DrillBit

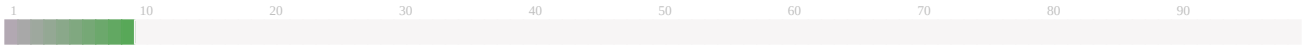
The Report is Generated by DrillBit Plagiarism Detection Software

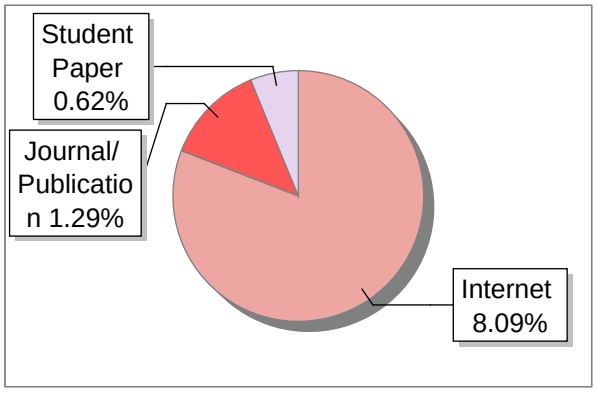
Submission Information

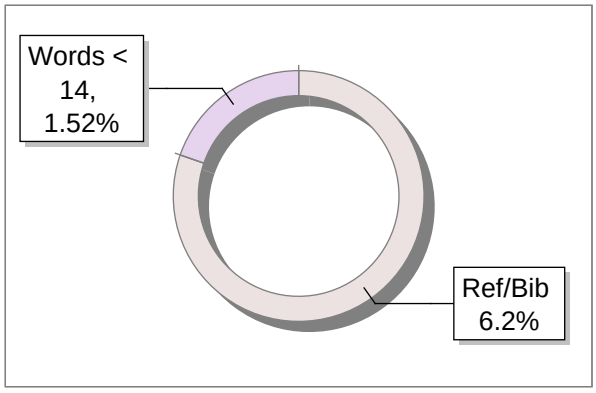
Author Name	Nuri Ari Efiana
Title	Improved mucoadhesive properties of self-nanoemulsifying drug delivery systems (SNEDDS) by introducing acyl chitosan
Paper/Submission ID	1524633
Submitted by	zulfa.erlin@staff.uad.ac.id
Submission Date	2024-03-13 07:49:48
Total Pages	7
Document type	Article

Result Information

Similarity **10 %**







Exclude Information

Quotes	Excluded
References/Bibliography	Excluded
Sources: Less than 14 Words %	Not Excluded
Excluded Source	**86 %**
Excluded Phrases	Not Excluded

Database Selection

Language	English
Student Papers	Yes
Journals & publishers	Yes
Internet or Web	Yes
Institution Repository	Yes

A Unique QR Code use to View/Download/Share Pdf File

DrillBit

DrillBit Similarity Report

10	10	A
SIMILARITY %	MATCHED SOURCES	GRADE

A-Satisfactory (0-10%)
B-Upgrade (11-40%)
C-Poor (41-60%)
D-Unacceptable (61-100%)

LOCATION	MATCHED DOMAIN	%	SOURCE TYPE
2	www.pharmaexcipients.com	<1	Publication
3	moam.info	1	Internet Data
4	REPOSITORY - Submitted to RKDF University on 2024-03-05 14-40	1	Student Paper
5	Mucus permeating self-emulsifying drug delivery systems (SEDDS) Abou, by Efiana, Nuri Ari P- 2017	<1	Publication
6	Alginate-caseinate based pH-responsive nano-coacervates to combat resistant bact by Niaz-2019	<1	Publication
7	moam.info	<1	Internet Data
8	biomedcentral.com	<1	Internet Data
9	Development of calcium pectinate-tamarind seed polysaccharide mucoadh, by Nayak, Amit Kumar - 2014	<1	Publication
10	Enhancement of the rectal absorption of sodium ampicillin by N-acylami by Whe-1987	<1	Publication
11	pubmed.ncbi.nlm.nih.gov	7	Internet Data

EXCLUDED SOURCES

1	eprints.uad.ac.id	86	Publication

International Journal of Pharmaceutics 519 (2017) 206–212

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Research paper

Improved mucoadhesive properties of self-nanoemulsifying drug delivery systems (SNEDDS) by introducing acyl chitosan

Nuri Ari Efiana[a,b], Arshad Mahmood[a], Hung Thanh Lam[a,c], Ožbej Zupančič[a], Gintare Leonaviciute[a], Andreas Bernkop-Schnürch[a,*]

[a] Department of Pharmaceutical Technology, Institute of Pharmacy, Leopold-Franzens- University of Innsbruck, Innrain 80/82, A-6020 Innsbruck, Austria
[b] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Ahmad Dahlan, Jl. Prof. Dr. Soepomo, S.H., Janturan, Warungboto, Umbulharjo, Yogyakarta, Indonesia
[c] Department of Pharmaceutical Technology, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam

ARTICLE INFO

Article history:
Received 26 October 2016
Received in revised form 5 January 2017
Accepted 6 January 2017
Available online 8 January 2017

Keywords:
Acyl chitosan
Fatty acid chloride
Mucoadhesion
SNEDDS

ABSTRACT

This study was aimed to improve the mucoadhesive properties of SNEDDS by the incorporation of acyl chitosan including octanoyl chitosan (OC), lauroyl chitosan (LC) and palmitoyl chitosan (PC). SNEDDS and acyl chitosan SNEDDS were characterized regarding droplet size and zeta potential. Their mucoadhesivity on porcine intestinal mucosa was evaluated by falling liquid film technique using Sudan Red G as marker. Degree of substitution of chitosan was determined to be 52.8%, 64.8 and 48.5% for OC, LC and PC, respectively. SNEDDS and acyl chitosan SNEDDS displayed a droplet size less than 50 nm and 80–300 nm as well as a zeta potential of −0.2 to −1.6 and 0.05 to 0.99 mV, respectively. Introducing 2% acyl chitosan into SNEDDS increased the residence time of SNEDDS on intestinal mucosa 2-fold. It is concluded that due to the incorporation of acyl chitosan into SNEDDS, their mucoadhesive properties can be increased.

1. Introduction

There is a great interest in developing mucoadhesive drug delivery systems due to the capability to increase drug accumulation on the mucosa by extending the residence time at the target site (Bernkop-Schnürch, 2013; Smart, 2005). Gastrointestinal transit time is one of the factors that impacts bioavailability of the drug (Sakloetsakun et al., 2010; Woodley, 2001). Chitosan has mucoadhesive properties due to its free amino groups causing a positive charge that can interact with negative charges of mucus (Bernkop-Schnürch and Dünnhaupt, 2012). Chemical modification of chitosan such as N-acylation can improve its mucoadhesivity (Shelma and Sharma, 2010). In this case, hydrophobic interactions play an important role in mucoadhesion (Sogias et al., 2008). In addition to electrostatic bonding and hydrophobic interactions, it has been shown that mucoadhesion can also occur through physical interlocking of polymer with mucin (Takeuchi et al., 2001).

SNEDDS have been declared as a potential drug carrier owing to their ability to preserve the drugs from the gastrointestinal environment (Leonaviciute and Bernkop-Schnürch, 2015) and diminish food effects (Sachan et al., 2010). Furthermore, nanoemulsion droplets generated spontaneously after SNEDDS dispersion in gastrointestinal medium have a high capacity to load poor water soluble drugs due to their lipophilic nature (Khan et al., 2012). As a result SNEDDS can be used to increase the bioavailability of lipophilic drugs (Chime et al., 2014). However, SNEDDS droplets displayed low mucoadhesivity (Leonaviciute et al., 2016). It is likely that the mainly negatively charged droplets of SNEDDS are repulsed from the also negatively charged mucus gel layer (Grießinger et al., 2015).

The aim of this study was therefore to improve the mucoadhesive properties of SNEDDS by the incorporation of acyl chitosan exhibiting a positive charge into SNEDDS. At present study, chitosan was modified using three kinds of fatty acid chlorides including palmitoyl chloride, lauroyl chloride and octanoyl chloride. It is expected that acyl groups of acyl chitosan are incorporated in the lipophilic core of SNEDDS droplets assembling on the surface of SNEDDS and consequently interacting with the mucus. Increasing the mucoadhesivity of SNEDDS is believed to prolong the contact time of the drug carrier with the mucosa.

* Corresponding author at: Center for Chemistry and Biomedicine, Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria.
E-mail addresses: andreas.bernkop@uibk.ac.at, a.bernkop@thiomatrix.com (A. Bernkop-Schnürch).

http://dx.doi.org/10.1016/j.ijpharm.2017.01.012
0378-5173/

2. Materials and methods

2.1. Materials

Capryol 90 and Labrafil M 1944 CS from Gattefosse (French), Imwitor 742 from IOI Oleo GmbH (Hamburg, Germany), Caprol 3GO and Captex 300 from ABITEC Corporation (Columbus, OH 43215 USA) were used as oil. Polyethylenglycol 400 (PEG 400) from Merck KGaA (Germany) and propylenglycol from Sigma Aldrich (Vienna, Austria) were used as cosolvent. Tween 20 and Kollipor EL from Sigma Aldrich (Vienna, Austria) were used as surfactant. Palmitoyl chloride, lauroyl chloride and octanoyl chloride from Acros Organics (New Jersey, USA) were used as acylating agent. Chitosan (from shrimp shells, low-viscosity (molecular weight 50.000–190.000 Da), degree of deacetylation 75-85%), triethylamine and Sudan Red G were obtained from Sigma Aldrich (Vienna, Austria). Porcine intestinal mucosa was taken freshly from slaughter house, Innsbruck, Austria. The intestinal mucosa was cleaned from debris and cut into small pieces (10 cm) and immediately used for mucoadhesion studies.

2.2. Methods

2.2.1. Synthesis of acyl chitosan

Low viscosity chitosan dispersion at concentration of 22 mg/mL was prepared using triethylamine-acetone (2:1) by stirring for 24 h at room temperature. Each fatty acid chloride including palmitoyl chloride, lauroyl chloride and octanoyl chloride was dissolved in acetone at concentration of 167 mg/mL. 30 mL of each fatty acid chloride solution was added dropwise into 90 mL of chitosan dispersion and thereafter stirred for two hours. Solid acyl chitosan resulting from such reaction was separated by filtration and afterwards washed using a 5% solution of sodium bicarbonate. Acetone traces in the precipitate were evaporated in the fume hood for two days. Thereafter, the remaining water in the precipitate was removed under oven at 30 °C overnight (Ma et al., 2011).

2.2.2. Characterization of acyl chitosan with FTIR and ninhydrin assay

FTIR (Perkin Elmer Spectrum 100 FTIR Spectrometers) was used to identify the alteration of functional groups of chitosan after the reaction with fatty acyl chlorides. Unscreambler x was used as software to record IR spectrum of products, including palmitoyl chitosan, lauroyl chitosan and octanoyl chitosan.

Ninhydrin assay was performed as follows. Acyl chitosan was dissolved in lactic acid (1 mg/mL) as stock solution. Various volumes of stock solution were diluted using deionized water and 4 M acetic buffer pH 5.5 to obtain sample solution at the concentration of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL. Concentration of acetic buffer in sample solution was set up of 2 M. Each sample solution was mixed with ninhydrin solution at twice volume prior to incubating at 90 °C for 15 min. Subsequently, the absorbance of each sample solution was measured using microreader Tecan Infinite M 200 at wavelength of 570 nm. All stages of work above were also conducted with D glucosamine as standard. Fraction of free amines from modified chitosan was calculated by dividing the slope of each sample from linear regression calculation with slope of standard D glucosamine by considering the molecular weight of sample and standard, the number of free amino groups in both and degree of deacetylation of chitosan (Curotto and Aros, 1993; Kador and Subramanian, 2011). Degree of substitution (DS) of chitosan was calculated using Eq. (1).

$$DS = (1 - \text{free amino group}) \times 100\% \quad (1)$$

2.2.3. Optimization of SNEDDS composition

Optimization of SNEDDS composition was conducted with different types and concentrations of oil as solvent (Capryol 90, Imwitor 742, Captex 300, Labrafil M 1944 CS and Caprol 3GO), surfactant (tween 20 and Kollipor EL) and co-solvent (PEG 400 and propylenglycol) as shown in Fig. 1. Each SNEDDS composition was prepared by mixing oil, co-solvent and surfactant using magnetic stirrer (Hotplate Stirrer Stuart) at the rate of 500 rpm at room temperature for 30 min. This mixture showed clear solution. SNEDDS were dispersed in 0.1 M phosphate buffered saline (PBS) pH 6.8 with a ratio of 1 in 100 by stirring at 50 rpm prior to be evaluated their droplet size and polydispersity index (PI) of nanoemulsion (Nicomp™ 380 ZLS Santa Barbara California USA). The smaller droplet size and its compatibility with modified chitosan were assigned as parameters of selection. The selected SNEDDS composition was further characterized regarding zeta potential.

2.2.4. Preparation and characterization of polymer SNEDDS

Acyl chitosan was dissolved in lactic acid. The initial concentration was varied to acquire final concentration of 1% and 2% in polymer SNEDDS composition. Each polymer SNEDDS composition was consisted of 25% acyl chitosan solution. Propylenglycol as cosolvent was added at the final concentration of 20% to increase

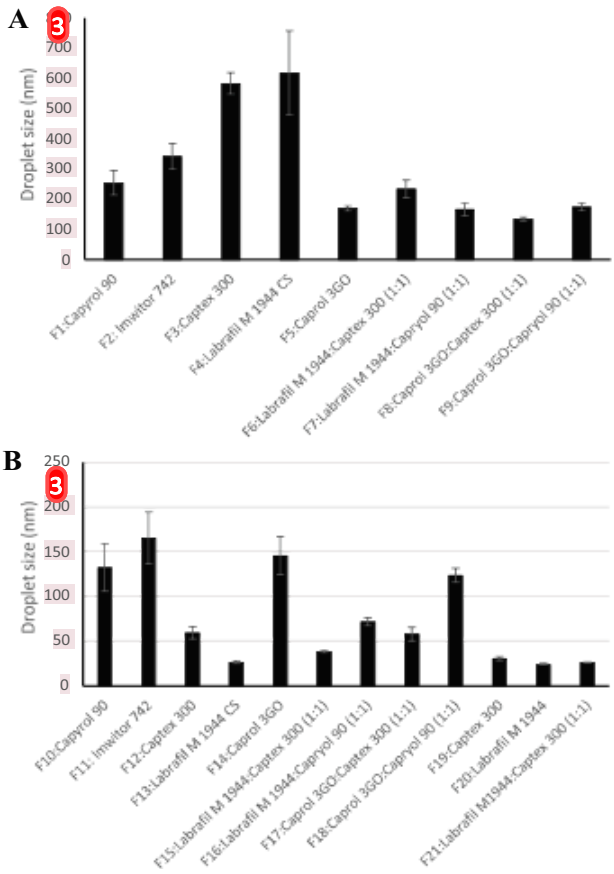


Fig. 1. Optimization of SNEDDS. Effect of indicated solvents on droplet size of SNEDDS containing Tween 20 (A) and Kolliphor EL (B) after dispersing in PBS pH 6.8 (1:100). F1 to F18 the concentration of solvent and surfactant are 37% and 45%, respectively. PEG-propylene glycol (1:1) are used as co-solvent. For F19 to F21 (B) the concentration of solvent and surfactant are 20% and 50%, respectively. Propyleneglycol is used as co-solvent. Indicated values are means ± SD (n = 3).

the compatibility of SNEDDS and polymer. As control, chitosan solution was used instead of acyl chitosan. Each mixture was stirred using magnetic bar at 500 rpm at room temperature for 30 min. Subsequently, it was sonicated for 3 h. In this way, clear solutions of chitosan SNEDDS (SC) serving as control, octanoyl chitosan SNEDDS (SOC), lauroyl chitosan SNEDDS (SLC) and palmitoyl chitosan SNEDDS (SPC) were obtained. The droplet size and PI of polymer SNEDDS were measured after dispersing the polymer SNEDDS in 0.1 M PBS pH 6.8 immediately and at 4 h. The polymer SNEDDS was also characterized regarding zeta potential.

Morphology of SNEDDS and acyl chitosan SNEDDS was characterized using transmission electron microscopy (TEM), microscope: Zeiss Libra 120 energy Filter Transmission Electron Microscope (Zeiss, Germany), camera: TRS 2kx2k high speed digital camera (Tröndle, Germany). Preparation of sample was done by transferring 5 μL dispersion of acyl chitosan SNEDDS as well as SNEDDS in demineralized water (1:100) to a 400 mesh Formvar-Carbon coated copper grid. Subsequently, it was dried and examined.

2.2.5. Mucoadhesion test

Mucoadhesive properties of SNEDDS and polymer SNEDDS were evaluated by falling liquid film technique (Gradauer et al., 2012). An inversed porcine intestinal mucosa with a size of 3×10 cm was mounted on a semicylindrical plastic tube with the mucosal side up. The experiment was performed in an incubator at a temperature of 37 °C. The intestinal mucosa was rinsed using 0.1 M PBS pH 6.8. SNEDDS, chitosan SNEDDS (as control) as well as acyl chitosan SNEDDS containing 2,5% of Sudan Red G were dispersed in 0.1 M PBS pH 6.8 at a ratio of 1:100. Then 1.0 mL of the dispersion was flowed drop wise onto the intestine. The overage of the dispersion was collected in a small tube and reapplied ten times. Afterwards, Sudan Red G on the mucosa was extracted using 10 mL ethanol 96% (v/v) with shaking incubator at a temperature of

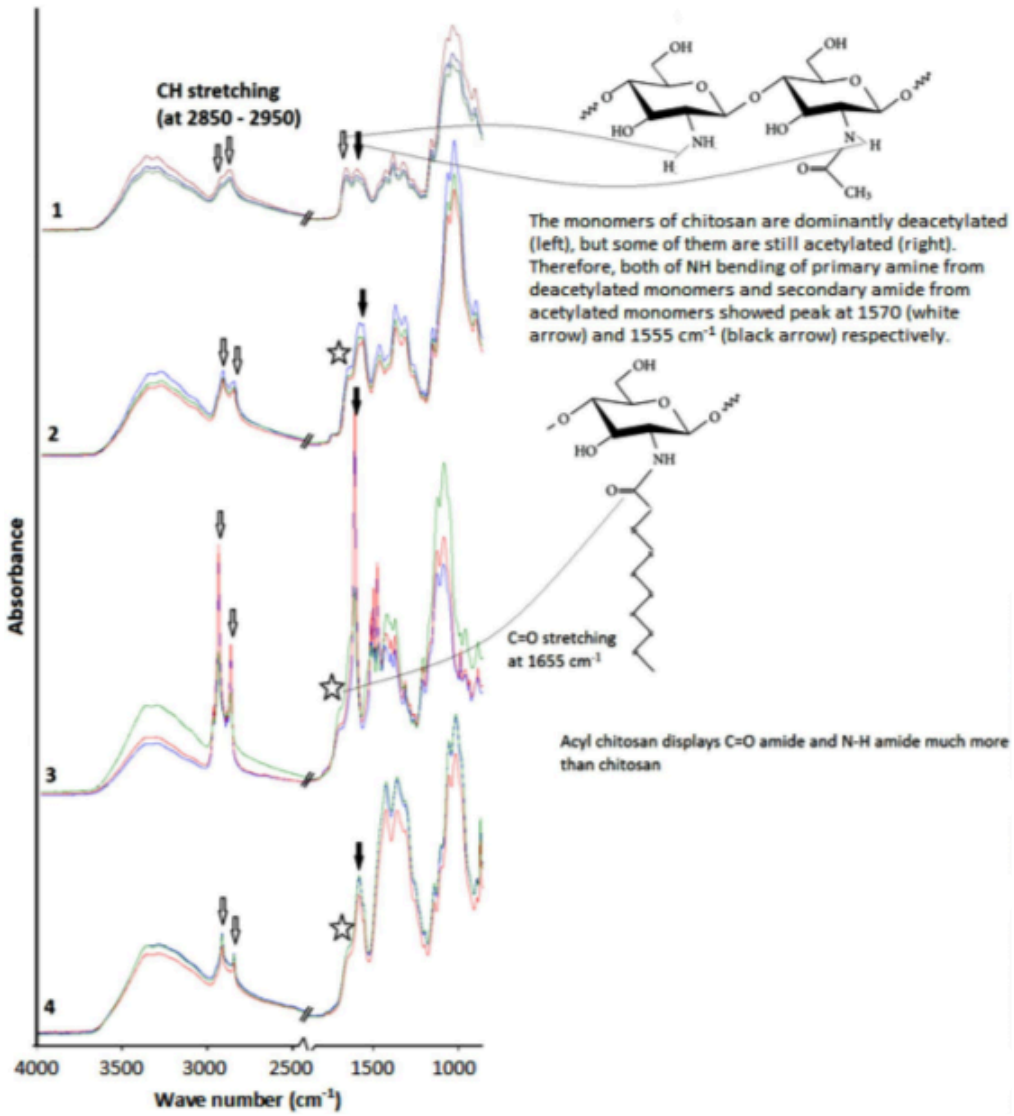


Fig. 2. FTIR spectrum of unmodified chitosan (1), lauroyl chitosan (2), octanoyl chitosan (3) and palmitoyl chitosan (4). In present study, chitosan has 85% degree of deacetylation. The functional groups represented by the peak are displayed bellow arrows or directed by curves connecting the peak to the functional group of the monomer of chitosan as well as acyl chitosan.

37 °C for 30 min, continued by centrifugation (Eppendorf, MiniSpin) at 10,000 rpm for 10 min prior to the absorbance measurement of Sudan Red G using microplate reader (Tecan infinite M200) at 500 nm of wavelength. As 100% control, the mixture of intestinal mucosa and 1 mL dispersion of corresponding sample was extracted using the same method. The mucoadhesivity was calculated by the ratio of absorbance after the experiment to the absorbance of associated 100% control.

2.2.6. Statistical data analysis

Independent sample *t*-test was used to analyze the effect of introducing chitosan and modified chitosan, the effect of chitosan acylation and the effect of difference concentration of polymer on physical characteristic as well as mucoadhesivity. Paired sample *t*-test was used to analyze the effect of time within dispersion of SNEDDS on droplet size and PI. Two-way Analysis of Variance (Two-way Anova) was used to analyze the effect of acyl chain length and polymer concentration on mucoadhesivity. All of statistical analysis was performed using SPSS 17 at 95% confidence level ($p < 0.05$).

3. Results and discussion

3.1. Characterization of acyl chitosan with FTIR and ninhydrin assay

In the present study, chitosan was modified by acylation using various fatty acid chlorides including palmitoyl chloride, lauroyl chloride and octanoyl chloride. Covalent bond was formed between the primary amino groups of chitosan and the carboxyl groups of fatty acid as identified by FTIR. The FTIR spectrum of chitosan and modified chitosan are shown in Fig. 2. An important signal of achievement in reaction process was the changing in C-H stretching. The acyl groups attached to chitosan contain CH_2 which is responsible for symmetric and asymmetric stretching (Mohan and Prabakaran, 1989) and displayed sharp duplet peak (white arrow) in the higher intensity, whereas the corresponding peak in chitosan is singlet as a result of CH groups.

Furthermore, ninhydrin assay was used to determine the percentage of hydrogen amino substituted by acyl from fatty acid chloride, referred to as degree of substitution. Degree of substitution could be denoted to peaks of CH stretching (wave number: 2850–2950 cm^{-1}) of fatty acid chain and NH bending of secondary amide groups (1555 cm^{-1}) (Fig. 2). As acylation of chitosan at free amino groups increases the number of amide and methylene groups, the intensity and sharpness of corresponding peaks is enhanced. According to Fig. 2, degree of substitution of lauroyl chitosan was the highest, followed by octanoyl chitosan and palmitoyl chitosan. In line with Fig. 2, Table 1 displays a similar ordered manner. Degree of substitution of lauroyl chitosan was 64.8%, exhibited higher intensity than octanoyl chitosan (52.8%) and palmitoyl chitosan (48.5%). High degree of substitution in present research gives a beneficial effect on improving the lipophilicity of polymer (Cho et al., 2012) triggering its capability to be integrated into SNEDDS droplets. Moreover, the ability of SNEDDS to attach on mucus of gastrointestinal membrane will increase.

Table 1
Degree of substitution of acyl chitosan having been modified by the fatty acyl chlorides: palmitoyl chloride, lauroyl chloride and octanoyl chloride.

No	Acyl chitosan	Degree of substitution (%)
1	palmitoyl chitosan	48.5
2	lauroyl chitosan	64.8
3	octanoyl chitosan	52.8

3.2. Optimization of SNEDDS composition

The optimization of SNEDDS composition aimed to select the appropriate oil (solvent), surfactant and cosolvent components. Fig. 1A shows that using tween 20 as surfactant, none of the preselected oil showed the high size of droplet SNEDDS (more than 100 nm), thus tween 20 was refused as a selected surfactant. Fig. 1B performs that using Kolliphor EL instead of tween 20 resulted Captex 300 and Labrafil M 1944 as a single or combination to be selected as oil component since they produced smaller droplet size (less than 100 nm). Decreasing oil concentration from 37% to 20% and eliminating PEG 400 from cosolvent component generated simple composition (F19, F20 and F21) with 50% Kolliphor EL as surfactant and 30% propylene glycol as cosolvent. Furthermore, this composition produced smaller droplet size than F12, F13 and F15. Based on compatibility of acyl chitosan with SNEDDS, F19 was selected for further experiments.

3.3. Preparation and characterization of polymer SNEDDS

Chitosan and modified chitosan could be quantitatively incorporated into SNEDDS in concentrations up to 2%. Droplet size and PI generated from SNEDDS and polymer SNEDDS are exhibited in Figs. 3 and 4. SNEDDS displayed a droplet size less than 50 nm, whereas acyl chitosan SNEDDS exhibited a droplet size between 80 and 300 nm. Either SNEDDS or polymer SNEDDS displayed a PI less than 0.5. Chitosan SNEDDS showed the higher droplet size than acyl chitosan SNEDDS. The droplet size of acyl chitosan SNEDDS was higher than SNEDDS as a result of droplet figuring by polymer as verified by TEM micrograph (Fig. 5). Such figuring is shown schematically in Fig. 6. Acyl chitosan featured SNEDDS droplet by incorporating acyl chain into SNEDDS droplets. Unmodified chitosan does not have a lipophilic tail to be incorporated into the droplet. It is assumed that electrostatic

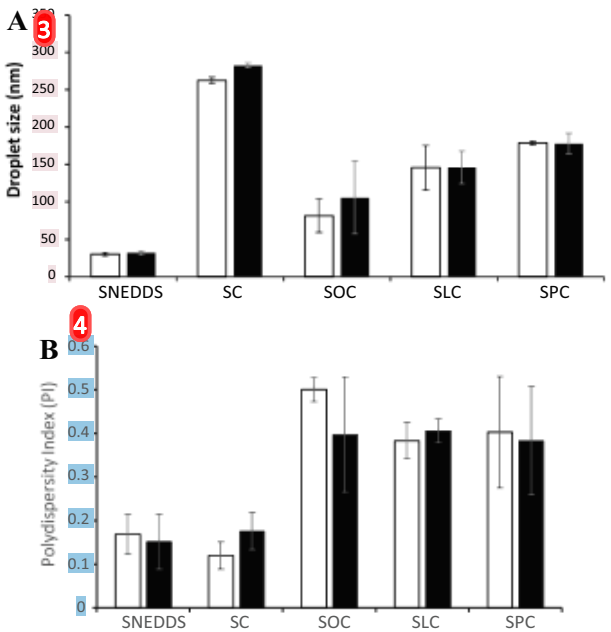


Fig. 3. Comparison of the droplet size (A) and PI (B) of SNEDDS and SNEDDS containing 1% indicated polymer after dispersing in PBS pH 6.8 at 0 (white bars) and 4 h (black bars). Indicated values are means ± SD of three experiments. SC (SNEDDS chitosan), SOC (SNEDDS octanoyl chitosan), SLC (SNEDDS lauroyl chitosan) and SPC (SNEDDS palmitoyl chitosan).

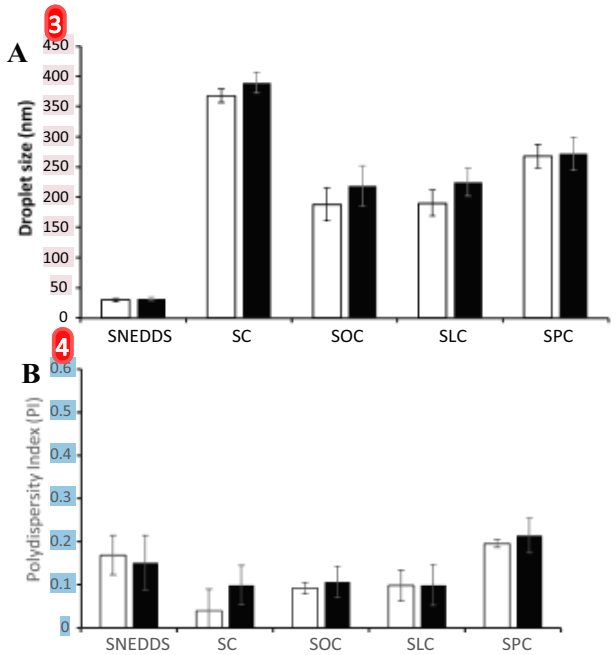


Fig. 4. Comparison of the droplet size (A) and PI (B) of SNEDDS and SNEDDS containing 2% indicated polymer after dispersing in PBS pH 6.8 at 0 (white bars) and 4 h (black bars). Indicated values are means ± SD of three experiments. SC (SNEDDS chitosan), SOC (SNEDDS octanoyl chitosan), SLC (SNEDDS lauroyl chitosan) and SPC (SNEDDS palmitoyl chitosan).

interactions between negatively charges of SNEDDS droplets with positively charges of chitosan are involved. This interaction is likely weaker than the integration of acyl tail of acyl chitosan into SNEDDS droplets.

By comparing the two polymer concentrations, it was shown that the augmentation of polymer concentration resulted in a significantly ($p < 0.05$) larger size of the nanoemulsion droplets. The droplet size of SNEDDS did not change significantly within 4 h of dispersion, whereas chitosan SNEDDS droplets in both concentrations increased significantly ($p < 0.05$). The droplet size of acyl chitosan SNEDDS at 1% concentration showed a constant droplet size within 4 h of dispersion (Fig. 3), whereas at 2% concentration the droplet size enhanced significantly ($p < 0.05$), except for palmitoyl chitosan SNEDDS (Fig. 4).

Monitoring the droplet size and PI of chitosan and acyl chitosan SNEDDS at 0 and 4 h of dispersion in 0.1 M PBS pH 6.8 was desirable to ensure that the droplets still remain in nanosize. Hence, the ability of droplets to adhere on the intestinal mucosa and permeate through the gastrointestinal membrane could increase. The stability of droplet size within 4 h means that oil in water emulsion in droplet nanosize could be reached during the absorption process in the gastrointestinal tract. Droplet size is a key factor governing the stability of emulsion (Sakloetsakun et al., 2010). In addition, PI plays a significant role associated with SNEDDS droplets stability. High value of PI indicates broad size of distribution of droplets ("ISO 22412," 2008) leading to coagulation.

Introducing acyl chitosan into SNEDDS changed the zeta potential to positive. In contrast, unmodified chitosan SNEDDS exhibited almost no charge as shown in Fig. 7. Increasing polymer concentration from 1% to 2% did not change the zeta potential significantly. The increasing of droplet size by the time after dispersing associated with zeta potential and concentration of polymer. Droplet size of chitosan SNEDDS increased within 4 h of dispersion. Since such compositions displayed zeta potential closed to zero, the electro-repulsion between droplets were not adequate to hinder the aggregation. At 2% concentration of polymer, the possibility of collision increased, as a result the droplet size enhanced.

3.4. Mucoadhesive properties of SNEDDS and acyl chitosan SNEDDS

Percentage of Sudan Red G bound on the mucosa was an indication for mucoadhesive properties of SNEDDS and modified SNEDDS. In the present study, introducing 1% of polymer increased SNEDDS mucoadhesivity by 1.2, 1.5, 1.6 and 1.8-fold for SC, SOC, SLC and SPC, respectively. Raising the polymer concentration to 2% resulted in enhancing SNEDDS mucoadhesivity by 1.2, 1.8, 2 and 2-fold for SC, SOC, SLC and SPC, respectively. Results are illustrated in Fig. 8.

The beneficial effect of chitosan modification on mucoadhesion is clearly shown by comparing the mucoadhesivity of SC as control with SOC, SLC and SPC at defined polymer concentration, which were increasing significantly ($p < 0.05$). According to Fig. 8, increasing the concentration in each polymer enhanced mucoadhesivity. Raising the carbon atom number of the acyl chain from 8 (octanoyl) to 12 (lauroyl) as well as 16 (palmitoyl) increased

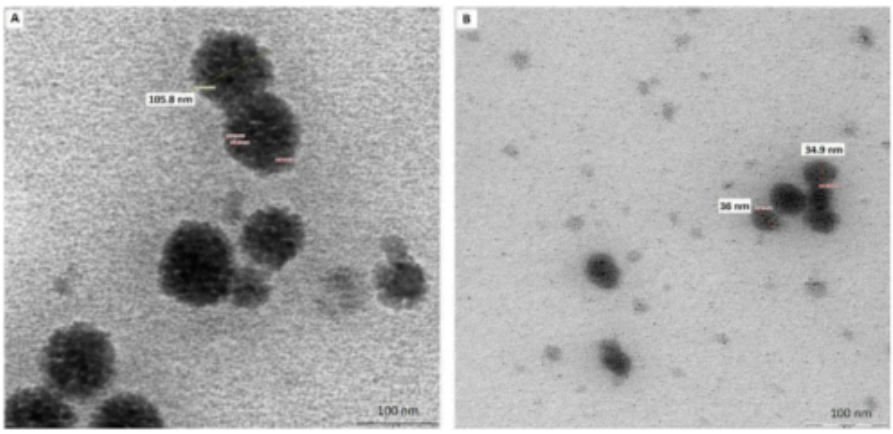


Fig. 5. Transmission electron microscopy (TEM) of acyl chitosan SNEDDS (A) and SNEDDS (B).

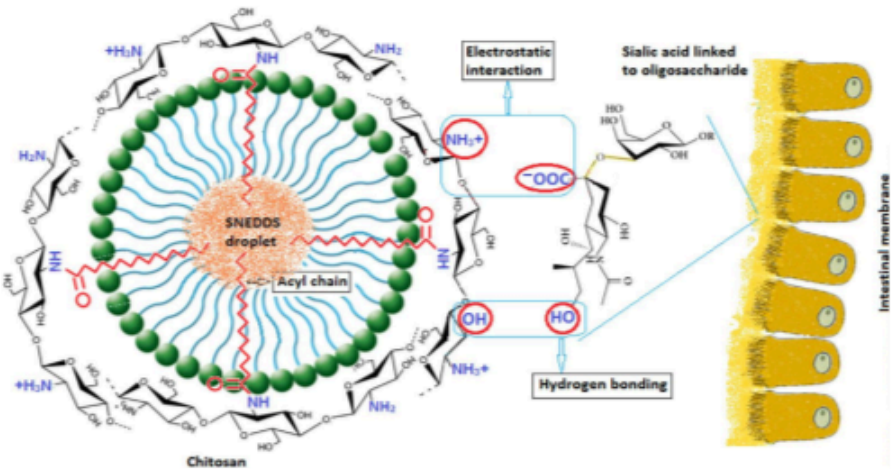


Fig. 6. Illustration of interactions of acyl chitosan SNEDDS with intestinal mucosa.

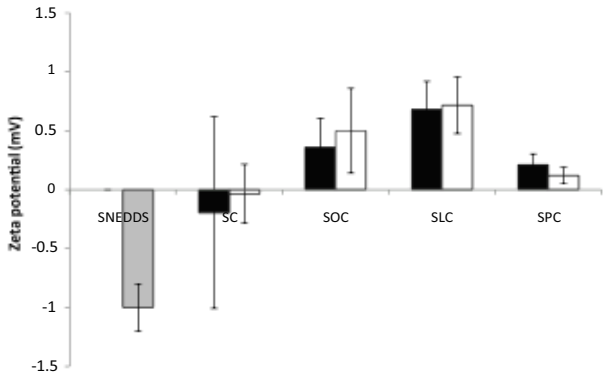


Fig. 7. Zeta potential of SNEDDS and modified SNEDDS containing 1% (black bars) and 2% (white bars) indicated polymer after dispersing in PBS pH 6.8. Indicated values are means ± SD of three experiments. SC (SNEDDS chitosan), SOC (SNEDDS octanoyl chitosan), SLC (SNEDDS lauroyl chitosan) and SPC (SNEDDS palmitoyl chitosan).

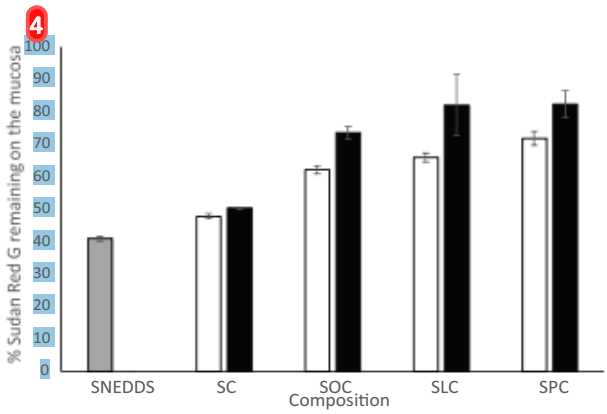


Fig. 8. Percentage of Sudan Red G remaining on the intestinal mucosa in case of SNEDDS and SNEDDS containing the indicated polymer at a concentration of 1% (white bars) and 2% (black bars) after having been dispersed in PBS pH 6.8. Data are means ± SD of three experiments. SC (SNEDDS chitosan), SOC (SNEDDS octanoyl chitosan), SLC (SNEDDS lauroyl chitosan) and SPC (SNEDDS palmitoyl chitosan).

mucoadhesivity significantly ($p < 0.05$). This observation might be explained on the one hand by an improved integration of chitosan into SNEDDS and on the other hand by improved hydrophobic interactions with mucins (Yang et al., 2012).

Generally, there are three possibilities for interactions between chitosan and mucins including electrostatic interaction, hydrogen bonding and hydrophobic effects (Sogias et al., 2008). In present research, acyl chains of modified chitosan will likely interact with lipophilic chains of surfactants in the core of nanoemulsion droplets, whereas the chitosan backbone will assemble on the polar surface of droplets interacting with the mucus via ionic interactions and hydrogen bonding (Fig. 6).

Positively charges of acyl chitosan SNEDDS droplets showed a greater mucoadhesivity in comparison to the negatively charged SNEDDS droplets and chitosan SNEDDS droplets. The likely reason for this observation are electrostatic interactions between positive charges of chitosan from acyl chitosan SNEDDS and negative charges of sialic acid from mucus (Millotti et al., 2010). By comparing the zeta potential in difference concentrations of acyl chitosan (Fig. 7) in relation to the mucoadhesive properties (Fig. 8), it is noticed that the electrostatic interaction was not the only one mechanism since a 2% concentration exhibits higher mucoadhesivity than 1%. It is predicted that hydrogen bonding take a significant place in mucoadhesion (Sogias et al., 2008)

Introducing chitosan and acyl chitosan into SNEDDS enhanced droplet size of nanoemulsion. Even if a smaller droplet size provides a larger effective surface area being available for interactions with the mucus, the ability to adhere on the mucosa is increased (Banik et al., 2012). In this case, the influence of droplet size was less than the zeta potential and the hydrogen bonding regarding the capability to attach to intestinal mucosa. Increasing the mucoadhesivity of SNEDDS is believed to expand the contact time of the drug carrier with the mucosa (Bernkop-Schnürch, 2005).

4. Conclusion

Acyl chitosan SNEDDS was developed in present study. SNEDDS incorporated with acyl chitosan displayed significantly ($P < 0.05$) higher mucoadhesivity in comparison with SNEDDS as well as chitosan SNEDDS. These findings suggest that acyl chitosan SNEDDS can be used as mucoadhesive carrier systems.

Acknowledgment

The authors would like to thank the ASEAN-European Academic University Network (ASEA Uninet), Austrian Federal Ministry of Science, Research and Economy (BMWFW) and the Ministry of Research, Technology and Higher Education Republic of Indonesia that have supported this project.

References

Banik, N., Hussain, A., Ramteke, A., Sharma, H.K., Maji, T.K., 2012. Preparation and evaluation of the effect of particle size on the properties of chitosan-montmorillonite nanoparticles loaded with isoniazid. RSC Adv. 2, 10519–10528. doi:http://dx.doi.org/10.1039/C2RA20702H.

Bernkop-Schnürch, A., Dünnhaupt, S., 2012. Chitosan-based drug delivery systems. Eur. J. Pharm. Biopharm. 81, 463–469. doi:http://dx.doi.org/10.1016/j.ejpb.2012.04.007.

Bernkop-Schnürch, A., 2005. Mucoadhesive systems in oral drug delivery. Drug Discov. Today Technol. 2, 83–87. doi:http://dx.doi.org/10.1016/j.ddtec.2005.05.001.

Bernkop-Schnürch, A., 2013. Nanocarrier systems for oral drug delivery: do we really need them? Eur. J. Pharm. Sci. 49, 272–277. doi:http://dx.doi.org/10.1016/j.ejps.2013.03.008.

Chime, S.A., Kenechukwu, F.C., Attama, A.A., 2014. Nanoemulsions — advances in formulation, characterization and alications in drug delivery. In: Sezer, A.D. (Ed.), Application of Nanotechnology in Drug Delivery. InTech, pp. 77–126.

Cho, Y., Kimb, J.T., Park, H.J., 2012. Size-controlled self-aggregated N-acyl chitosan nanoparticles as a vitamin C carrier. Carbohydr. Polym. 88, 1087–1092.

Curotto, E., Aros, F., 1993. Quantitative determination of chitosan and the percentage of free amino groups. Anal. Biochem. 211, 240–241. doi:http://dx.doi.org/10.1006/abio.1993.1263.

Gradauer, K., Vonach, C., Leitinger, G., Kolb, D., Fröhlich, E., Roblegg, E., Bernkop-Schnürch, A., Prassl, R., 2012. Chemical coupling of thiolated chitosan to preformed liposomes improves mucoadhesive properties. Int. J. Nanomed. 7, 2523–2534. doi:http://dx.doi.org/10.2147/IJN.S29980.

Grießinger, J., Dünnhaupt, S., Cattoz, B., Griffiths, P., Oh, S., Gómez I, S.B., Wilcox, M., Pearson, J., Gumbleton, M., Abdulkarim, Pereira de Sousa I, M., Bernkop-Schnürch, A., 2015. Methods to determine the interactions of micro- and nanoparticles with mucus. Eur. J. Pharm. Biopharm. 96, 464–476. doi:http://dx.doi.org/10.1016/j.ejpb.2015.01.005.

ISO 22412, 2008. Particle Size Analysis ? Dynamic Light Scattering (DLS) [WWW Document]. ISO URL http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=40942 (accessed 10.23.16).

Kador, K.E., Subramanian, A., 2011. Selective modification of chitosan to enable the formation of chitosan-DNA condensates by electron donator stabilization. Int. J. Carbohydr. Chem. 2011 (e146419), 1–11. doi:http://dx.doi.org/10.1155/2011/146419.

Khan, A.W., Kotta, S., Ansari, S.H., Sharma, R.K., Ali, J., 2012. Potentials and challenges in self-nanoemulsifying drug delivery systems. Expert Opin. Drug Deliv. 9, 1305–1317. doi:http://dx.doi.org/10.1517/17425247.2012.719870.

Leonaviciute, G., Bernkop-Schnürch, A., 2015. Self-emulsifying drug delivery systems in oral (poly)peptide drug delivery. Expert Opin. Drug Deliv. 12, 1703–1716. doi:http://dx.doi.org/10.1517/17425247.2015.1068287.

Leonaviciute, G., Trivic Adamovic, N., Thanh Lam, H., Rohrer, J., Partenhauser, A., Bernkop-Schnürch, A., 2016. Self-emulsifying drug delivery systems (SEDDS): proof-of-concept how to make them mucoadhesive. Eur. J. Pharm. Biopharm. Off. J. Arbeitsgemeinschaft Pharm. Verfahrenstechnik EV 112, 51–57. doi:http://dx.doi.org/10.1016/j.ejpb.2016.11.019.

Ma, G., Liu, Y., Kennedy, J.F., Nie, J., 2011. Synthesize and properties of photosensitive organic solvent soluble acylated chitosan derivatives (2). Carbohydr. Polym. 84, 681–685. doi:http://dx.doi.org/10.1016/j.carbpol.2010.11.013.

Millotti, G., Samberger, C., Fröhlich, E., Sakloetsakun, D., Bernkop-Schnürch, A., 2010. Chitosan-4-mercaptobenzoic acid: synthesis and characterization of a novel thiolated chitosan. J. Mater. Chem. 20, 2432–2440. doi:http://dx.doi.org/10.1039/B916528B.

Mohan, S., Prabakaran, A., 1989. Infrared and laser raman spectra of polythylene and its normal coordinat analysis. Asian J. Chem. 1, 196–205.

Sachan, R., Khatri, K., Kasture, S., 2010. Self-emulsifying drug delivery system a novel approach for enhancement of bioavalibility. Int. J. Pharm. Technol. Res. 2, 1738–1745.

Sakloetsakun, D., Perera, G., Hombach, J., Millotti, G., Bernkop-Schnürch, A., 2010. The impact of vehicles on the mucoadhesive properties of orally administrated nanoparticles: a case study with chitosan-4-thiobutylamidine conjugate. AAPS PharmSciTech 11, 1185–1192. doi:http://dx.doi.org/10.1208/s12249-010-9479-8.

Shelma, R., Sharma, C.P., 2010. Acyl modified chitosan derivatives for oral delivery of insulin and curcumin. J. Mater. Sci. Mater. Med. 21, 2133–2140. doi:http://dx.doi.org/10.1007/s10856-010-4073-x.

Smart, J.D., 2005. The basics and underlying mechanisms of mucoadhesion. Adv. Drug Deliv. Rev. 57, 1556–1568. doi:http://dx.doi.org/10.1016/j.addr.2005.07.001.

Sogias, I.A., Williams, A.C., Khutoryanskiy, V.V., 2008. Why is chitosan mucoadhesive? Biomacromolecules 9, 1837–1842. doi:http://dx.doi.org/10.1021/bm800276d.

Takeuchi, H., Yamamoto, H., Kawashima, Y., 2001. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv. Drug Deliv. Rev. 47, 39–54.

Woodley, J., 2001. Bioadhesion: new possibilities for drug administration? Clin. Pharmacokinet. 40, 77–84. doi:http://dx.doi.org/10.2165/00003088-200140020-00001.

Yang, X., Forier, K., Steukers, L., Van Vlierberghe, S., Dubruel, P., Braeckmans, K., Glorieux, S., Nauwynck, H.J., 2012. Immobilization of pseudorabies virus in porcine tracheal respiratory mucus revealed by single particle tracking. PLoS One 7 (e51054), 1–9. doi:http://dx.doi.org/10.1371/journal.pone.0051054.