled release in our previous study (Woraphatphadung et al., 2016).

Characterization of curcumin-loaded polymeric micelles

The loading efficiency and loading capacity of curcumin in OSCS polymeric micelles are presented in Fig. 1. The X-axis represents the initial amount of curcumin used in the preparation (percentage of curcumin), and the Y-axis represents the percentage of curcumin loaded into the micelles (Fig. 1a) and loading capacity (Fig. 1b). The pH sensitive OSCS micelles were able to entrap curcumin by dialysis and evaporation methods. It can be seen that the different methods and weight

ratios of drugs to polymer (10 and 20% to polymer) had a great effect on the loading efficiency and loading capacity (Yang et al., 2012, Woraphatphadung et al., 2015). The loading capacity of both methods increased with the increase in the initial curcumin loading from 10% to 20% while loading efficiency had a tendency to decrease. At 20% to polymer, the curcumin-loaded OSCS micelles by dialysis method showed loading efficiency (22.75±5.54%) and loading capacity (78.42±5.94 µg/mg) higher than evaporation method (loading efficiency 7.80±1.46%; loading capacity 15.59±2.91 µg/mg). This result revealed that the self-aggregated micelles could improve drug solubility with high incorporation efficiency. The particle sizes and size distribution of curcumin-loaded OSCS polymeric micelles are displayed in Table 1. The mean particle sizes of the curcumin-loaded micelles increased with an increase in the weight ratio of the drug to polymer. The large particle size of curcumin-loaded into polymeric micelles might be due to the aggregation of micelles (Ngawhirunpat et al., 2009). Moreover, the particle size of micelles prepared by dialysis method (193-260 nm) was smaller than that by evaporation method (310-354 nm). The results indicated that the different methods and the initial drug concentration influenced the particle size of the micelles.

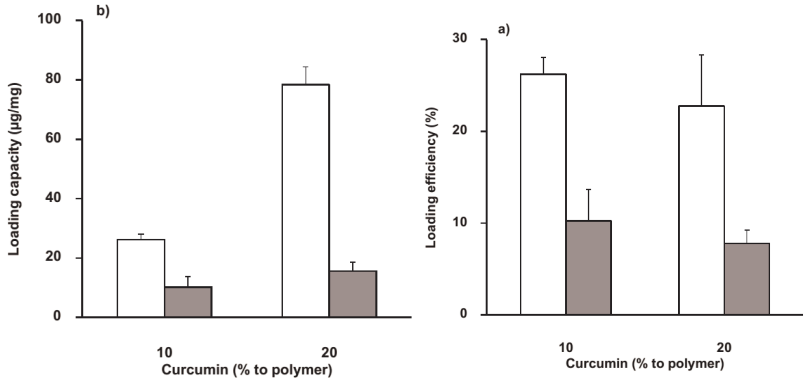

Figure 1 Effect of the incorporation technique; dialysis method (white bar graph) and evaporation method (shaded bar graph); and initial drug concentration (10 and 20 % to polymer) on (a) the loading efficiency, (b) loading capacity of curcumin-loaded OSCS micelles. Data are plotted as the average ± SD of three measurements

Table 1 Particle size of curcumin-loaded OSCS polymeric micelles

Method	Curcumin (% to polymer)	Particle size (nm)	PDI
Dialysis	10	192.60±3.50	0.471
	20	259.70±5.51	0.404
Evaporation	10	309.07±8.57	0.358
	20	353.40±8.94	0.452

In vitro cytotoxicity

The majority requirement of polymers is used to prepare self-assembly micelle that should be non-toxic. Thus, the cytotoxicity of OSCS micelles was determined by quantitative evaluation of cell viability using Caco-2 cells. Fig. 2 shows the Caco-2 cells viability after treatment with various the concentrations (0.01–5 mg/mL) of the copolymer for 24h. The IC_{50} value was calculated to be 2.95±0.06 mg/mL. This indicated that the OSCS copolymer micelles had low cytotoxicity and would be safe *in vivo*.

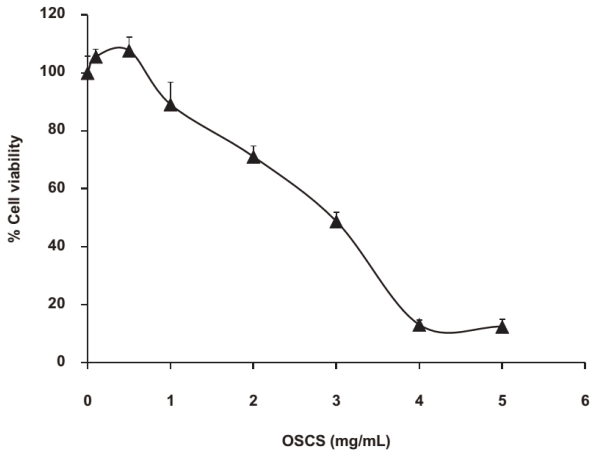

Figure 2 The percent cell viability in Caco-2 cells at varying concentrations of OSCS micelles. Each value represents the average ± S.D. of five wells.

In vitro release

Polymeric micelle drug delivery systems are advantageous for their wide applicability in delivering hydrophobic drugs. This study, pH sensitive OSCS polymeric micelles carrier was developed for colon delivery. As it is known, the pH levels in GI tract vary according to the site; in the stomach the pH is 1-2; in the small intestine the pH is 5.1-7.5 and in the colon the pH is 7-7.5 (Li et al., 2009; Yang et al., 2012). Thus, the release profile of curcumin at the fixed amount of the drug from the polymeric micelles and curcumin free drug were evaluated at 37°C in three-different pH media (Simulated gastric fluid (SGF) pH 1.2, simulated intestinal fluid (SIF) pH 6.8 and simulated colonic fluid (SCF) pH7.4) approach to mimic the GI tract, as shown in Fig. 3. The time interval for three different stages was at 1-2 h in SGF, then 3-5 h in SIF and 6-8 h in SCF. In SGF medium, the released rate of curcumin from pH sensitive curcumin-loaded OSCS micelles was about 20% of the amount of curcumin released after 2 h. This may be due to poor solubility of the drug. Afterward, the amount of curcumin was released

increase in SIF (approximately 50-55%; 5 h) and SCF (approximately 60-70%; 8 h) because of the ionization of pendant carboxyl groups in the succinic acid moiety of the self-assembled micelles. The accumulative release of curcumin from curcumin-loaded OSCS micelles in SCF was significant higher than the cumulative release of curcumin from free drug (approximately 20%). The results indicated that pH sensitive OSCS polymeric micelles may be a prospective candidate as a colon delivery carrier for the efficient administration of curcumin drug.

Discussions and Conclusion

The OSCS was formed micelles via self-assembly in aqueous media and successfully loaded curcumin in the hydrophobic inner core. The curcumin-loaded OSCS micelles by dialysis showed the high loading efficiency and curcumin release from the micelles could be adjusted by changing the pH values that follow as GI tract. Therefore, the OSCS polymeric micelle presents interest to develop desirable pH-sensitive carriers for colon-targeted oral drug delivery.

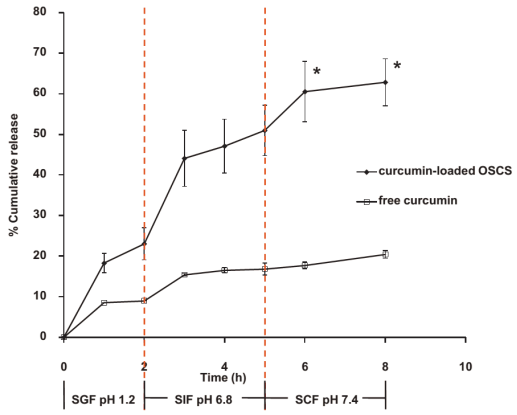

Figure 3 cumulative drug releases from curcumin-loaded OSCS polymeric micelles and free curcumin. Data are plotted as the average ± SD of three measurements.
* Statistically significant ($p < 0.05$)

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