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Formulation of Span 80 niosomes modified with SDS for lactic acid entrapment

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ABSTRACT

This study examines the effects of different formulation parameters on the physico-chemical properties of niosomes containing Span 80 (sorbitan monooleate), cholesterol and/or SDS (sodium dodecyl sulfate), and lactic acid for the future use of formulated niosomes as lactic acid extraction agents in aqueous solutions. Niosomes were prepared by direct ultrasonication of the aqueous samples containing all the aforementioned components. Results revealed that SDS acts as a niosome stabilizer that can be used as a substitute of cholesterol, because it increased the zeta potential absolute value while decreased the particle size. Additionally, SDS also increased the lactic acid entrapment efficiency, which indicates that Span 80 niosomes modified with SDS can be used as selective extraction agents for lactic acid present in aqueous solutions at low concentrations. The best formulation, based on niosome stability and maximum lactic acid entrapment efficiency, was obtained for 20 mol/m^3 of Span 80 + 2 mol/m^3 SDS + 10 mol/m^3 lactic acid, leading to niosomes with 36% of lactic acid entrapment efficiency, −47 mV of zeta potential, and 156 nm of hydrodynamic size.

Keywords: Niosomes; Ultrasonication; Lactic acid; Span 80; SDS; Centrifugal ultrafiltration

1. Introduction

Niosomes are vesicles formed by one or more non-ionic surfactant bilayers enclosing an aqueous inside cavity. Usually, they are thermodynamically unstable and require energy input for their formation. Niosomes, like liposomes, are able to encapsulate both hydrophobic and hydrophilic compounds inside their core and in the bilayer, respectively. Nevertheless, from a technical point of view, niosomes are preferred because of their greater chemical stability, high purity, low cost, content uniformity, and their easy handling and storage, which overcome the drawbacks associated with liposomes [1–4]. Another advantage is the simple method for the large-scale production of niosomes without the use of unacceptable solvents, so they are widely used in pharmaceutical, cosmetic, and, to a lesser extent, food applications [5–9].

There are large numbers of non-ionic surfactants available, which are non-toxic and relatively low-cost materials for niosome design, greatly increasing the

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attractiveness of these vesicles for industrial production [10,11]. Encapsulation efficiency depends on several factors such as the type of structure (multilamellar, unilamellar, or multivesicular), the length of the hydrophobic group of surfactant forming the bilayer, the nature and size of the hydrophilic head, pH and composition of the formulation medium, and the nature of the solute (hydrophobicity, polarity, molecular weight, and structure). Several methods are used for niosome formation which mainly depends on solute to encapsulate [8,12]. The most used methods are hydration of a dry lipid film, phase inversion, and sonication, which is the method used in the present work.

In general, surfactants with a high hydrophilic-lipophilic balance (HLB) are likely to be micelle-forming surfactants and need additives to achieve suitable molecular geometry and hydrophobicity for bilayer vesicle formation [13–15]. Membrane additives are substances added to the formulation in order to stabilize the niosomes. The most common additive found in niosomal systems is cholesterol, which is known to influence the physical properties and structure of niosomes because of its interaction with the hydrophobic portion of the amphiphile [16,17]. The most important effect is the modulation of the mechanical strength of the bilayers and their permeability to water. Several non-ionic surfactants form vesicles only after cholesterol addition. The amount of cholesterol to be added depends on the HLB value of the surfactant and the loaded solute, and it needs to be evaluated case by case.

Vesicle formation without cholesterol addition only occurred for surfactants with a low HLB at a relatively high surfactant concentration. Non-ionic surfactants with long alkyl chains (C_{18}) but a high HLB, such as Tween 61, cannot form vesicles without cholesterol because of their large hydrophilic head groups. However, the addition of cholesterol 1:1 molar ratio yields high hydrophobic moiety as well as high hydrophilic moiety, which are excellent conditions for the entrapment of hydrophilic substances [18]. Non-ionic surfactants such as Tween with long ethylene oxide chain lengths are more likely to be well hydrated, leading to an increased permeability to solutes, while more hydrophobic surfactants such as Span form more compact niosomes when hydrated in the presence of cholesterol [19,20]. In a recent work, we have studied the entrapment efficiencies of lactic acid and citric acid by Tween 80/cholesterol niosomes modified with TOMAC (tri-n-octylmethylammonium chloride), where the higher citric acid than lactic acid entrapment efficiency is explained in terms of interactions within the solubilization sites of the niosomes [21].

Another niosome stabilization method is to add a charged molecule to the bilayer. Normally, the charged molecule is added in a niosomal formulation at an amount lower than 5 mol%, because the high concentration of charged molecules could inhibit the formation of niosomes [22–24]. Particle aggregation is less likely to occur for charged particles due to their electric repulsion. Zeta potential absolute values higher than 30 mV are usually needed for full electrostatic stabilization; zeta potentials between 5 and 15 mV represent the region of limited flocculation, whereas between 3 and 5 mV correspond to the maximum flocculation [9].

In this work, lactic acid was used as a solute model because of its importance in biotechnology and food industry [25,26]. It is used as a food preservative, antioxidant, and flavor enhancer in many foods [27–29]. Furthermore, it is also used in the synthesis of new biodegradable polymers with biomedical applications. In the cosmetics sector, it is a substitute for glycerin and is added to creams and gels because of its ability to improve skin texture and tone. Lactic acid is usually obtained by fermentation, and its recovery from waste streams or fermentation broths is usually made by solvent extraction [30]. However, research on new extractants for increasing the selectivity and lowering the cost of separation is needed. Lactic acid extraction from aqueous solutions can be performed using surfactants as extraction agents and membrane technology; these processes are considered to be clean technologies as they have the advantages of large-scale continuous separation without phase change, avoiding the use of organic solvents.

In previous works, we studied the recovery of several biocompounds [31,32] including lactic acid and citric acid with SDS by micellar-enhanced ultrafiltration [33]. Based on these previous results, in this work we investigate the feasibility of using niosomes modified with SDS as extraction agents of lactic acid in aqueous solutions. This work shows the results of the first step of the research, where the effect of different niosome formulations including Span 80 (sorbitan monooleate) as the encapsulating surfactant with and without cholesterol, sodium dodecyl sulfate (SDS) as a membrane modifier, and lactic acid as a loaded solute has been investigated. In the second step, kinetics and equilibrium capacities of niosomes for lactic acid extraction under different medium conditions are now being investigated and will be soon published.

2. Materials and methods

2.1. Materials

Lactic acid (2-hydroxypropionic acid, >99.5% purity) was supplied by Fluka (Germany). Cholesterol

(>95%), the non-ionic surfactant Span 80 (sorbitan monooleate, >99%), and the anionic surfactant SDS (>99%) were provided by Sigma-Aldrich (Germany). Other chemicals such as methanol (HPLC grade, HiPerSolv Chromanorm), ethanol (HPLC grade, Lichrosolv), maleic acid (>99%, Fluka), phosphoric acid (>85%, Aldrich), disodium hydrogen phosphate dodecahydrate (>98%, Panreac), and potassium dihydrogen phosphate (>99.5%, Merck) were used throughout the experiments. Laboratory-grade chemicals without further purification were used as supplied in all cases. Milli-Q water (Millipore, USA) was used to prepare all formulations. Fig. 1 shows the molecular structure of the surfactants used in this work.

2.2. Niosome preparation

Niosomes were prepared by direct ultrasonication of the aqueous samples. A high-intensity ultrasonic processor (Vibra-Cell VCX 500, Sonics & Materials Inc., USA) was used. Experiments were performed with a 3-mm-diameter titanium alloy bicylindrical probe over a 5-min effective time, with pulses every 5 s (5 s on and 5 s off, 60 cycles; 30% amplitude, 500 W) to avoid overheating of the sample. Exceptionally, some samples were treated for 10 min. Samples were prepared in an Erlenmeyer flask, weighing out the exact amounts on an analytical balance (Sartorius, accurate to ±0.0001 g), with the addition of deionized water up to the final volume of 10 cm^3. The samples were shaken until completely dissolved and stopped for one day. Their formulations are shown in Tables 1 and 2. For the ultrasonication process, samples were placed in round-based polystyrene tubes, 115 mm in height and 29 mm in diameter, supplied by Labbox (Spain). The 1 cm skirt at the base of tubes assisted homogeneous probe positioning in all samples. Throughout the ultrasonication process, the samples were immersed in an ice bath to prevent chemical degradation. Temperature of the process was lower than 70˚C, well above the gel–liquid phase transition temperature. Following ultrasonication, the sample was left to stand for an hour to avoid any creaming or coalescence effect. The final volume was then measured in order to consider the evaporation of the sample for the efficiency calculation. Subsequently, the sample was centrifuged (Eppendorf 5804 centrifuge) in 15 cm^3 polystyrene centrifuge tubes for 45 min at 9,000 rpm in order to remove traces of metal detached from the probe.

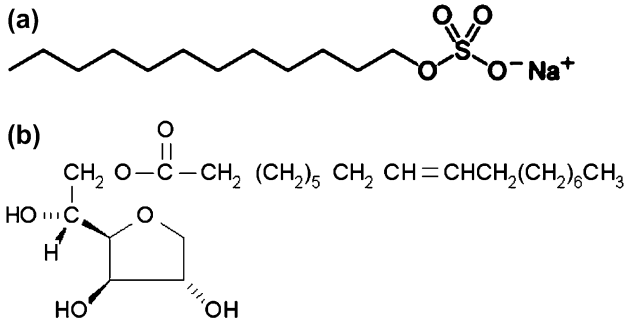

Fig. 1. Molecular structure of surfactants: (a) SDS and (b) Span 80.

2.3. pH measurement

The pH was measured with a glass pH electrode (Crison, Spain). The pH measurement was performed at 25˚C with an error of ±0.01 pH units.

2.4. Particle size measurement

The particle size distribution, the mean hydrodynamic diameter, and the polydispersity index (PDI) of the samples were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS apparatus (Malvern Instruments Ltd., UK). The apparatus was equipped with a He–Ne laser emitting at 633 nm and with a 4.0 mW power source. It was set for backscattering detection at a scattering angle of 173º. Samples (2 cm^3) were diluted 1:100 to avoid multiple scattering effects, and measurements were performed in DTS0012 square disposable polystyrene cells at 25˚C. The path length of the light was automatically set by the apparatus in accordance with the turbidity of each sample. The equipment software uses the cumulative method for a polynomial fit of the correlation function and for the calculation of the translational diffusion coefficient of the particle. The hydrodynamic diameter was calculated using the Stokes–Einstein equation, on the assumption that the particles were monodisperse spheres [34].

Three replicates, each of 20 runs, were performed for each sample. The values shown in the figures and tables are the average values of the three replicates with the relative measurement error. The PDI is a dimensionless measure of the width of the size distribution ranging from 0 to 1, a higher value being indicative of a broader distribution of particle size [35].

2.5. Zeta potential measurement

Zeta potential measurements were performed with the aforementioned Zetasizer Nano ZS apparatus

Table 1
Formulations and results of the characterization of samples containing Span 80, cholesterol, SDS, and lactic acid in pH 7 phosphate buffer, and treated with 5 min of ultrasounds. EE is the lactic acid entrapment efficiency

Formulation					Results of characterization			
Experiment	Span 80 ± 0.1 (mol/m^3)	Cholesterol ± 0.1 (mol/m^3)	SDS ± 0.1 (mol/m^3)	Lactic acid (mol/m^3)	EE (%)	Particle size (nm)	PDI	Z Potential (mV)
1	10.3	0	0	7.38 ± 0.03	3.93 ± 1.45	202.67 ± 0.99	0.37 ± 0.01	−36.01 ± 2.35
2	10.6	0	4.5	7.35 ± 0.03	10.84 ± 0.10	101.10 ± 1.34	0.22 ± 0.01	−55.38 ± 2.07
3	10.4	10.0	0	0	0	283.16 ± 3.04	0.11 ± 0.03	−68.46 ± 1.06
4	10.1	10.1	0	7.27 ± 0.27	6.52 ± 0.35	286.50 ± 3.64	0.06 ± 0.01	−68.30 ± 1.62
5	20.1	0	0	8.20 ± 0.04	5.94 ± 0.26	183.80 ± 2.83	0.32 ± 0.01	−25.05 ± 1.39
6	20.4	0	4.2	8.32 ± 0.22	23.31 ± 0.36	99.40 ± 2.65	0.13 ± 0.02	−55.29 ± 2.12
7	20.2	10.0	0	8.59 ± 0.15	12.39 ± 0.18	267.93 ± 9.30	0.06 ± 0.02	−61.30 ± 0.73
8	20.3	10.0	4.1	8.47 ± 0.43	22.40 ± 0.53	124.93 ± 2.35	0.09 ± 0.02	−57.15 ± 1.71
9	20.4	7.3	0	8.20 ± 0.06	8.34 ± 0.13	281.60 ± 8.32	0.05 ± 0.03	−60.33 ± 1.52
10	20.2	5.1	0	8.21 ± 0.08	9.10 ± 0.64	268.13 ± 8.91	0.10 ± 0.03	−59.02 ± 2.48

using the Laser Doppler Velocimetry technique. In this technique, an electrical current is applied across a pair of electrodes placed at both ends of a DTS1061 disposable folded capillary cell containing the particle dispersion. Charged particles are attracted to the oppositely charged electrodes and their velocity is measured and expressed per unit of field strength in terms of electrophoretic mobility. Then, the zeta potential is calculated by using Henry's equation and the Smoluchowski approximation, which considers that the double layer thickness is much smaller than the particle size [36]. Six replicates of 11 measurements were performed for each sample at 20°C. Figures and tables show average values of the six runs with the relative measurement error.

2.6. Morphological analysis

Morphological analysis of niosomes was performed by negative staining transmission electron microscopy, using a JEOL-2000 EX-II TEM operating at 160–180 kV, with an image resolution of 1 nm, located at the University of Oviedo (Spain). A drop of the selected niosome formulation was placed on a carbon-coated copper grid, and the sample excess was removed using a piece of filter paper. Then, a drop of phosphotungstic acid solution (2% w/v) was applied to the carbon grid and left for 2 min. Once the excess of staining agent was removed by absorbing with the filter paper, the sample was air-dried and the thin film of the stained niosomes was observed by TEM.

2.7. Lactic acid entrapment efficiency measurement

Free components were separated from niosomes by centrifugal ultrafiltration (5 min, 7,500 rpm) using Amicon Ultra-4 units (Millipore) with 10 kDa cutoff-regenerated cellulose membranes. Lactic acid concentrations in the initial samples and permeates were determined by high-performance liquid chromatography using a HPLC Beckman System Gold model. A reverse-phase column ACE 5C18 (ACE HPLC columns) and a UV–vis detector at 216 nm were used. The mobile phase was an aqueous solution of 0.17 vol% phosphoric acid and 0.16 wt% potassium dihydrogen phosphate with a flow rate of 1 mL/min. Maleic acid was used as an internal standard. Ten standards between 0.5 and 16 mol/m^3 of lactic acid concentration were prepared for the calibration and were analyzed by triplicate. The detection limit of the method was 0.005 mol/m^3. Samples were measured by triplicate, and the analytical error was lower than ±0.001 mol/m^3.

Lactic acid entrapment efficiency was estimated as the ratio between the amount of the acid entrapped in the niosomes and its total amount in the formulation, and it was calculated by considering a total retention of niosomes by the membrane by the following equation:

$$\mathrm{EE}\ (\%) = \left(\frac{(C_r - C_p)\frac{V_r}{V_{ini}} V_{US}}{C_F V_F} \right) \times 100 \quad (1)$$

where C_F, C_r, and C_p are the lactic acid concentrations in the initial formulation (before ultrasonication), the retentate and the permeate after centrifugal ultrafiltration, respectively, and V_F, V_{US}, V_{ini}, and V_r correspond to the initial volume of the sample (10 cm^3), its volume after ultrasonication, aliquots of supernatant (4 cm^3) for centrifugal ultrafiltration, and retentate volume after ultrafiltration, respectively.

Table 2
Formulations and results of the characterization of samples containing Span 80, SDS, and lactic acid in deionized water (samples without cholesterol), and treated with 5 min of ultrasounds. EE is the lactic acid entrapment efficiency

Formulation					Results of characterization				
Experiment	Span 80 ± 0.1 (mol/m^3)	SDS ± 0.1 (mol/m^3)	Lactic acid ± 0.01 (mol/m^3)	pH ± 0.01	Lactic acid entrapped (mol/m^3)	EE (%)	Particle size (nm)	PDI	Z Potential (mV)
1	20.1	0	10.20	2.77	0.05 ± 0.00	0.44 ± 0.01	207.40 ± 14.21	0.197 ± 0.044	−41.20 ± 3.14
2	20.1	0	20.40	2.57	0.22 ± 0.00	1.68 ± 0.00	206.60 ± 2.21	0.209 ± 0.200	−30.50 ± 2.32
3	20.0	0	30.60	2.46	1.35 ± 0.00	4.20 ± 0.00	215.10 ± 4.45	0.133 ± 0.010	−33.90 ± 1.97
4	20.2	0	40.80	2.37	3.78 ± 0.00	14.68 ± 0,00	176.10 ± 2.48	0.174 ± 0.000	−25.20 ± 1.33
5	20.1	0	61.19	2.26	8.46 ± 0.00	22.73 ± 0.00	185.10 ± 1.77	0.140 ± 0.004	−20.40 ± 1.04
6	20.0	0	81.59	2.20	13.49 ± 0.00	24.27 ± 0.00	198.30 ± 3.25	0.190 ± 0.001	−22.30 ± 2.73
7	20.2	2.0	9.88	2.45	2.27 ± 0.01	36.32 ± 0.06	156.00 ± 0.26	0.222 ± 0.004	−47.50 ± 0.90
8	20.2	2.1	19.75	1.71	1.63 ± 0.00	13.20 ± 0.00	146.60 ± 0.92	0.237 ± 0.023	−43.50 ± 0.37
9	20.0	2.0	29.63	1.50	0.54 ± 0.00	2.89 ± 0.01	143.80 ± 1.42	0.233 ± 0.005	−41.40 ± 1.96
10	20.1	2.1	39.51	1.42	0.34 ± 0.00	1.36 ± 0.00	149.00 ± 0.64	0.234 ± 0.009	−42.30 ± 2.34
11	20.1	2.1	59.26	1.39	0.39 ± 0.00	1.05 ± 0.00	139.40 ± 2.19	0.238 ± 0.009	−43.50 ± 0.95
12	20.1	2.2	79.01	1.36	0.05 ± 0.00	0.11 ± 0.00	152.10 ± 0.21	0.229 ± 0.033	−45.80 ± 1.86
13	20.0	4.1	10.17	3.22	0.87 ± 0.00	13.47 ± 0.02	142.30 ± 0.77	0.296 ± 0.040	−30.40 ± 0.30
14	20.1	4.2	20.34	2.35	0.22 ± 0.00	1.71 ± 0.01	192.20 ± 4.92	0.393 ± 0.035	−32.20 ± 1.99
15	20.0	4.0	30.51	2.15	0.32 ± 0.00	1.69 ± 0.01	140.60 ± 2.47	0.311 ± 0.030	−31.10 ± 0.99
16	20.0	4.0	40.69	2.12	0.05 ± 0.00	0.20 ± 0.00	140.10 ± 2.16	0.322 ± 0.056	−30.40 ± 2.69
17	20.2	4.0	61.03	1.92	0.56 ± 0.00	1.45 ± 0.00	156.70 ± 2.19	0.419 ± 0.030	−34.70 ± 1.50
18	20.1	4.1	81.37	1.80	0.16 ± 0.00	0.32 ± 0.00	139.60 ± 0.21	0.324 ± 0.056	−31.10 ± 3.83
19	20.0	10.0	9.72	2.63	0.55 ± 0.00	8.76 ± 0.00	129.90 ± 3.39	0.434 ± 0.006	−36.80 ± 2.48
20	20.1	10.0	19.44	1.71	0.08 ± 0.00	0.66 ± 0.00	158.40 ± 0.56	0.298 ± 0.028	−31.70 ± 3.14
21	20.1	10.1	29.16	1.65	0.13 ± 0.01	0.68 ± 0.03	143.40 ± 2.83	0.340 ± 0.045	−33.40 ± 3.26
22	20.0	10.2	38.88	1.51	0.07 ± 0.01	0.29 ± 0.02	137.30 ± 0.21	0.301 ± 0.003	−31.80 ± 2.53
23	20.0	10.2	58.32	1.43	0.23 ± 0.00	0.59 ± 0.00	142.10 ± 1.27	0.289 ± 0.008	−32.80 ± 0.90
24	20.2	10.1	77.76	1.33	0.09 ± 0.00	0.18 ± 0.00	154.00 ± 0.56	0.345 ± 0.036	−34.30 ± 3.41

The lactic acid concentration in the retentate after centrifugal ultrafiltration, C_r, was estimated by the following mass balance:

$$C_r = \left(\frac{\frac{V_{ini}}{V_{US}} C_F V_F - C_p V_p}{V_r} \right) \quad (2)$$

Substitution of the above equation in Eq. (1) yields the following equation for the estimation of the lactic acid entrapment efficiency:

$$EE\,(\%) = \left(1 - \frac{C_p V_{US}}{C_F V_F} \right) \quad (3)$$

2.8. Conductivity measurements

Niosomes were prepared by 10 min of ultrasounds on formulations containing Span 80 (0–1 molar fraction) and SDS (0–1 molar fraction) in Milli-Q deionized water (conductivity <1 μS/cm). The total concentration of surfactants in all aqueous samples was 20 mol/m^3. Conductivity measurements were determined at room temperature using a Crison Basic 30 conductimeter (Crison, Spain) with a cell constant of 1 cm. Five measurements were made for each sample, and the error was lower than 0.01 mS/cm. Conductivity experimental data were normalized by subtracting the conductivity of water used in each experiment.

3. Results and discussion

The best formulation for lactic acid-loaded niosomes will be those leading to stable niosomes with the maximum lactic acid concentration entrapped inside them. As entrapment efficiency depends on the initial lactic acid used in formulation, according to Eq. (3), it was also included as a characterization parameter. Niosome size is of paramount importance because it defines the mass transfer area in the lactic acid extraction process, which is the final objective of these formulation experiments. The homogeneity of the population size is expressed by the PDI. A low PDI value indicates a narrow distribution of niosome sizes, while high PDI values indicate a niosome population of heterogeneous sizes with tendency to particle aggregation. Zeta potential is an indicator of the stability of niosomes by repulsion between negatively charged particles, in this case. For these reasons, the optimal formulation will be those leading to maximize the lactic acid concentration entrapped inside niosomes and its entrapment efficiency, and also to minimize the niosome size, PDI, and zeta potential (or to maximize the zeta potential absolute value).

3.1. SDS vs. cholesterol in niosomes

First, we analyze the effect of cholesterol (0 and 10 mol/m^3) and SDS (0 and 4 mol/m^3) presence in formulations containing Span 80 (20 mol/m^3) and lactic acid (8 mol/m^3) in pH 7 phosphate-buffered medium (experiments 5–10 in Table 1). Fig. 2(a) shows that 4 mol/m^3 SDS addition increased the lactic acid entrapment efficiency from 6 to 23% in formulations without cholesterol (experiments 5 and 6 in Table 1) and from 12 to 22% in formulations with 10 mol/m^3 cholesterol (experiments 7 and 8 in Table 1). Fig. 2(b) shows that 4 mol/m^3 SDS presence decreased the size of niosomes formulated without and with cholesterol. PDI values were lower than 0.2 for all formulations shown in Table 1, indicating a uniform-size niosome population, except for the sample formulated without cholesterol or SDS which has a PDI higher than 0.3. Negative zeta potential values were obtained in all samples (Fig. 2(c)) as a consequence of the net negative charge at the niosome surface. Comparison of the two formulations without cholesterol (experiments 5 and 6 in Table 1) shows that the zeta potential absolute value was significantly higher in the formulation with 4 mol/m^3 of SDS. A similar trend is observed in formulations with 10 mol/m^3 of Span 80 (experiments 1 and 2 in Table 1) indicating that the anionic nature of the Span 80 niosomes does not affect the adsorption of the SDS anionic surfactant, and hence hydrophobic and van der Waals forces are responsible for the interactions between the SDS and Span 80 in the bilayer.

The effect of different cholesterol concentrations on formulations of Span 80 (20 mol/m^3) and lactic acid (8 mol/m^3) without SDS is also observed in Fig. 2 (experiments 5, 7, 9, and 10 in Table 1). Cholesterol increased the lactic acid entrapment efficiency, niosome size, and the zeta potential absolute value, which varied from 6%, 181 nm, and −25 mV in formulations without cholesterol (experiment 5) to 12%, 260 nm, and −61 mV in the 10 mol/m^3 cholesterol formulation (experiment 7), respectively. These results are in agreement with those obtained by other researchers for the entrapment of hydrophilic solutes in niosomes, such as calcein [1,18], salicylic acid [3], mannitol [37], cromolyn sodium [38], and atenolol [39].

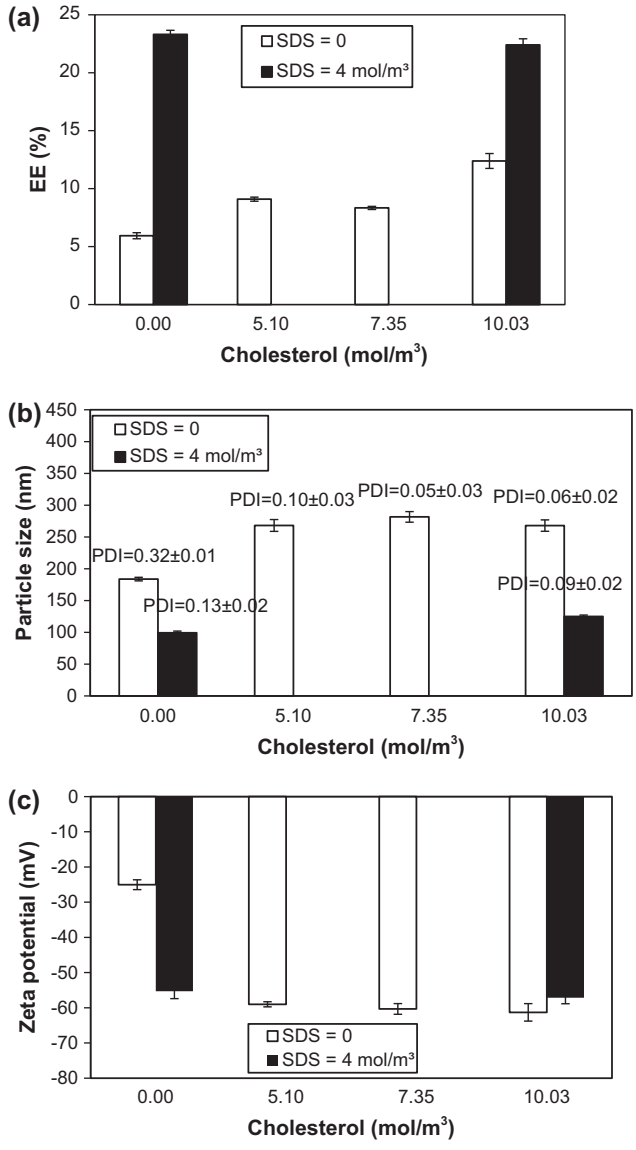

Fig. 2. Effect of cholesterol and SDS addition to formulations of Span 80 (20 mol/m^3) + cholesterol (0–10 mol/m^3) + SDS (0–4 mol/m^3) + lactic acid (8 mol/m^3) in pH 7 phosphate buffer: (a) Lactic acid entrapment efficiency, (b) mean diameter and PDI, and (c) zeta potential (Experiments 5–10 in Table 1).

3.2. Effect of Span 80 concentration

The effect of the Span 80 concentration is shown in Fig. 3 where the basic formulations of Span 80 (10 or 20 mol/m^3) and lactic acid (7–8 mol/m^3) (experiments 1 and 5 in Table 1) are compared to those having cholesterol (experiments 4 and 7) or SDS (experiments 2 and 6). As it is shown in Fig. 3(a), the highest lactic acid entrapment efficiency (23.31%) was obtained for the formulation of 20 mol/m^3 Span 80 with 4 mol/m^3 SDS (experiment 6). Fig. 3(b) and (c) shows that cholesterol or SDS addition stabilized the niosomes as it decreased the PDI and increased the zeta potential absolute value, thus increasing the stability of niosomes against coalescence; however, cholesterol increased and SDS decreased the average diameter of the formulated niosomes.

Experimental results depicted in Figs. 2 and 3 lead us to conclude that SDS has a stabilizing effect on lactic acid-loaded niosomes, and it can be used instead of cholesterol in the formulations. SDS also has a synergistic effect on lactic acid entrapment efficiency, being Span 80 (20 mol/m^3) and SDS (4 mol/m^3) an adequate formulation for its future use in lactic acid extraction from aqueous solutions.

3.3. Effect of the lactic acid and SDS concentrations

Next, the influence of the lactic acid and SDS concentrations was investigated in formulations with Span 80 (20 mol/m^3) and SDS (0–10 mol/m^3) in deionized water. It is well known [40] that the presence of salts decreases the CMC (critical micelle concentration) of both ionic and non-ionic surfactants. At concentrations sufficiently below the CMC of SDS, the formation of the niosomal bilayer is facilitated in buffered medium, as used in experiments presented in Table 1, which provides more uniform particles in size (PDI < 0.2). However, in this work, once the formulation of Span 80 (20 mol/m^3) with SDS as a substitute of cholesterol was selected, the following experiments were performed using deionized water, as it must be considered that, according to the goal of this work, simplification of the formulation medium is a key factor for the economy of the extraction process.

Niosomes of Table 2 were prepared by 5 min direct ultrasonication, as described in Section 2.2. Fig. 4 shows the scatter plots. It is observed in Fig. 4(a) that formulations with SDS had smaller particle size than those without SDS, having the 2 mol/m^3 SDS formulations, the highest absolute zeta potentials. Fig. 4(b) shows that SDS presence in formulations increased the PDI, indicating a more heterogeneous-size population of niosomes. Fig. 4(c) shows that the highest values of lactic acid concentration entrapped in niosomes corresponded to formulations without SDS and high initial lactic acid content; however, the lactic acid concentration entrapped is higher for 2 mol/m^3 SDS formulations and low initial lactic acid content, having these formulations of high absolute zeta potentials. A similar trend is observed in Fig. 4(d) for the lactic acid entrapment efficiency.

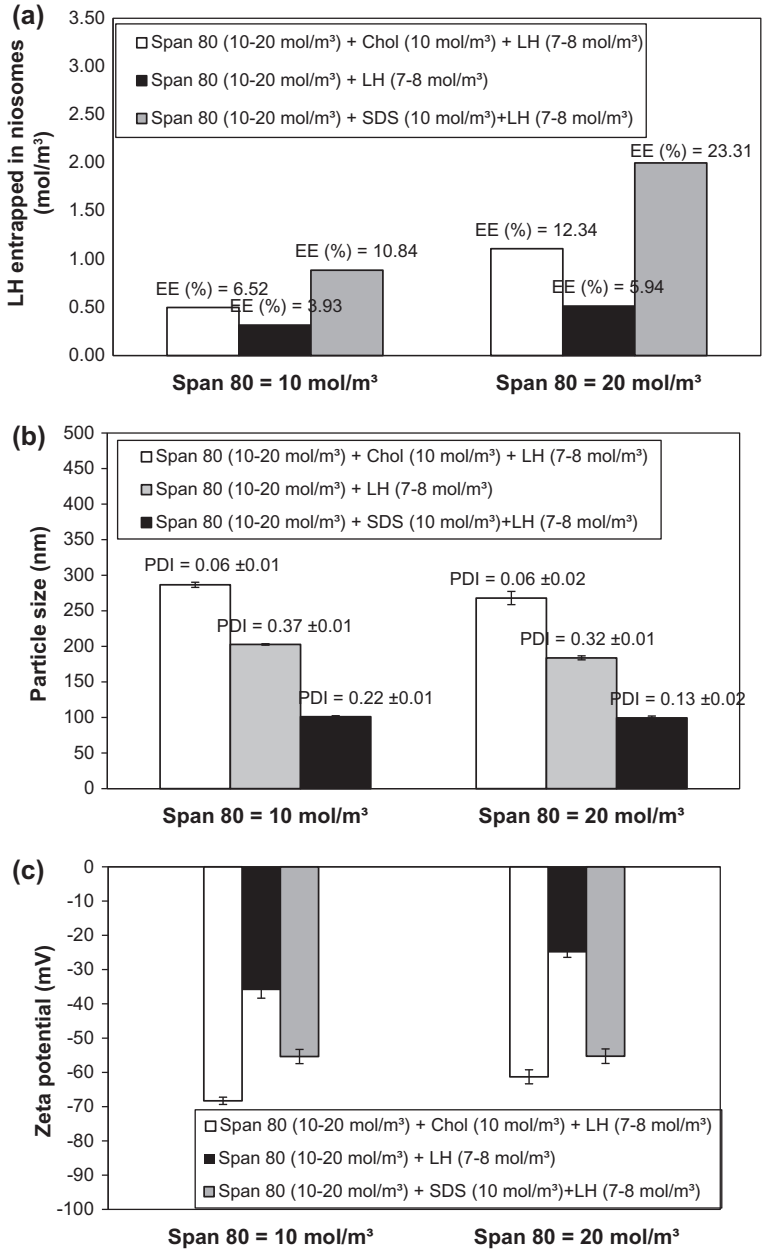

Fig. 3. Effect of Span 80 concentration in formulations of Span 80 (10 and 20 mol/m^3) + cholesterol (0 and 10 mol/m^3) + SDS (0 and 4 mol/m^3) + lactic acid (7 mol/m^3): (a) Lactic acid concentration entrapped in niosomes (entrapment efficiency is also indicated), (b) niosome mean diameter and PDI, and (c) zeta potential (Experiments 1, 2, 4, 5, 6, and 7 in Table 1).

The effect of the initial lactic acid concentration is more clearly observed in Fig. 5, where the entrapment efficiency increased with the lactic acid concentration in systems without SDS and decreased in systems with SDS. It is also observed that an increase of SDS content from 2 to 10 mol/m^3 results in a decrease of the lactic acid entrapment efficiency.

Taking into account that SDS molecules bind to the vesicles by its hydrophobic moiety [14] and that the pH of all formulations of Table 2 is lower than the pKa (pKa = 3.84 for lactic acid), the increased lactic acid entrapment efficiency for niosomes modified with SDS in formulations with low lactic acid concentration can be attributed to the complex formation at the external interface by hydrogen bonds formation between the

SDS adsorbed on the niosome surface and the lactic acid protonated species. These results indicate that niosomes of Span 80 (20 mol/m^3) modified with SDS at low concentration (2 and 4 mol/m^3) could be good systems for lactic acid extraction from aqueous solutions at low acid concentrations. Such preferential hydrogen bonds between SDS and lactic acid could modify the structure of the niosome membrane bilayer hindering the entrapment of the lactic acid in its inside cavity, as occurring in niosomes without SDS (experiments 1–6 in Table 2) where the entrapment efficiency increases with the lactic acid concentration in the solution, as shown in Fig. 5.

Differences in zeta potential values are observed between formulations of Tables 1 and 2. As it was mentioned in Section 3.1 for formulations of Table 1 in the absence of cholesterol (experiments 1–2 and 5–6), the zeta potential absolute values were significantly higher in the formulations with 4 mol/m^3 of SDS. However, this behavior was not observed for formulations 1–13 and 3–15 in Table 2 and differences can be attributed to the formulation medium. At pH 7 (experiments in Table 1), the lactic acid is present as a lactate ion and it is entrapped into the internal aqueous cavity of niosomes. However, experiments in Table 2 were made at a natural pH (<pKa) where the entrapment of protonated species of the lactic acid occurs on the surface of niosomes. The different sites for lactic acid entrapment affect the configuration of the Stern layer surrounding the particles, and therefore the zeta potential values.

3.4. Effect of the SDS concentration

As the results of the influence of SDS concentration were inconclusive, conductivity assays were performed for lactic acid-unloaded niosomes formulated with Span 80 and SDS in all ranges of their concentrations, from 0 to 1 in molar fraction. The total concentration of both surfactants in all water samples was

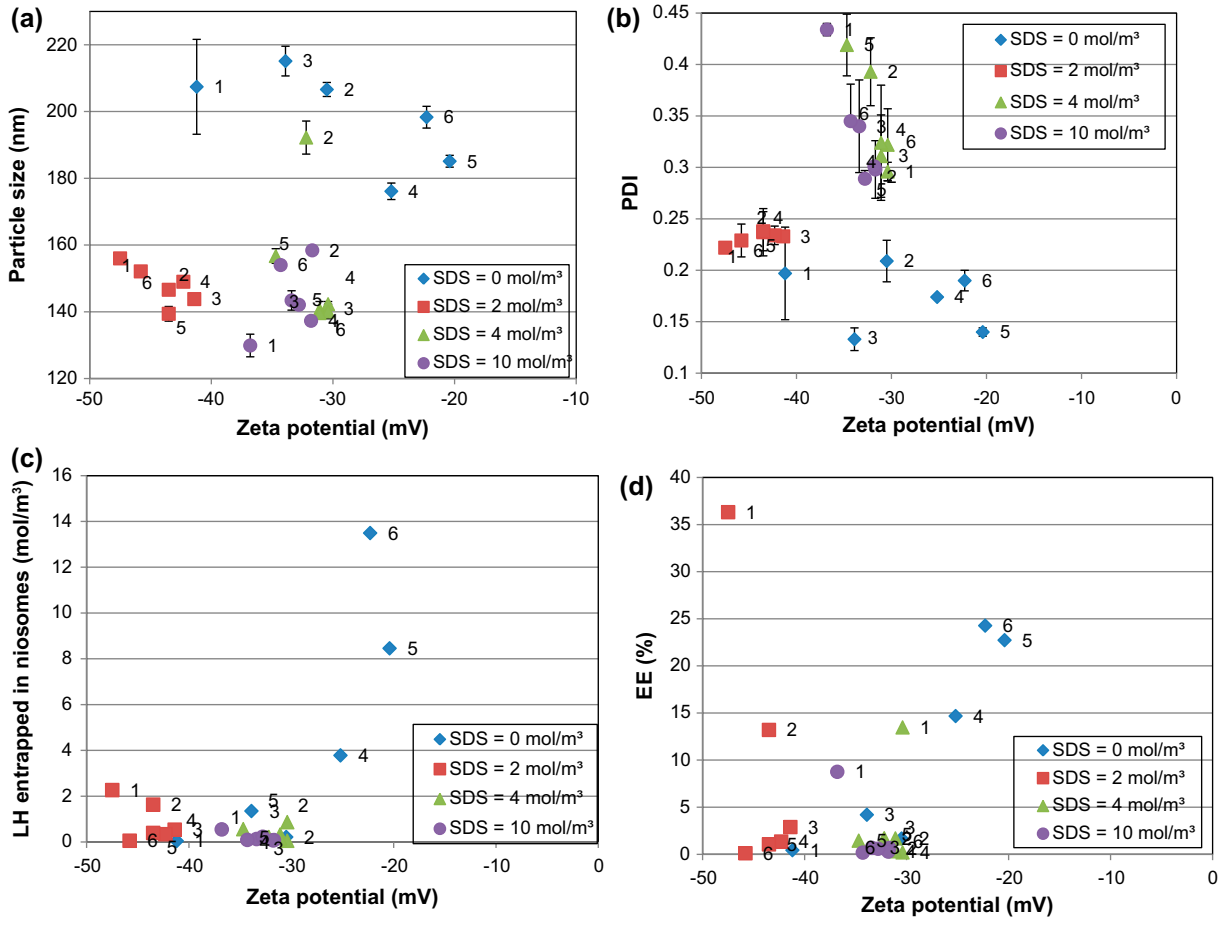

Fig. 4. Scatter plot of the characterization results of formulations shown in Table 2 vs. zeta potential: (a) Niosome mean diameter, (b) PDI, (c) lactic acid concentration entrapped in niosomes, and (d) lactic acid entrapment efficiency.

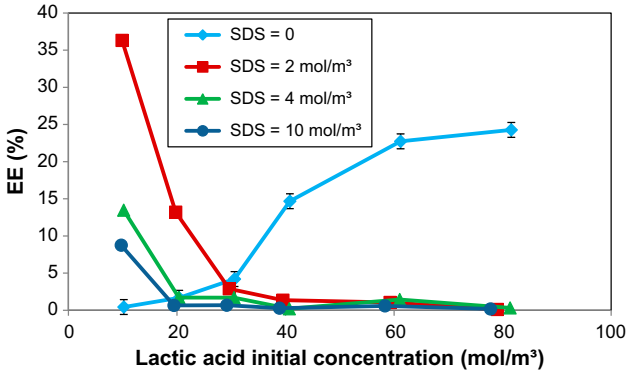

Fig. 5. Effect of the initial lactic acid concentration on the lactic acid entrapment efficiency for Span 80 + SDS formulations shown in Table 2.

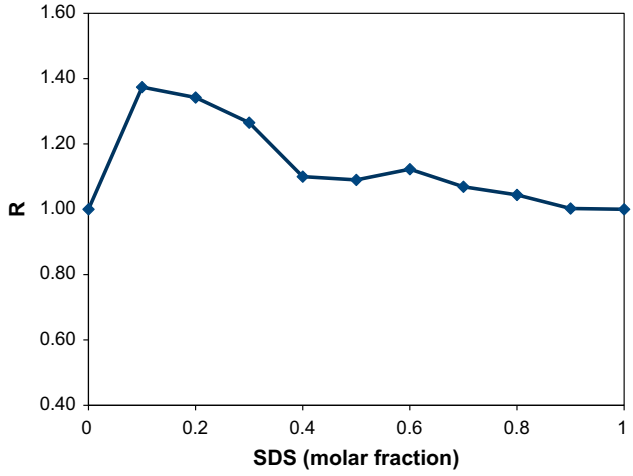

Fig. 6. Ratio between the experimental and theoretical conductivities (R) vs. the molar fraction of SDS in water formulations of Span 80 + SDS with 20 mol/m^3 of total concentration of both surfactants.

20 mol/m^3. The aim of these experiments was to check the surfactant compositions leading to a higher interaction between both molecules in the niosome bilayer.

The theoretical conductivity for a surfactant mixture can be estimated from the conductivity obtained for single surfactant solutions using the ideal mixing rule by the following expression:

$$\Delta_{id} = x_1\Delta_1 + x_2\Delta_2 \quad (4)$$

where x_1 and x_2 are the molar fractions of the two surfactants, and Δ_1 and Δ_2 are the conductivities of surfactants in single aqueous solutions. The ratio (R) between the experimental and theoretical conductivities is shown in Fig. 6. A significant synergistic effect on conductivity is observed for formulations with a SDS molar fraction lower than 0.4. Synergism defines the conditions when the properties of the mixture are better than those obtained with the single surfactants.

It should be noted that estimation of the theoretical conductivity calculated by Eq. (4) uses the experimental conductivity values of single solutions of Span 80 and SDS, both of 20 mol/m^3, well above their CMC, so that a value of $R = 1$ represents a system with a conductivity equal to that which would provide a system consisting of two types of discrete structures of Span 80 niosomes and SDS micelles. Assuming that the degree of interaction of SDS and Span 80 depends exclusively on the formulation, values of $R > 1$ must necessarily be attributed to the increased conductivity of Span 80 niosomes modified with SDS. The presence of SDS at low concentration affects the composition of the niosome bilayer, decreasing its size and increasing its surface charge and so, its conductivity. However, the addition of SDS amounts in a molar fraction higher than 0.4 can destabilize the niosome bilayer by increasing the micelle formation. The liposome solubilization process by micellization due to the addition of surfactants has been studied by Lichtenberg et al. [41] and roughly sketched by the following three-stage model: gradual incorporation of surfactant into the lipid bilayer up to saturation, progressive transformation of mixed bilayer into lipid-rich mixed micelles (coexistence of surfactant-saturated vesicles and lipid-saturated micelles), and finally, presence of all the phospholipid as mixed micelles (micellization point). Liposome interaction with SDS has been studied by Deo and Somasundaran [42] showing that the SDS/lipid molar ratio and interaction times are the governing parameters in the liposomes solubilization process. They also determined that in the presence of liposomes, the CMC of SDS shifted toward higher concentrations depending on the amount of liposomes, indicating that as the liposomes concentration is increased, a higher SDS concentration is required to reach the micellization point. Although micellization point was not determined in this work, it is clear from Fig. 6 that when the SDS surfactant concentration is lower than 0.4 molar fraction, SDS is found to be associated with the niosome bilayers without any disruption of the bilayer structure, which corresponds to the first stage of the Lichtenberg et al. model. Note that a SDS molar fraction of 0.4 corresponds to 8 mol/m^3, which is the CMC of the pure SDS in water (8.30 ± 0.04 mol/m^3) [33,40].

Niosomes formation and morphology were confirmed by TEM measurements. Fig. 7 shows two negative stain micrographs for niosomes obtained by

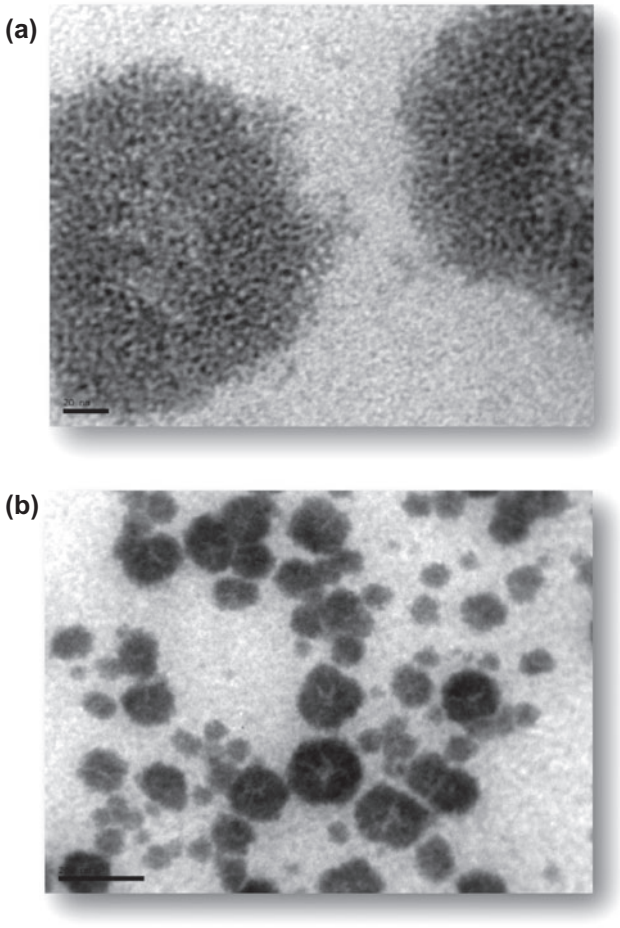

Fig. 7. TEM micrographs of niosomes obtained by ultrasonication (5 min) of Span 80 (20 mol/m^3) and SDS (4 mol/m^3) in aqueous solution. Scale bars: (a) 20 nm and (b) 200 nm.

5 min of ultrasounds on formulations of Span 80 (20 mol/m^3) and SDS (4 mol/m^3), without lactic acid. Dark structures shown in these micrographs correspond to spherical niosomes, with diameters ranging from 100 to 160 nm, which agree with sizes measured by DLS.

4. Conclusions

Direct ultrasonication in pulses of 5 s of the aqueous formulations over a 5-min effective time, using a 500 W high-intensity ultrasonic processor at 30% amplitude, has proven to be an effective, fast, and low-cost technique for Span 80 niosome formulation.

Span 80 is a non-ionic surfactant capable of forming niosomes in the absence of cholesterol; however, its ability to encapsulate lactic acid was very low, with efficiencies of 4 and 6% in formulations containing 7–8 mol/m^3 of lactic acid and 10 or 20 mol/m^3 of Span 80, respectively. The addition of 4 mol/m^3 SDS increased the lactic acid entrapment efficiency up to values of 11 and 23% for Span 80 concentrations of 10 and 20 mol/m^3, respectively. The presence of cholesterol in formulations of 20 mol/m^3 Span 80 with 4 mol/m^3 SDS increased the niosomes size from 99 to 124 nm in the samples without and with 10 mol/m^3 cholesterol, respectively, with little variation in the lactic acid entrapment efficiency (about 23%) and zeta potential (about −55 mV).

SDS can be used as a membrane modifier instead of cholesterol because it stabilizes the niosomal bilayer, similar to cholesterol but with lower concentrations, is not toxic, facilitates sample preparation in aqueous phase, and is cheaper.

SDS also has a synergistic effect on the entrapment efficiency of lactic acid, being Span 80 (20 mol/m^3) and SDS (<4 mol/m^3) adequate formulations for lactic acid extraction from aqueous solutions at low concentrations. The increased lactic acid entrapment in the SDS-modified niosomes may be attributed to the complex formation at the external interface by means of hydrogen bonds between the SDS adsorbed in the niosome surface and the lactic acid protonated species. The niosome bilayer modified with SDS hinders the entrapment of lactic acid in its inside cavity.

The results of this study shed light on further investigation of these systems regarding their possible use as selective extraction agents of hydrophilic compounds in aqueous solutions at low concentrations.

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