Evaluation of Sucrose Esters as Alternative Surfactants in Microencapsulation of Proteins by the Solvent Evaporation Method

Submitted: February 26, 2003; Accepted: April 29, 2003; Published: June 25, 2003

Bi-Botti C. Youan,¹ Alamdar Hussain,¹ and Nga T. Nguyen¹

¹Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center (TTUHSC) School of Pharmacy, Amarillo, TX 79106

ABSTRACT

Sucrose esters (SE) are surfactants with potential pharmaceutical applications because of their low toxicity, biocompatibility, and excellent biodegradability. The objective of the study was to investigate SE as alternative surfactants in stabilizing emulsions for the preparation of protein-loaded microparticles. To achieve this goal, using bovine serum albumin as model protein and 75/25 poly(d,l-lactide-co-glycolide) as polymer carrier, we have investigated the influence of the following formulation variables on particle characteristics: (1) SE concentration from 0.01% to 1% (wt/vol), (2) hydrophile-lipophile balance (HLB) value of SE from 6 to 15, and (3) the nature of emulsion stabilizer. The formulations were characterized using ATR-FTIR spectroscopy, bicinchoninic acid protein assay, optical microscopy and SDS-PAGE. Results showed that at 0.05% (wt/vol) surfactant concentration, SE with HLB of 6 to 15 provided discrete and spherical microparticles with the highest encapsulation efficiency compared with controls polyvinyl alcohol (PVA) and poloxamer 188. These results may be explained by the difference in critical micelle concentration, diffusion, and partition coefficient among the tested surfactants. HLB values were consistent with SE spectral data. The protein molecular weight was preserved after the encapsulation process. The effective SE concentration was far less (20- to 200-fold) than that is usually required for PVA in microencapsulation of proteins. However, the encapsulation efficiency was relatively lower (~13.5%). These preliminary results suggest that it may be desirable to optimize such formulations in vitro and in vivo for SE to be eventually used as alternative surfactants in the development of microparticulate systems for parenteral delivery of protein and gene medicines.

Corresponding Author: Bi-Botti C. Youan, Department of Pharmaceutical Sciences, TTUHSC School of Pharmacy, 1300 Coulter, Amarillo, TX 79106; Tel: (806) 356-4015, ext 236; Fax: (806) 356-4034; Email: beyouan@ama.ttuhsu.edu.

KEYWORDS: proteins, microencapsulation, solvent evaporation, sucrose ester, surfactants

INTRODUCTION

Microencapsulation is a technology devoted to entrapping solids, liquids, or gases inside one or more polymeric coatings.¹ This technology has both proven and potential pharmaceutical applications including (1) masking unpleasant tastes and odors; (2) protecting drugs against environmental stress (eg, pH, humidity, enzymes) to enhance drug stability; (3) possibly allowing time-dependent drug delivery to increase patient compliance; and (4) possibly targeting drugs using nanosized particles covalently attached to a specific antibody to reduce undesirable side effects. Therefore, this technology is a promising alternative in addressing the current challenges of drug delivery resulting from advances in biotechnology, genetic engineering, bioinformatics, and the human genome project.

Surfactants are not only important for stabilizing emulsions during microencapsulation, but they are also important in preventing aggregation and contributing to stabilization of proteins, if judiciously selected. For instance, peroxide residues in surfactant have been implicated in oxidations of protein.² For parenteral administration, only nonionic surfactants are generally used.²⁻⁵ Beside phospholipids, the group of acceptable surfactants for this purpose has been limited to polyoxyethylene-based surfactants. These observations are consistent with knowledge that ionic surfactants are less structure-perturbing compared with ionic surfactants because of their low monomer concentration and their less directional interactions (no electrostatic binding).⁵ Moreover, today most microencapsulation processes performed by solvent evaporation technique make use of polyvinyl alcohol (PVA),⁶⁻⁸ a surfactant that is unacceptable for parenteral application because of its relative toxicity. PVA has been reported to be carcinogenic.⁹ Studies in rats have also shown that 5% PVA aqueous solutions injected subcutaneously can cause anemia and infiltrate various organs and tissues. The LD₅₀ (mouse, oral) is 14.7 g/kg.¹⁰ Therefore, several washing

Table 1. Physicochemical Properties of the Raw Materials*

Product Abbrev.	Composition and MW	HLB	cmc at 25°C	Surface tension (mN/m)	Solubility
DK- F160	Mono:Di:Tri: Poly-ester: 72:23:5:0 (659Da) [†]	15 [†]	NR	34.50 (0.1% solution) [†]	W-soluble at 80°C [†]
DK-F140	61:30:7:2 (690Da) [†]	13 [†]	NR	35.00 (0.1% solution) [†]	W-soluble at 80°C [†]
DK-F110	52:36:10:2 (723Da) [†]	11 [†]	NR	36.70 (0.1% solution) [†]	W-soluble at 80°C [†]
DK-F70	39:45:12:4 (756Da) [†]	8 [†]	NR	40.60 (0.1% solution) [†]	W-soluble at 80°C [†]
DK-F50	34:46:17:3 (777Da) [†]	6 [†]	NR	46.80 (0.1% solution) [†]	Partly W-soluble at 80°C [†]
SML	Sucrose monolaurate (NR)	NR	$3.4 \times 10^{-4} \text{ M}^{19}$	NR	NR
SMO	Sucrose monooleate (NR)	NR	$5.1 \times 10^{-6} \text{ M}^{19}$	NR	NR
POL-188	Poloxamer 188 (9.95kDa) [‡]	NR	0.1% ²⁰	NR	35% in MC, W-soluble [‡]
PVA-10k	Polyvinyl alcohol (10kDa)	NR	NR	NR	W-soluble at 90°C, Insoluble in organic solvents ¹⁰
PVA-70k	Polyvinyl alcohol (70kDa)	NR	NR	NR	W-soluble at 90°C, Insoluble in organic solvents ¹⁰
PVA-100k	Polyvinyl alcohol, (100kDa)	NR	NR	NR	W-soluble at 90°C, Insoluble in organic solvents ¹⁰
W	Water: H ₂ O (18 Da)	N/A	N/A	71.99 ²¹	Insoluble in organic solvents ¹⁰
MC	Methylene chloride: CH ₂ Cl ₂ (84.93 Da)	N/A	N/A	39.05 ²¹	Soluble with 50 parts of W ²²

*CMC indicates critical micelle concentration; HLB, hydrophile-lipophile balance; MC, methylene chloride; MW, molecular weight; N/A, not applicable; NR, not reported; POL, poloxamer; PVA, polyvinyl alcohol; W, water; SML, sucrose monolaurate; SMO, sucrose monooleate.

[†]Data reported from manufacturer product data sheet.²³

[‡]Data reported from manufacturer product data sheet.²⁴

processes are usually required during the development of microparticles when PVA is used, leading to loss of expensive biotechnology-derived products intended to be encapsulated. There are also some environmental concerns of using presently accepted surfactants as they are mostly manufactured from petrochemical products.³ Among alternative formulation strategies to existing surfactants, which are gaining interest, are avoiding surfactants using amphiphilic cyclodextrins¹¹ and using novel biocompatible surfactants.^{3,12-18} Among the latter strategies, there is increasing interest in using sucrose ester in pharmaceutical formulations.^{3,12-16,18} Sucrose-based surfactants (**Figure 1** and **Table 1**) are biodegradable surfactants whose hydrophilic and lipophilic properties can be adjusted by varying fatty acid chain lengths. They have potential pharmaceutical applications because of their low toxicity, biocompatibility, and excellent biode-

gradability. They offer an attractive alternative to the generally more convenient ethylene oxide (EO)-based non-ionic surfactants. Sugar-based surfactants are prepared from natural and renewable resources that allow researchers to devise manifold structural combinations suited for modulating surfactant properties. Furthermore, the phase behavior of this class of nonionic surfactant is much less influenced by temperature than the phase behavior of typical EO-based nonionic surfactants.¹⁵ In the present work, we intend to evaluate sucrose esters (SE) as an alternative surfactant to PVA in stabilizing the emulsion for the preparation of drug-loaded microparticles. To achieve this goal, using bovine serum albumin (BSA) as model protein and 75/25 poly(d,l-lactide-co-glycolide) as polymer carrier, we have mainly investigated the influence of SE concentration and hydrophile-lipophile balance (HLB) and the nature of emulsion stabilizer on particle characteristics.

Figure 1. Chemical structure of sucrose ester. Sucrose ester is a nonionic surface active agent manufactured from a pure sugar and vegetable oils. It is an ester compound consisting of sucrose and fatty acids. Mono-ester is a sucrose ester that combines 1 mol of fatty acid with 1 mol of sucrose; Di-ester is 2 mol of fatty acid; and tri-ester is 3 mol fatty acid. Di-ester and tri-ester combinations include 1 mol of sucrose. R = alkyl group.

MATERIALS AND METHODS

Materials

The following chemicals were used from suppliers without further modification or purification. SE, namely DK-Ester F-50, F-70, F-110, F-140, and F-140 (Dai-Ichi Kogyo Seiyaku Co, Tokyo, Japan) were kindly provided by Montello Inc (Tulsa, OK). For simplicity, these SE are hereafter referred to as DK-F50, DK-F70, DK-F110, DK-F140, and DK-F160, respectively. The physicochemical properties of these SE are summarized in **Table 1**, and their chemical structure is shown in **Figure 1**. Poloxamer 188 (Lutrol F68), hereafter referred to as POL-188, was kindly provided by BASF Corp (Washington, NJ). Native BSA (~67 kDa), used as model protein, and PVAs of molecular weight (MW) 9-10 kDa, 30-70 kDa, and 70-100 kDa were purchased from Sigma Chemical Co (St Louis, MO). These PVAs are hereafter referred to as PVA-10k, PVA-70k, and PVA-100k, respectively. The model polymer carrier, 75/25 poly(D,L-lactide-co-glycolide) (PLGA) of inherent viscosity 0.80 dL/g and MW of 98 kDa (Resomer RG 756), was purchased from Boehringer, Ingelheim Chemicals Inc (Petersburg, VA). Methylene chloride (MC), high-performance liquid chromatography (HPLC) grade, used as organic phase, was obtained from Fisher Scientific (Houston, TX). Deionized water was used as aqueous medium. The bicinchoninic acid (BCA) protein assay kit was purchased from Pierce Chemical (Rockford, IL). All other chemicals were reagent grade.

Preparation of Microcapsules

The microparticles were prepared by double emulsion solvent-evaporation method as previously described.⁷ Typically, 0.5 mL of BSA solution (40 mg/mL, W_1 phase) was dispersed into 2.5 mL of 200 mg PLGA containing MC solution (O phase). The W_1 and O phases were vigorously mixed for 30 seconds to form a simple O/W_1 emulsion using a vortex (Touch mixer model 231, Fisher Scientific) at maximum speed (2800 rpm). This first emulsion was then immediately dispersed into the surfactant solution (50 mL, W_2 phase) and homogenized at 1200 rpm for 5 minutes for formation of microdroplets using the mixer model L4RT (Silverson Machines Inc, East Longmeadow, MA). This double emulsion ($W_1/O/W_2$) underwent a subsequent mixing at magnetic stirring speed (model Ro15 power, IKA Works, Wilmington, NC) at 700 rpm for 4 hours to allow solvent evaporation at room temperature and under the chemical hood. The solidified microparticles were recovered by centrifugation at 3000 rpm for 10 minutes and were freeze-dried for 12 hours. Unless otherwise stated, the particles were not washed with deionized water before freeze-drying. The influence of the following formulation variables on particle characteristics was investigated: SE concentration and HLB values and nature of emulsion stabilizers. Unless otherwise stated, the percentage (%) concentrations are %wt/vol. All experiments were performed in triplicate.

Morphological Analysis

This analysis was conducted as previously described.⁸ Briefly, microparticles were suspended in a few droplets of water and their surface, shape, and size were analyzed by light microscopy at magnification of $\times 400$. The light microscope Nikon Eclipse TE2000 (Nikon, Japan) used was connected to a digital image analyzer.

Determination of Protein Loading and Encapsulation Efficiency

Fifty milligrams of protein-loaded microparticles were accurately weighed, and the polymeric carrier was allowed to dissolve in 1 mL of methylene chloride. Then, the protein was extracted by 2 mL of phosphate buffer pH 7.4. The amount of protein extracted in the supernatant was assayed using a BCA kit adapted to a microplate reader (Model Tecan SpectraFluor Plus, Phoenix Research Products, Hayward, CA) at 570 nm according to the manufacturer's protocol. Control protein unloaded microparticles (empty or blank microparticles) were analyzed in the same conditions. Calibration samples prepared under the same conditions were similarly analyzed. These data allowed the

determination of protein loading and encapsulation efficiency as defined elsewhere.⁷ All experiments were performed in triplicate. Data were compared by one-way analysis of variance (ANOVA) using Instat, version 3.0 for Windows (GraphPad Software, San Diego, CA). A probability level (*P* value) of <0.05 was considered to be statistically significant.

Sodium Dodecyl Sulphate–polyacrylamide Gel Electrophoresis Studies

To evaluate the effects of processing conditions on the molecular weight of BSA, the protein-loaded microcapsules were suspended in phosphate-buffered saline (PBS), pH 7.4. After 1 hour, the samples were centrifuged, and the protein that was released into the supernatant was separated from the microparticles. The protein sample solutions were directly loaded into the sample well of a discontinuous sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) using a Mini-Protein 3 dual slab cell (Bio-Rad Laboratories, Hercules, CA). The study was conducted according to the manufacturer's protocol. The gels were stained with Coomassie blue R-250 dye, and pictures were taken by a scanner.

Attenuated Total Reflection–Fourier Transform Infrared Spectroscopy

Attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectra were recorded on Nicolet Nexus 470 spectrometer (Thermo Nicolet Corp, Madison, WI) using the Smart Miracle ATR accessory. An approximately 100- μ g portion of sucrose ester solid sample was deposited on the sample holder. Interferograms recorded at a spectral resolution of 4 cm^{-1} were averaged for each sample after 32 scans. Interferograms were processed using Happ-Genzel apodization, followed by automatic baseline correction.

RESULTS AND DISCUSSION

The interfacial tension of water/methylene chloride was reported to be 27.84 mN/m.²⁵ This value may be different if the water contains a protein such as BSA (W_1 phase) or a surfactant (W_2 phase) and if methylene chloride contains PLGA (O phase). This speculation is supported by decrease in surface tension of 0.1% SE solutions reported in **Table 1**. The presence of 0.1% SE significantly decreases the surface tension of water (34.50–46.80 mN/m) compared with the original value of water

surface tension (71.99 mN/m). The foregoing observations allow us to postulate that the addition of SE to the water phase may be thermodynamically beneficial for dispersion stability. At this stage of investigations, in our complex formulation conditions, the different surface tensions and interfacial tensions were not determined or reported in order to be able to compute spreading coefficients and optimize the formulation on thermodynamic standpoint. Future investigations will be required to optimize such formulations in this respect. Therefore, we hereafter rely on observed experimental results for subsequent discussions.

Figure 2 shows the influence of the concentration of SE on the protein content of microparticles. The HLB 15 (DK-F160) was selected for this experiment because it was within the reported HLB range for O/W emulsification^{8–15} (1). All parameters remaining the same, the concentration of 0.05% SE led to the highest protein content ($1349.30 \pm 81.98 \mu\text{g}/100 \text{ mg}$, $n = 3$) and encapsulation efficiency (13.49 ± 0.82 , $n = 3$). When the later results of 0.05% SE were compared with those of other SE concentrations, the *P* value was even less than 0.001. These data were therefore considered extremely significant. These observations may be explained by the low critical micelle concentration (CMC) of the SE based on available SE CMC data (**Table 1**). The average molecular weight of DK-F160 is 659Da (**Table 1**). The concentration of 0.05%, which is equivalent to 0.758 mM of SE, is slightly above the reported CMC of some sucrose esters¹⁹ (**Table 1**). It is postulated that at the concentration lower than 0.05%, the surfactant was preferentially located at water/air interface and therefore could not stabilize the emulsion. And at the concentration superior to 0.05% (too far above the CMC), microemulsion leading to the formation of nanoparticles after hardening. Such nanoparticles were not probably recovered by the low centrifugation speed and duration (3000 rpm/10 minutes) leading to apparent low encapsulation efficiency. The latter speculation is based on previous publications showing that SE could be used to formulate microemulsion for use in food science and for topical applications^{13–15} and to prepare reverse micelles in microencapsulation.¹⁷ The importance of the concentration of emulsion stabilizers (eg, PVA) in microencapsulation of protein has also been demonstrated.²⁶

Figure 3 shows the influence of the HLB of the SE from 6 to 15 on protein content of microparticles. The more mono-ester contained in SE, the higher the hydrophilic property it shows, and the more di-ester, tri-ester, and poly-ester, the higher the lipophilic property (**Table 1**). The HLB data were consistent with the ATR–FTIR spectra (**Figure 4**). The absorbance intensities of the $-\text{CH}_3$ and $-\text{CH}_2$ stretching, which are known to occur from 2975 to 2950 cm^{-1} and from 2885 to 2865 cm^{-1} , increased when

Figure 2. Influence of sucrose ester concentration of HLB 15 (DK-F160) on protein content of polymeric microparticles. The model protein and polymeric carrier were bovine serum albumin and 25/75 poly(lactide-co-glycolide), respectively. The particles were prepared in triplicate by double emulsion solvent evaporation method as described in the materials and methods section. Results are expressed as average $\pm$ SEM for $n = 3$.

Figure 3. Influence of sucrose ester HLB value on protein content of polymeric microparticles. The model protein and polymeric carrier were bovine serum albumin and 25/75 poly(lactide-co-glycolide), respectively. The HLB 6, 8, 11, 13, and 15 corresponded to DK-F50, DK-F70, DK-F110, DK-F140, and DK-F160, respectively. In all cases, the concentration of the sucrose ester used was 0.05%. The particles were prepared in triplicate by double emulsion solvent evaporation as described in the materials and methods section. Results are expressed as average $\pm$ SEM for $n = 3$.

the HLB decreased thus providing evidence of increase in the hydrophobic moieties. In contrast, the primary alcohol (C–O stretching and –OH deformation) at 1050 cm^{-1} increased when HLB increased, indicating the increased hydrophilicity and hydrogen bonding capability of the SE of higher HLB. From **Figure 3**, it appears that

the protein content increased slightly with increase of HLB of SE. However, the P value was $0.1395 > 0.05$, when results were compared. These data were therefore considered not statistically significant. The slight difference in protein content among the SE modification was consistent with reported difference in surface tension of 0.1% SE solution from 48.80 to 34.50 mN/m when HLB varied from 6 to 15 (**Table 1**). Hayashi et al¹⁷ and Perumal et al¹⁶ demonstrated that the HLB of emulsifiers influenced the dispersion stability. For instance, Perumal et al¹⁶ were only successful in encapsulating the nonsteroidal anti-inflammatory drug ibuprofen in methacrylic polymers by emulsion solvent diffusion method using 0.025% sucrose ester DK-F70 with a HLB value of 8. However, it is difficult to objectively compare this study's results with those of these authors because operating conditions and data analysis methods were not identical in terms of active ingredients, polymer carrier, method of preparation, etc.

Figure 5 shows the influence of the nature of the emulsion stabilizers used. In this study, SE of HLB 15 was compared with poloxamer 188 and PVA of average MW 10, 70, and 100 kDa. Poloxamer 188 (POL-188) was selected as control because it is widely used as dispersing and wetting agent, emulsion stabilizer, and solubilizer in parenteral applications. PVA was selected for the following reasons. PVA is used primarily in topical pharmaceutical and ophthalmic formulations. It is used (at concentration 0.25%–3%) as a stabilizing agent for emulsions¹⁰ and has been used extensively for the last decade in microencapsulation technology.⁶ **Figure 5** shows that the highest protein content was obtained with SE of HLB 15 (DK-F160). The P value was even less than 0.001 when the later results of the SE were compared with those of other surfactants such as PVAs and POL-188. These data were therefore considered extremely significant. The obtained results may be fundamentally explained by 3 hypotheses: difference in CMC of surfactants, their partition (K), and diffusion coefficient (D).

The difference in CMC hypothesis may be derived from **Table 1**. For example, the reported CMC of poloxamer 188 (0.1%) is less than the operating concentration (0.05%) for this experiment. In these conditions, most poloxamer 188 molecules may be preferentially located at the water/air interface therefore leading to poor emulsion stabilization. PVA is a hydrophilic colloid and may stabilize emulsion by steric repulsion.^{27,28} Concentrations of PVA of up to 10% have been used in microencapsulation of proteins.²⁶ It is also postulated that the tested PVA concentration (0.05%) was not favorable for PVA emulsion stabilization efficiency.

The difference in partition coefficient hypotheses may be adapted from the work of Schurtenberger et al.²⁹ The ef-

Figure 4. ATR-FTIR spectra of sucrose esters of different average degree of esterification: DK-ester F160 (1.23), DK-ester F140 (1.35), DK-ester F110 (1.48), DK-ester F70 (1.60) and DK-ester F50 (1.69). The proportions of mono, di-, and tri-ester are given in **Table 1**.

fect of the surfactant concentration on the stabilization of the microdroplets prior to microparticle hardening may be better understood in the light of an adaptation of the structural surfactant effects in terms of the partition equilibrium and its corresponding partition coefficient (K) defined in Equation 1.

$$K = \frac{S_B}{S_w L} \quad (1)$$

In this case, S_B is the concentration of surfactant incorporated into the oil/water interface; S_w is the surfactant monomer concentration in the aqueous phase; and L is the lipid (or oil phase) concentration. According to the suggested model, K may be used to predict the stability of the dispersion and subsequent leakage of protein from the microdroplets prior to particle hardening. Ideally, the surfactant should be effective if it can be preferentially located at the oil/water interface. Therefore, the lipophilicity or hydrophilicity of the surfactant (expressed as

term of partition coefficient K) is critical for emulsion stabilization at the oil/water interface. Hence, surfactants with high affinity to the oil phase such as poloxamer 188 (solubility in methylene chloride approximately 35% according to manufacturer's product data sheet) (**Table 1**), which are relatively lipophilic, led to very low protein content and encapsulation efficiency (**Figure 5**). Assuming that no poloxamer 188 was soluble in water, calculations showed that, at 0.05% concentration used, the amount of poloxamer 188 in presence of methylene chloride is 1%, which is far less than the 35% solubility limit. Therefore, in addition to the preferential water/air interface location, the surfactant solubilization in the methylene chloride phase may be disadvantageous for the emulsion droplet stabilization that should occur at the oil/water interface. This hypothesis may also explain the slight difference in results among PVAs of different MW (with slight difference in hydrophilicity) and of the overall lower protein content with PVAs since PVAs seems to be less interface active than the SE.

Figure 5. Influence of the nature of the emulsion stabilizer on protein content of polymeric microparticles. The model protein and polymeric carrier were bovine serum albumin and 25/75 poly(lactide-co-glycolide), respectively. The abbreviations of emulsion stabilizers DK-F160, PVA10k, PVA70k, PVA-100k, POL-188, WPVA-70k referred to DK-ester F160, Polyvinyl alcohol of MW 10, 70 and 100KDa, poloxamer 188 and PVA formulated particle after washing, respectively. In all cases, the concentration of the sucrose ester used was 0.05%. The particles were prepared in triplicate by double emulsion solvent evaporation as described in the materials and method section. Results are expressed as average $\pm$ SEM for $n = 3$.

The difference in diffusion coefficient hypotheses may be derived from Stokes-Einstein equations.²⁷ If the colloidal particles can be assumed to be approximately spherical, the following equation (Equation 2) can be used to normally obtain the particle radius, weight, MW, and diffusion coefficient:

$$D = \frac{kT}{6\pi\eta r} \quad (2)$$

in which D is the diffusion coefficient obtained from Flick's law, k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the solvent, r is the radius of the spherical particle (related to particle size and MW).

From **Table 1**, different surfactants tested varied widely in MW from 659 Da to 100 kDa, and the viscosity of the medium (aqueous surfactant containing the aqueous phase) also visually varied. Therefore, it can reasonably be postulated that the surfactants tested have different diffusion coefficient in the aqueous phase toward the oil/water interface. The smallest size surfactant (SE of HLB 15) with probably the highest diffusion coefficient was most effective at lower concentration. These observations were also consistent with surface tension data among 0.1% SE solutions provided in **Table 1**. From a theoretical standpoint, such double emulsion systems are

too complex for any reasonable estimate of repulsive and attractive force to apply the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory.^{27,30} More mechanistic studies involving techniques such as droplet size determination, transmission microscopy-freeze fracture electron microscopy (FFEM), small angle X-ray scattering (SAXS), small angle neutron scattering (SANS), static light scattering (SLS) or intensity light scattering (ILS), dynamic light scattering (DLS), electrophoretic light scattering (ELS), and rheological and chemometric determination of micelle formations³¹ will be required to confirm or optimize these preliminary results.

Figure 6. Typical photomicrograph (magnification $\times 400$) of microparticles prepared using sucrose ester of HLB 16 (DK-F160).

Figure 6 shows the photomicrograph of the microparticles prepared using SE of HLB 15, namely, DK-F160. This picture provides visual evidence of formation of spherical and discrete microparticles with average diameter of 50 μm . **Figure 7** shows the SDS-PAGE of protein sample released from microparticles using sucrose ester of HLB 15. A similar band for BSA was observed for analyzed sample, native BSA, and BSA containing MW standard or markers. This allowed us to conclude that within the limit of detection of Coomassie blue staining, the MW (or primary structure) of the model protein was preserved throughout the preparation process. It is important to point out that further characterizations of the structure and assembly state by techniques such as gel permeation chromatography, FTIR spectroscopy, and/or circular dichroism of different proteins are necessary before any generalizations on protein conformation stability can be made based on these preliminary results. Such analysis should not only

Figure 7. SDS-PAGE of BSA. From left to right lanes. Std = molecular weight markers from 20.1 to 205kDa (bottom to top). Before = native bovine serum albumin (BSA). After = BSA extracted from microparticles prepared using sucrose ester of HLB 15 (DK-F160).

be applied to protein that is released from microparticles but also to protein that does not get released. These considerations are important because proteins' structure is related to their bioactivity; much of the protein could form aggregates (noncovalent and covalent), and the structure of the protein within the particle may be nonnative (unfolded).

CONCLUSION

SE of HLB 6 to 15 led to the obtainment of discrete protein-loaded microparticles with a relatively low encapsulation efficiency (~13.5%). These SE were most effective at a concentration of 0.05% wt/vol (20-200 times less than other approved surfactants). There was a slight increase in protein content with HLB, which was not statistically significant. These results were consistent with ATR-FTIR spectra showing increasing hydrogen-bonding capability with HLB and available surface tension data. The difference in CMC, partition, and diffusion coefficient hypotheses may be used to explain the observed results. These preliminary results suggest that it would be desirable to investigate such surfactants in vitro (synthesis of more surface effective SE and mechanistic studies) and in vivo (eg, hemolytic effect) in order to eventually establish sucrose esters as alternative surfactants for the development of a controlled-release system or for new parenteral formulations for protein and gene medicine.

ACKNOWLEDGEMENTS

This work was supported by financial aid from Texas Tech University Health Sciences Center (TTUHSC), Amarillo, TX. We are grateful to Montello Incorporated, Tulsa, OK, and BASF Corporation, Washington, NJ, for kindly providing the sucrose esters and poloxamer 188, respectively. The photomicrograph was provided by Dr. Girish V. Shah, TTUHSC School of Pharmacy, Amarillo, Texas.

REFERENCES

1. Mathiowitz E, Kreitz MR, Brannon-Peppas L. Microencapsulation. In: Mathiowitz E, ed. *Encyclopedia of Controlled Drug Delivery*. New York, NY: John Wiley & Sons, Inc; 1999:493-546.
2. Botempo JA. *Development of Biopharmaceutical Parenteral Dosage Forms*. New York, NY: Marcel Dekker, Inc; 1997:248.
3. Soderlind E, Wollbratt M, von Corswant C. The usefulness of sugar surfactants as solubilizing agents in parenteral formulations. *Int J Pharm*. 2003;252:61-71.
4. Nema S, Washkuhn RJ, Brendel RJ. Excipients and their use in injectable products. *PDA J Pharm Sci Technol*. 1997;51:166-171.
5. Wang Y-CJ, Hanson MA. Parenterals formulations of proteins and peptides: stability and stabilizers. *J Parenter Sci Technol*. 1987;42:S5-S26.
6. Gupta RK, Singh M, O'Hagan DT. Poly(lactide-co-glycolide) microparticles for the development of single-dose controlled-release vaccines. *Adv Drug Deliv Rev*. 1998;32:225-246.
7. Youan BB, Benoit MA, Baras B, Gillard J. Protein-loaded poly(epsilon-caprolactone) microparticles. I. Optimization of the preparation by (water-in-oil)-in water emulsion solvent evaporation. *J Microencapsul*. 1999;16:587-599.
8. Youan BB, Jackson TL, Dickens L, Hernandez C, Owusu-Ababio G. Protein release profiles and morphology of biodegradable microcapsules containing an oily core. *J Control Release*. 2001;76:313-326.
9. Lacasse FX, Filion MC, Phillips NC, Escher E, McMullen JN, Hildgen P. Influence of surface properties at biodegradable microsphere surfaces: effects on plasma protein adsorption and phagocytosis. *Pharm Res*. 1998;15:312-317.
10. *Pharmaceutical Excipients 2000* [book on CD-ROM]. Washington, DC: American Pharmaceutical Association and Pharmaceutical Press; 2000. Based on: Kibbe AH. *Pharmaceutical Excipients 2000*. Washington, DC: American Pharmaceutical Association and Pharmaceutical Press; 2000.
11. Memisoglu E, Bochot A, Sen M, Charon D, Duchene D, Hincal AA. Amphiphilic beta-cyclodextrins modified on the primary face: synthesis, characterization, and evaluation of their potential as novel excipients in the preparation of nanocapsules. *J Pharm Sci*. 2002;91:1214-1224.
12. Lerk PC, Sucker HH, Eicke HF. Micellization and solubilization behavior of sucrose laurate, a new pharmaceutical excipient. *Pharm Dev Technol*. 1996;1:27-36.
13. Bolzinger MA, Carduner TC, Poelman MC. Bicontinuous sucrose ester microemulsion: a new vehicle for topical delivery of niflumic acid. *Int J Pharm*. 1998;176:37-39.

14. von Rybinski W, Hill K. Alkyl polyglycosides. In: Holmberg K, ed. *Novel Surfactants: Preparation, Applications, and Biodegradability*. New York, NY: Marcel Dekker, Inc; 1998:31-85.
15. Bagwe RP, Kanicky JR, Palla BJ, Patanjali PK, Shah DO. Improved drug delivery using microemulsions: rationale, recent progress, and new horizons. *Crit Rev Ther Drug Carrier Syst*. 2001;18:77-140.
16. Perumal D, Dangor CM, Alcock RS, Hurbans N, Moopanar KR. Effect of formulation variables on in vitro drug release and micromeritic properties of modified release ibuprofen microspheres. *J Microencapsul*. 1999;16:475-487.
17. Hayashi Y, Yoshioka S, Aso Y, Li Wan Po A, Terao T. Entrapment of proteins in poly(L-lactide) microspheres using reversed micelle solvent evaporation. *Pharm Res*. 1994;11:337-340.
18. Uchebgu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int J Pharm*. 1998;172:33-70.
19. Herrington TM, Sahi SS. Temperature dependence of the micellar aggregation number of aqueous solutions of sucrose monolaurate and sucrose monooleate. *Colloids Surf*. 1986;17:103-113.
20. Katakam M, Bell LN, Banga AK. Effect of surfactants on the physical stability of recombinant human growth hormone. *J Pharm Sci*. 1995;84:713-716.
21. Lide DR. *Handbook of Chemistry and Physics*. 80th ed. Boca Raton, FL: CRC Press LLC; 1999.
22. Merck & Co. I. *The Merck Index*. 13th ed. Whitehouse Station, NJ: Merck & Co, Inc; 2001.
23. Dai-Ichi Kogyo Seyaku Co Ltd. DK Ester. Sucrose fatty acid ester. [material data sheet/technical note]. Tokyo, Japan: Dai-Ichi Kogyo Seyaku Co Ltd; 1985:13.
24. BASF Corporation. Lutrol F 68. Poloxamer 188 for the pharmaceutical industry. [material data sheet]. Ludwigshafen, Germany: BASF Corporation; 1997:4.
25. Sah H. Protein behavior at the water/methylene chloride interface. *J Pharm Sci*. 1999;88:1320-1325.
26. Jeffery H, Davis SS, O'Hagan DT. The preparation and characterization of poly(lactide-co-glycolide) microparticles. II. The entrapment of a model protein using a (water-in-oil)-in-water emulsion solvent evaporation technique. *Pharm Res*. 1993;10:362-368.
27. Martin A, Bustamante P, Chun AHC. *Physical Pharmacy*. 4th ed. Philadelphia, Pa: Lippincott, Williams & Wilkins; 1993:622.
28. Rosen MJ. *Surfactants and Interfacial Phenomena*. New York, NY: John Wiley & Sons, Inc; 1989:429.
29. Schurtenberger P, Mazer N, Kanzig W. Micelle to vesicle transition in aqueous solutions of bile salt and lecithin. *Phys Chem*. 1985;89:1042-1049.
30. Florence AT, Whitehill D. The formulation and stability of multiple emulsions. *Int J Pharm*. 1982;11:277-308.
31. Tenjarla S. Microemulsions: an overview and pharmaceutical applications. *Crit Rev Ther Drug Carrier Syst*. 1999;16:461-521.