Vol. 19, No. 1 (2020) 49-58
Revista Mexicana de Ingeniería Química

DEVELOPMENT OF A SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) FROM AN INSULIN COMPLEX WITH MODIFIED PHOSPHATIDYLCHOLINE AND MUCOADHESIVE POLYSACCHARIDE COATING AS A POTENTIAL NONE-INVASIVE TREATMENT FOR DIABETES

DESARROLLO DE UN SISTEMA DE LIBERACIÓN DE FÁRMACOS AUTO-NANOEMULSIFICANTES (SNEDDS) PARA UN COMPLEJO DE INSULINA CON FOSFATIDILCOLINA MODIFICADA Y POLISACÁRIDOS MUCOADHESIVOS COMO POTENCIAL MEDIO TRATAMIENTO NO INVASIVO CONTRA DIABETES MELLITUS

M.O.F. Muñoz-Correa[1], D.A. Bravo-Alfaro[1], H.S. García[1], R. García-Varela[2]*

[1] *UNIDA, Tecnológico Nacional de México/Instituto Tecnológico de Veracruz. M.A. de Quevedo # 2779, Col. Formando Hogar, Veracruz, Ver. 91897, México*

[2] *Department of Medicine, Hematology/Oncology, UW Carbone Cancer Center, University of Wisconsin at Madison, School of Medicine and Public Health, Madison, WI, USA.*

Received: January 9, 2019; Accepted: March 8, 2019

Abstract

The present work depicts the development of a self-nanoemulsifiable drug delivery system (SNEDDS) for the administration of insulin, in which an insulin complex was formed with enzymatically modified soy phosphatidylcholine, by means of the solvent elimination technique. In addition, sodium alginate and guar gum were added to this system to provide mucoadherence capacities and to increase insulin protection against gastric conditions. SNEDDS were developed from mixtures of surfactant, co-surfactant and oil phase: Cremophor EL, Labrafil M1944CS and Lauroglycol FCC, respectively: obtaining particle sizes in the range of 27.36-50.53 nm and a monomodal distribution. Mucoadhesives were added by orbital agitation of 100 rpm and particle sizes between 53.1-83.2 nm were obtained. Finally, nanoemulsions were submitted to an *in vitro* gastric simulation system, were insulin bioavailability was increased to 46.3% in systems that included mucoadhesive coating. These results show that the developed systems can possibly be used for the administration of insulin by oral route as a potential non-invasive treatment for diabetes.

Keywords: diabetes, insulin, modified phosphatidylcholine, mucoadhesive polysaccharide, nanoemulsions.

Resumen

En el presente trabajo se muestra el desarrollo y estudio de un sistema de entrega de fármacos auto-nanoemulsificante (SNEDDS) para la administración de insulina, en el que se formó un complejo de insulina con fosfatidilcolina de soya modificada enzimáticamente, mediante la técnica de eliminación de solventes. Adicionalmente, dicho sistema fue adicionado con alginato de sodio y goma guar como mucoadhesivos y para aumentar la protección de la insulina contra las condiciones gástricas. Los SNEDDS se desarrollaron a partir de mezclas de surfactante, co-surfactante y fase oleosa: Cremophor EL, Labrafil M1944CS y Lauroglicol FCC respectivamente, obteniendo tamaños de partícula de 27.36 - 50.53 nm y comportamientos monomodales. Los mucoadhesivos se agregaron por medio de agitación orbital a 100 rpm obteniendo tamaños de partícula entre 53.1-83.2 nm. Por último, al someter las nanoemulsiones a un sistema de simulación gástrica *in vitro*, se logró aumentar la bioaccesibilidad de la insulina hasta en 46.3% en los sistemas que incluían recubrimiento mucoadhesivo. Estos resultados muestran que los sistemas desarrollados pueden ser utilizados en la administración de insulina por vía oral como posible tratamiento no invasivo contra diabetes mellitus.

Palabras clave: diabetes, insulina, fosfatidilcolina modificada, polisacáridos mucoadhesivos, nanoemulsiones.

* *Corresponding author. E-mail*: garciavarela@wisc.edu
https://doi.org/10.24275/rmiq/Bio454
issn-e: 2395-8472

1 Introduction

Diabetes mellitus is a chronic and degenerative disease; in 2017 approximately 425 million adults (ages 20-79) were living with diabetes and caused 4 million deceases (International Diabetes Federation, 2017), making one of the leading causes of death. The main treatment consists on the application of the exogenous hormone insulin, of which the most common forms of administration are subcutaneous and intravenous injections (Libman, 2009). Endogenous insulin is produced and released into the bloodstream by the pancreas, in response to increased blood glucose levels; once in the bloodstream it is transported to the target cells, where insulin binds to specific membrane receptors activating a cascade of signals that allow glucose to enter the cell. Subcutaneously administered insulin passes through capillaries into the bloodstream where it is transported in the same manner as endogenous insulin (Atkinson *et al*., 2014; Kanzarkar *et al*., 2015). However, these are methods that can generate complications due to the long periods in which the injections are applied. Among the most common aggravations are lipodystrophy that causes a decrease in the absorption of the compound, possible infections produced by injection punctures, and the absorption of the hormone will depend on the site of application of the injection (Heinemann, 2002; Nawaz *et al*., 2017). An alternative to this invasive treatment is a non-invasive oral administration strategy (Li *et al*., 2014; Özer *et al*., 2017). It is important to note that given the nature of the digestive tract, this administration route suffers of an important problem of low bioavailability due to degradation, providing this strategy with major challenges (Al Rubeaan *et al*., 2016; Granillo-Guerrero *et al*., 2017; Olivares-Romero *et al*., 2018).

Currently self-nanoemulsifiable drug delivery systems SNEDDS have shown promising potential in the pharmaceutical industry for the administration of different drugs (Cavazos Garduño *et al*., 2014; Dwivedi *et al*., 2014). A nanoemulsion is a system composed of a continuous and a dispersed phase, in addition to a surfactant or emulsifier that helps to stabilize the system (Alizadeh-Sani *et al*., 2018).

The implementation of phospholipids enzymatically modified with medium chain fatty acids in nanoemulsions has shown a better stability than the use of native phospholipids, and also an increase in bioavailability of the encapsulated compound, most likely because these fatty acids cause an enhancement in permeability of the mucosal tissue (Lindmark *et al*., 1998; Ochoa-Flores *et al*., 2017; Vikbjerg *et al*., 2006). Mucoadhesives have been incorporated in different drug delivery systems (Sakloetsakun *et al*., 2013); these compounds interact with the intestinal mucosal membrane, increasing the interaction time between the drug and the organism. Among the most recent applications of mucoadhesives are their incorporation into nanoemulsions (Carvalho *et al*., 2010; Li *et al*., 2011). Therefore, the objective of this work was to formulate a SNEDDS with mucoadhesive coating in order to encapsulate an insulin-phosphatidylcholine complex; to increase the oral bioavailability of the hormone; to develop a potential alternative for the treatment of diabetes. In order to do so, human insulin powder was subjected to the formation of a complex with soy phosphatidylcholine by means of the solvent elimination technique, it was then incorporated into SNEDDS with sodium alginate and guar gum, its bioavailability when passing the nanoemulsion by an *in vitro* gastric simulation system was measured. Bioavailability was expected to increase due to the incorporation of the hormone into the SNEDDS that function as a first protection barrier against the organism's environment.

2 Materials and methods

2.1 Materials

Lauroglycol FCC oil and Labrafil M1944CS co-surfactant, for SNEDDS formation, were donated by Gattefossé (Saint-Priest, France). Soybean phosphatidylcholine (PC) with 95% purity was obtained from Avanti Polar Lipids (Alabaster, AL). Phospholipase PLA1 (Lecitase® Ultra) was a donation by Novozymes (Barcelona, Spain) and Duolite A568 was a gift from Rohm and Haas (Barcelona, Spain). Octanoic acid, Cremphor EL, recombinant human insulin, streptozotocin, mucin porcine, bovine albumin, pepsin, lipases, sodium alginate and guar gum were purchased from Sigma-Aldrich (Mexico City). Glucose determination kits (Bio-insulin®) and blood insulin (Bio-insulin®) were purchased from Grupo MEXLAB (Mexico). HPLC grade solvents and reagents used in this work were acquired from Teqsiquim (Mexico City).

2.2 Acidolysis reaction

The synthesis of enriched PC with octanoic acid, a medium chain fatty acid (MCFA), was carried out by an enzymatic reaction of acidolysis, using Phospholipase A1 Lecitase® Ultra immobilized on Duolite A568 according to the methodology described by Esperón-Rojas *et al*. (2017). Briefly, soy phosphatidylcholine was dissolved in hexane by ultrasonication in a ratio of 4 mL solvent per gram of phospholipid, it was carried out under the following conditions: 50°C, immobilized enzyme concentration of 10%, molar ratio of substrates (PC:MCFA) 1:16 and magnetic stirring at 300 rpm for 24 h. Samples from the acidolysis reaction were withdrawn at times 0, 4, 12, 24 and 48 h.

The incorporation of octanoic acid to soybean phospholipase was determined by gas chromatography (GC). Esterified fatty acids were derivatized to methyl esters by alkaline methylation (Garcia *et al*., 2008); 1 ?L was injected into a Hewlett-Packard Model 6890 Gas Chromatograph equipped with a flame ionization detector, using a HP-INNOWAX Polyethylene Glycol capillary column (60 mm X 0.25 mm X 0.25 mm). The temperature program was: an initial temperature of 190°C for 1 minute followed by a ramp of 4°C per minute up to a final temperature of 210°C. Total analysis time was 30 minutes. The injection port was maintained at 200°C and the detector at 230°C. High purity nitrogen was used as a carrier gas at a constant flow rate of 1 mL/min (Esperón-Rojas *et al*., 2017).

2.3 Preparation of insulin-PC complex

The formation of insulin complexes with native PC and modified PC (PCM) was carried out following the method proposed by Zhou *et al*. (2012), using human insulin powder with 27 IU/mg. A mass ratio of 1:8 insulin-phospholipid (modified or unmodified) was used in accordance to published data where a ratio of 1:7.5 or higher was employed, the association of phospholipids to the insulin is near 100%. Each component was placed in a round flask and dissolved in a proper solvent, insulin in 10 mL of methanol acidified with 0.1% trifluoroacetic acid, and PC/PCM in ethyl ether at a 1:9 ratio. Once dissolved independently, they were placed in a flask and then mixed together; afterwards, the solvents were removed by rotary evaporation for 30 minutes in a water bath at 40 °C and under a vacuum of 850 mbar. The complex was completely dried with nitrogen, to remove solvent residues, and left in a vacuum for 12 hours. Finally, the complex was collected and stored in an amber glass bottle and refrigerated at 4°C, until added to the oil phase of the nanoemulsion.

2.4 Preparation of the SNEDDS

First, a pre-formulation of the SNEDDS was prepared considering the results reported by Bravo-Alfaro (2018) and Karamanidou *et al*. (2015): 12 different compositions were formulated as shown in Table 1. In these SNEDDS, Lauroglycol FCC constituted the oil phase, Cremophor EL the surfactant and Labrafil M1944CS the co-surfactant. These systems were characterized by measuring their particle size, and a ternary phase diagram was made using the software SigmaPlot®.

Table 1. SNEDDS compositions and formulations.

System	Oil Lauroglycol FCC (mg)	Sufactant Cremophor EL (mg)	Co-Surfactant Labrafil M1944 (mg)
1	100	300	150
2	100	300	250
3	100	500	150
4	100	500	250
5	100	700	150
6	100	700	250
7	300	300	250
8	300	500	250
9	500	500	250
10	500	700	50
11	500	700	150
12	500	700	250

Table 2. Treatment experimental design.

Treatments	Phospholipid	Sodium alginate	Guar gum
1	PC	0%	0%
2	PC	0%	0.10%
3	PC	0.05%	0%
4	PC	0.05%	0.10%
5	PCM	0%	0%
6	PCM	0%	0.10%
7	PCM	0.05%	0%
8	PCM	0.05%	0.10%

The formulations found in the emulsification zone were selected by means of the resulting ternary phases diagram and by having a monomodal distribution. Monodispersity or monomodal distribution is of great importance since narrow size distribution has been improved to produce higher stability against droplet coalescence: additionally, particle sizes under 400 nm are easily absorbed by organisms (McClements, 2013).

2.5 *Incorporation of the complex in the SNEDDS*

To determine the solubility capacity of the complex in the oil phase (Labarafil M1944 CS), 2.5, 5 and 10% of the complex was tested and integrated into one of the formulations to which particle size was measured.

2.6 *Formation of SNEDDS with mucoadhesive coating*

The mucoadhesive polysaccharides were initially dissolved in phosphate buffer pH 7. The final system volume ratio was 1:1 polysaccharide solution:SNEDDS; solutions were mixed at 100 rpm at room temperature for 60 minutes. For treatments with sodium alginate, 1 mL of 20 mM $CaCl_2$ solution was added. Subsequently, pH was adjusted to 5 with HCl 0.1 M, in order to promote the absorption of the mucoadhesive polymers; and finally, filtered using 0.45 μm PVDF membrane filter. For this experimental stage, a 23 statistical design was carried out, of which factors and levels shown in Table 2.

2.7 *in vitro digestion simulation*

This model simulates the main events that occur during digestion, using the conditions described by McClements and Li (2010). The simulator consisted of two reactors: 1) simulates the mouth and stomach conditions and 2) the small intestine conditions; an infusion pump adds gastric fluids solutions while stirring to provide temperature and peristaltic movements simulations. At the end of each stage a sample was taken and particle size was measured. Bioavailability was calculated by determining the amount of insulin in the final small intestine sample by an insulin-specific ELISA test.

2.8 *Statistical analysis*

The results obtained from the designs were analyzed by analysis of variance (ANOVA) and comparison of means using Tukey´s test, with a level of significance of $p<0.05$ using the statistical software STATGRAPHICS® Centurion XVI (The Plains, VA).

3 Results and discussion

3.1 *Determination of MCFA incorporation to phosphatidylcholine*

The highest percentage of MCFA incorporation (49.69% in molar relation) was attained after 24 hours of reaction, as shown in Fig. 1. These results are higher than those reported by Vikbjerg *et al*. (2006) of 27.3-37%, using the same fatty acid as in this work, but by means of the enzyme PLA2. Previous research in which PLA1 was used, like those form Ochoa *et al*. (2013) and García *et al*. (2008) obtained incorporation results between 37-41% in molar basis. The difference between the data is probably attributed to the use of a solvent medium, since in the presence of hexane the migration of acyl groups can occur, allowing the incorporation of the fatty acid in the sn-1 and sn-2 position of the PC molecule (Schmid *et al*., 1998); this agrees with data reported by Bravo-Alfaro

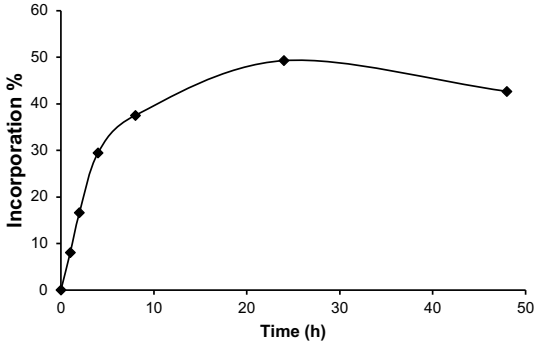

Fig. 1. Percent Molar PC incorporation, of MCFA into PC. The reaction is first order respect to the MCFA, this substrate must be in higher relation than the PC, in order to promote the use of this fatty acids instead of the free fatty acids results of the first stage of the reaction.

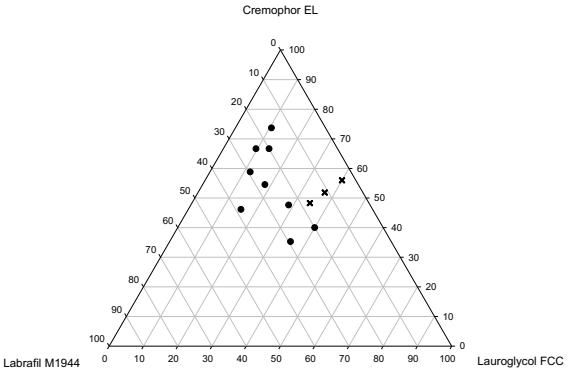

Fig. 2. Ternary phase diagram. Dots indicate zones of spontaneous emulsification with monomodal behaviors. Crosses indicate areas where spontaneous emulsification did not occur.

(2018), who obtained an incorporation of 57.44%, under similar reaction conditions. It is also important to note that in the present work, a smaller amount of enzyme was used at the same reaction time than in work of Ochoa *et al*. (2013), where 16% more enzyme was used and a lesser incorporation was obtained.

3.2 Formulation of the SNEDDS

Following the SNEDDS formulations described by Karamanidou *et al*. (2015), and Bravo-Alfaro (2018), we proceeded to test those that produced smaller particle sizes and monomodal size distribution; 12 different composition systems shown in Table 1 in the Materials and Methods section were tested. nanoemulsions. The factors like stability, solubilizing capacity of surfactants in water for an efficient self-nanoemulsification, a ratio surfactant/co-surfactant should be considered in the formulation and selection of SNEDDS; for that, a ternary phases diagram was obtained (Fig. 2). The ternary phases diagram was related to the particle size distribution and spontaneous emulsification of the formulation systems. It was observed that the systems formed multimodal nanoemulsions, but also the spontaneous emulsification may pose difficulties and the systems require more agitation. This formulation contained the highest amount of the surfactant Cremophor which may cause the formation of particles with multiple sizes and the concomitant difficulty of assembly with the other components. The SNEDDS in the multimodal nanoemulsions area were discarded because this distribution may produce coalescence and low stability in the nanoemulsion.

Particle size, D90 and PDI of 9 monomodal systems selected from the ternary phases diagram, are shown in Table 3. These data coincide with that reported by Bravo-Alfaro (2018). Those treatments with the best results were selected to add the mucoadhesive coating. For the next stage of the study, systems 2, 6 and 9 were selected because of their smaller particle size and PDI values (Ochoa *et al*., 2016), and different compositions, which allowed us to observe their behavior in the incorporation of mucoadhesives.

3.3 Incorporation of the insulin-phospholipid complex in SNEDDS

Solubility of the complex in the oil phase was tested at 2.5, 5 and 10%. This test was performed in system 2, unlike that reported by Bravo-Alfaro (2018), where solubility was found up to 10%, although the particle size increased with the percent of incorporated complex, sizes were obtained below 100 nm with monomodal distribution. This difference can be attributed to the method by which the complex was prepared, since in Bravo-Alfaro (2018) it was by freeze-drying, having to use several freezing cycles to achieve the final complex, which allowed a larger size. Although the particle size did not differ significantly, greater sedimentation and less repeatability in distribution was observed. It was determined that the 2.5% complex was the one to be used as it produced the smallest particle size.

Table 3. SNEDDS particle size.

System	Average size (nm)	D90 (nm)	PDI
1	23.53 ± 0.92	27.36 ± 0.39	0.17 ± 0.05
2	27.90 ± 1.07	33.95 ± 1.98	0.08 ± 0.01
3	22.43 ± 0.61	25.11 ± 0.27	0.23 ± 0.02
4	21.59 ± 0.86	26.03 ± 0.94	0.12 ± 0.03
5	21.85 ±1.02	24.1 ± 0.43	0.37 ± 0.03
6	18.56 ± 0.51	22.43 ± 0.87	0.10 ± 0.01
7	43.71 ± 1.52	50.53 ± 3.17	0.13 ± 0.01
8	26.97 ± 1.72	31.43 ± 2.68	0.17 ± 0.03
9	33.66 ± 1.37	39.68 ± 2.76	0.11 ± 0.02

Data Mean ± SD (n=6).

Table 4. Particle sizes for systems with mucoadhesives.

Treatment	Average size (nm)	D90 (nm)	PDI
S2 T1	27.90 ± 1.07	33.95 ± 1.98c	0.08 ± 0.01a
S2 T2	80.70 ± 0.36	83.2 ± 1.82a	0.10 ± 0.01a
S2 T3	57.10 ± 2.31	67.06 ± 2.72a	0.16 ± 0.02b
S2 T4	47.50 ± 0.19	53.1 ±2.09a	0.20 ± 0.01b
S6 T1	18.56 ± 0.51	22.43 ± 0.87c	0.10 ± 0.01a
S6 T2	31.80 ± 5.32	24.6 ± 1.71a	0.49 ± 0.015c
S6 T3	21.54 ± 0.21	25.7 ±0.26a	0.15 ± 0.01b
S6 T4	23.04 ± 0.13	26.33 ± 0.12a	0.22 ± 0.01b
S9 T1	33.66 ± 1.37	39.68 ± 2.76c	0.11 ± 0.02a
S9 T2	117.93 ± 1.21	3271 ± 2759.82b	0.26 ± 0.02b
S9 T3	97.64 ± 5.04	155 ± 39.05b	0.23 ± 0.01b
S9 T4	103.23 ± 1.47	100.66 ± 5.86b	0.23± 0.01b

3 selected SNEDDS were used to represent particle size. Data mean ± SD (n=6). Letters represent significant difference ($p<0.05$).

3.4 Incorporation of mucoadhesives in SNEDDS

According to Table 2, different treatments were assessed in the 3 selected systems from section 3.2; treatments 1-4 have a representative behavior of the treatments (data not shown). An increase in size can be observed in comparison to the free systems depicted in Table 4; this increase is attributed to the coating generated when mucoadhesives were added to the nanoemulsion oil particle surface; consistent with the data reported by Choi *et al.* (2011). System 6 (S6) did not show a considerable increase in average size or D90, meaning that this system did not achieve polysaccharide incorporation, possibly because of its composition, containing a high quantity of Cremophor EL, which generates different interactions or repulsions between the carboxyl groups in polysaccharides and the particle surface. Data corresponds to the report by Guzey and McClements (2006); for this reason S6 was discarded. System 9 (S9) was also discarded because it produced particle sizes and D90 values around 100 nm and small coated particle sizes.

System 2 (S2) was employed to perform digestive tests adding the PC and PCM complex, this system was chosen because it required the lowest surfactant-cosurfactant ratio and had a low PDI value, ensuring less dispersed particle sizes. Treatments 4 and 8 were rejected because they produced bimodal size distributions, in which the second peak (2000 nm) represents 3% of the measurements.

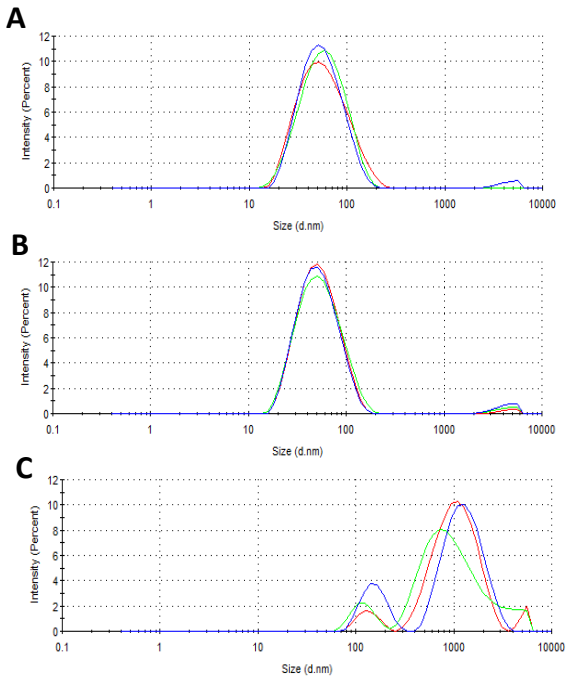

Fig. 3. Change in the particle size distribution in an *in vitro* digestive simulation system for T3 treatment, with sodium alginate: A) Mouth, B) Stomach, C) Small intestine.

3.5 *Evaluation of the bioaccessibility in an in vitro digestion simulation*

The treatments, excluding 4 and 8, were subjected to the conditions described in the methodology (section 2.7). In all treatments a change in particle size was observed after passing through each phase. In the mouth stage, micrometer-size particles were observed. It has been suggested that these particles are aggregates that are formed by the interaction with mucin that is present in this phase (Zhang *et al*., 2017). In the stomach stage, the particle size also increased; although not significantly, and aggregates were also observed. In this case that may occur by the interaction with the enzymes and salts present, some particles can be affected by the environmental conditions and produce the rupture and free the insulin, which is hydrolyzed by pepsin and denatured by the pH. The largest increase in size occurred in the small intestine stage, where sizes up to 1500 nm were attained in treatments where one of the mucoadhesives was used. This increase has been associated with the activity of the enzymes, pH value and peristaltic movement in mucoadhesives, causing the structure to be lost and triggered the release of oily particles.

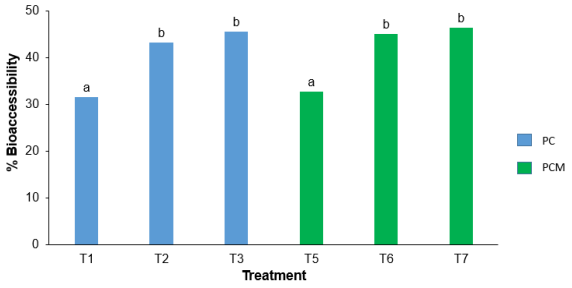

Fig. 4. Treatment bioaccesibility in *in vitro* assays. Value mean ± SD. n=3. Letters determine significant difference ($p < 0.05$).

Also, the basic pH would cause the dissociation of the insulin-PC complex (Li *et al*., 2011; McClements, 2015; Zhang *et al*., 2017). In the treatments that did not include mucoadhesives (T1 and T5), the particle size increased to 66-230 nm in the small intestine stage. Fig. 3 shows the change in particle size distribution in a system with sodium alginate. The percent of bioaccessibility calculated with the sample taken at the end of the small intestine phase is shown in Figure 4, where it can be observed a significant difference that may be caused by the mucoadhesive, with greater bioavailability in the treatments that used any of the mucoadhesives. The obtained values were between 40-46.3% with respect to the amount of insulin present in the initial phase. This value was higher than that reported by Sakloetsakun *et al*. (2013), who reported that after 4 hours only 10-8% of insulin remained. This means that the developed system better protected insulin from gastric conditions, and suggests the possibility using these systems as reliable insulin delivery strategies.

In an *in vivo* scenario, the nanoemulsion droplets and their digestion products can be absorbed once in the gastrointestinal tract; hence, exact absorption points have not been completed established, different authors have hypothesized that absorption is carried out by epithelial cells and M-cells and are highly particle size dependent. Also, nanoparticles could be the appropriate size to pass between epithelial cell tight junctions or absorbed by passive or active cell membrane transport (McClements & Rao, 2011).

Conclusions

Insulin was successfully incorporated into SNEDDS through a complex with native soy PC and PC

modified with octanoic acid. It was demonstrated that the SNEDDS developed with the insulin complex and the mucoadhesive polysaccharides coating can protect the protein from the degradation caused by gastric conditions, including enzymes such as pepsin. Additionally, it was observed that systems including mucoadhesives achieved higher levels of bioavailability compared with free insulin systems. It was determined that the T7 treatment displayed the best profile regarding resistance when exposed to the gastrointestinal simulations. These results propose that SNEDDS systems with mucoadhesives can be considered as viable oral insulin delivery systems. However, further research is required in order to characterize by microscopy these nanoemulsion and determine their pharmacokinetic parameter using *in vivo* models to further assess their application in diabetic patients.

Acknowledgements

The authors wish to thank CONACYT for scholarship number 621437 for the first author and the financial support through the grant number 250784.

Nomenclature

SNEDDS	self-nanoemulsifying drug delivery system
PC	native soy phosphatidylcholine
PCM	modified soy phosphatidylcholine
GC	gas chromatography
IU	international units of insulin
MCFA	medium chain fatty acids
D90	percentile 90 of the particle size distribution
PDI	polydispersity index
S	system
T	treatment

References

Al Rubeaan, K., Rafiullah, M., and Jayavanth, S. (2016). Oral insulin delivery systems using chitosan-based formulation: a review. *Expert Opinion on Drug Delivery 13*, 2233-237. https://doi.org/10.1517/17425247.2016.1107543

Alizadeh-Sani, M., Hamishehkar, H., Khezerlou, A., Azizi-Lalabadi, M., Azadi, Y., Nattagh-Eshtivani, E., Fasihi, M., Ghavami, A., Aynechi, A. and Ehsani, A. (2018). Bioemulsifiers derived from microorganisms: Applications in the drug and food industry. *Advanced Pharmaceutical Bulletin 8*, 191.199. https://doi.org/10.15171/apb.2018.023

Atkinson, M. A., Eisenbarth, G. S., and Michels, A. W. (2014). Type 1 diabetes. *The Lancet 383*, 69-82.

Bravo-Alfaro, D. A. (2018). Desarrollo de un sistema auto-nanoemulsificable (SNEDDS) para la administración de insulina por vía oral. Tecnológico Nacional de México/Instituto Tecnológico de Veracruz.

Carvalho, F. C., Bruschi, M. L., Evangelista, R. C., & Gremião, M. P. D. (2010). Mucoadhesive drug delivery systems. *Brazilian Journal of Pharmaceutical Sciences 46*, 1-17.

Cavazos Garduño, A., Ochoa Flores, A. A., Serrano-Niño, J. C., Beristain, C. I., and García, H. S. (2014). Operating and compositional variables for preparation of betulinic acid nanoemulsions. *Revista Mexicana de Ingeniería Química 13*, 689-703.

Choi, A. J., Kim, C. J., Cho, Y. J., Hwang, J. K., and Kim, C. T. (2011). Characterization of capsaicin-loaded nanoemulsions stabilized with alginate and chitosan by self-assembly. *Food and Bioprocess Technology 4*, 1119-1126. https://doi.org/10.1007/s11947-011-0568-9

Dwivedi, P., Khatik, R., Khandelwal, K., Srivastava, R., Taneja, I., Raju, K. S. R, Dwivedi, H., Shukla, P., Gupta, P., Singh, S., Tripathi, R., Paliwal, S. K., Wahajuddin., Dwivedi, A. K., and Mishra, P. R. (2014). Self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of arteether: pharmacokinetics, toxicity and antimalarial activity in mice. *RSC Advances 4*, 64905-64918. https://doi.org/10.1039/C4RA09267H

Esperón-Rojas, A. A., Baeza-Jiménez, R., Cano-Sarmiento, C., and García, H. S. (2017). Structured mono- and diacylglycerols with a high content of medium chain fatty acids. *Journal of Oleo Science 66*, 991-996. https://doi.org/10.5650/jos.ess17010

Garcia, H. S., Kim, I. H., Lopez-Hernandez, A., and Hill, C. G. (2008). Enrichment

of lecithin with n-3 fatty acids by acidolysis using immobilized phospholipase A1. *Grasas y Aceites 59*, 368-374. https://doi.org/10.3989/gya.2008.v59.i4.531

Granillo-Guerrero, V. G., Villalobos-Espinosa, J. C., Alamilla-Beltrán, L., Téllez-Medina, D. I., Hernández-Sánchez, H., Dorantes-Álvarez, L., & Gutiérrez-López, G. F. (2017). Optimization of the formulation of emulsions prepared with a mixture of vitamins D and E by means of an experimental design simplex centroid and analysis of colocalization of its components. *Revista Mexicana de Ingeniería Química 16*, 861-872

Guzey, D. and McClements, D. J. (2006). Formation, stability and properties of multilayer emulsions for application in the food industry. *Advances in Colloid and Interface Science 128-130*, 227-248. https://doi.org/10.1016/j.cis.2006.11.021

Heinemann, L. (2002). Variability of insulin absorption and insulin action. *Diabetes Technology & Therapeutics 4*, 673-682.

International Diabetes Federation. (2017). IDF Worldwide table 2017. www.diabetesatlas.org, 1-2. https://doi.org/http://www.diabetesatlas.org/. (accessed 7 December 2015)

Kanzarkar, M., Pathak, P. P., Vaidya, M., Brumlik, C., and Choudhury, A. (2015). Oral insulin-delivery system for diabetes mellitus. *Pharmaceutical Patent Analyst 4*, 29-36.

Karamanidou, T., Karidi, K., Bourganis, V., Kontonikola, K., Kammona, O., and Kiparissides, C. (2015). Effective incorporation of insulin in mucus permeating self-nanoemulsifying drug delivery systems. *European Journal of Pharmaceutics and Biopharmaceutics 97*, 223-229. https://doi.org/10.1016/j.ejpb.2015.04.013

Li, P., Tan, A., Prestidge, C. A., Nielsen, H. M., and Müllertz, A. (2014). Self-nanoemulsifying drug delivery systems for oral insulin delivery: *in vitro* and *in vivo* evaluations of enteric coating and drug loading. *International Journal of Pharmaceutics 477*, 390-398. https://doi.org/10.1016/j.ijpharm.2014.10.039

Li, Y., Hu, M., Du, Y., and McClements, D. J. (2011). Controlling lipid nanoemulsion digestion using nanolaminated biopolymer coatings. *Journal of Microencapsulation 28*, 166-175. https://doi.org/10.3109/02652048.2010.544417

Libman, I. M. (2009). Epidemiología de la diabetes mellitus en la infancia y adolescencia: Tipo 1, tipo 2 y ¿diabetes ?doble?? *Revista Argentina de Endocrinologia y Metabolismo 46*, 22-36.

Lindmark, T., Kimura, Y., and Artursson, P. (1998). Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in caco-2 cells. *The Journal of Pharmacology and Experimental Therapeutics 284*, 362-369.

McClements, D. J. (2013). Edible lipid nanoparticles: digestion, absorption, and potential toxicity. *Progress in Lipid Research 52*, 409-423.

McClements, D. J. (2015). Encapsulation, protection, and release of hydrophilic active components: Potential and limitations of colloidal delivery systems. *Advances in Colloid and Interface Science 219*, 27-53. https://doi.org/10.1016/j.cis.2015.02.002

McClements, D. J. and Li, Y. (2010). Review of *in vitro* digestion models for rapid screening of emulsion-based systems. *Food and Function 1*, 32-59. https://doi.org/10.1039/c0fo00111b

McClements, D. J. and Rao, J. (2011). Food-grade nanoemulsions: Formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical Reviews in Food Science and Nutrition 51*, 285-330. https://doi.org/10.1080/10408398.2011.559558

Nawaz, M. S., Shah, K. U., Khan, T. M., Rehman, A. U., Rashid, H. U., Mahmood, S., Khan, S. and Farrukh, M. J. (2017). Evaluation of current trends and recent development in insulin therapy for management of diabetes mellitus. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews 11*, S833-S839. https://doi.org/10.1016/j.dsx.2017.07.003

Ochoa-Flores, A. A., Hernández-Becerra, J. A., Cavazos-Garduño, A., Soto-Rodríguez, I., Sanchez-Otero, M. G., Vernon-Carter, E. J., and García, H. S. (2017). Enhanced bioavailability of curcumin nanoemulsions stabilized with phosphatidylcholine modified with medium chain fatty acids. *Current Drug Delivery 14*,

377-385. https://doi.org/10.2174/1567201813666160919142811

Ochoa, A. A., Hernández-Becerra, J. A., Cavazos-Garduño, A., García, H. S., and Vernon-Carter, E. J. (2013). Phosphatidylcholine enrichment with medium chain fatty acids by immobilized phospholipase A1-catalyzed acidolysis. *Biotechnology Progress 29*, 142-148. https://doi.org/10.1002/btpr.1648

Ochoa, A., Hernández, J., Cavazos, A., Vernon-Carter, E. J., and García, H. (2016). Preparation and characterization of curcumin nanoemulsions obtained by thin-film hydratation emulsification and ultrasonication methods. *Revista Mexicana de Ingeniería Química 15*, 79-90

Olivarez-Romero, R., Faustino-Vega, A., Miranda-Calderon, J. E., González-Vázquez, R., & Azaola-Espinosa, A. (2018). Microencapsulation of lactobacillus acidophilus la-5 increases relative survival under simulated gastrointestinal tract stress. *Revista Mexicana de Ingeniería Química 17*, 641-650

Özer, S., Kerimoglu, O., and Ugurlu, T. (2017). Nanocarriers: Novel approaches to oral delivery of insulin/nanotasiyicilar: Oral insulin tedavisine Yeni Yaklasimlar. *Clinical and Experimental Health Sciences 7*, 115-123.

Sakloetsakun, D., Dünnhaupt, S., Barthelmes, J., Perera, G., and Bernkop-Schnürch, A. (2013). Combining two technologies: Multifunctional polymers and self-nanoemulsifying drug delivery system (SNEDDS) for oral insulin administration. *International Journal of Biological Macromolecules 61*, 363-372. https://doi.org/10.1016/j.ijbiomac.2013.08.002

Schmid, U., Bornscheuer, U. J., Soumanou, M. M., McNeill, G. P., and Schmid, R. D. (1998). Optimization of the reaction conditions in the lipase-catalyzed synthesis of structured triglycerides. *Journal of the American Oil Chemists? Society 75*, 1527-1531. https://doi.org/10.1007/s11746-998-0089-5

Vikbjerg, A. F., Rusig, J. Y., Jonsson, G., Mu, H., and Xu, X. (2006). Comparative evaluation of the emulsifying properties of phosphatidylcholine after enzymatic acyl modification. *Journal of Agricultural and Food Chemistry 54*, 3310-3316. https://doi.org/10.1021/jf052665w

Vikbjerg, A. F., Rusig, J. Y., Jonsson, G., Mu, H., and Xu, X. (2006). Strategies for lipase-catalyzed production and the purification of structured phospholipids. *European Journal of Lipid Science and Technology 108*, 802-811. https://doi.org/10.1002/ejlt.200600138

Zhang, Z., Zhang, R., and McClements, D. J. (2017). Control of protein digestion under simulated gastrointestinal conditions using biopolymer microgels. *Food Research International 100*, 86-94. https://doi.org/10.1016/j.foodres.2017.08.037

Zhou, C., Xia, X., Liu, Y., and Li, L. (2012). The preparation of a complex of insulin-phospholipids and their interaction mechanism. *Journal of Peptide Science 18*, 541-548.