MICROEMULSION-BASED STRATEGY FOR THE CAPILLARY ELECTROPHORETIC ANALYSIS OF SOME ANTIDIABETIC DRUGS

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Abstract. Microemulsions are systems of interest to the pharmaceutical science. Important applications are found in the separation techniques, being used as running media in microemulsion electrokinetic chromatography (MEEKC). The paper surveys the possibility to include resonance light scattering (RLS) spectrometry as a preliminary step in the development of microemulsion electrokinetic chromatography method. Thus the scope of the RLS technique is extended. Microemulsion of heptane in borate buffer (pH = 9), prepared using natrium dodecyl suphate as surfactant and n-butanol as cosurfactant, and solutions of two antidiabetic drugs (metformin and glibenclamide) were studied. Results were correlated with molecular modeling and physical-chemical parameters (viscosity, conductivity, relative permittivity). The ability to form ion pairs of the studied molecules, in ionized form, with the surfactant molecules in the aqueous phase and the extent to which the antidiabetics in molecular form are distributed in the oil phase was also investigated. Thus for glibenclamide a strong retention at the surface of the oil droplet was suggested. Metformin forms ion pairs with low association constant anionic SDS molecules. MEEKC of the two antidiabetics proved an electrophoretic behavior in accordance with the mechanisms proposed.

Key words: microemulsion, resonance light scattering, metformin, glibenclamide, capillary electrophoresis.

INTRODUCTION

Microemulsions are transparent dispersions of oil and water, stabilized by a surfactant and/or a co-surfactant, mixed together in defined ratios and sometimes in a predefined order of mixing. These systems are thermodynamically stable; the

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tendency to phase separation appears over a considerably longer time than conventional emulsions. Microemulsions are prone to disintegration upon dilution [14].

An important characteristic of microemulsions is the higher solubilization capacity of the hydrophobic substances. This feature is attributed to the lower interfacial tension, with values of 10^{-3}–10^{-6} mN/cm^{-1}, that indicate the absence of direct oil-water contact [1].

Microemulsion electrokinetic chromatography (MEEKC) is a separation technique that uses microemulsions as running media and thus combines electrophoretic and chromatographic principles. Understanding the mechanism of interaction between analyte and considered pseudostationary phase and establishing the optimum microemulsion composition are steps that slower the optimization of a MEEKC method [12].

Light scattering techniques are useful in the study of microemulsions structure, by giving information about particle sizes, interaction forces and hydrodynamic interactions [3]. They provide informations that are not directly accessible by other techniques such as absorption, fluorescence or circular dichroism spectroscopy. Scattering, similarly to absorption, is a process that removes energy from the incident light beam. This energy is redirected as scattered light, while, in the absorption process the removed energy is converted to heat. Thus, in light scattering experiments, for scatterers which both absorb and scatter light, extinction is measured [15]. In resonance light scattering (RLS) technique the incident and scattered wavelengths are identical. The spectrum obtained by synchronous scanning of both monochromators is a result of the resonance phenomena between absorbed and scattered light and it has two components: absorption and scattering. Studies indicate that useful information about size, structure and interaction mechanisms can be obtained if the absorption is not too strong and the particle size is sufficiently large [6].

Most of the RLS methods published investigate interactions between substances that contain chromophores [6, 7]. Few studies have been focused on the interaction between colorless molecules [9, 10]. The technique has not been reported yet for the investigation of microemulsion systems.

The aim of the present study was to assess the extent to which RLS can be used as preliminary step in developing a MEEKC method. Microemulsion of heptane in borate buffer (pH = 9), prepared using natrium dodecyl sulphate (SDS) as surfactant and n-butanol as co-surfactant, and solutions of two antidiabetic drugs (metformin and glibenclamide) were studied.

Metformin (N,N dimethylbiguanide) is a hydrophilic compound, with two pKa values, pK_1 = 2.8 and pK_2 = 11.6 [2]. Glibenclamide (also known as glyburide) is a very lipophilic sulfonylurea compound, with one pKa, with reported value 5.3 [13].

The interaction mechanisms of the analytes with the components of the microemulsion system were investigated also *in silico*, by molecular modeling. Complementary determinations of physical-chemical parameters (viscosity, relative permittivity, conductivity) were performed.

MATERIALS AND METHODS

MATERIALS AND REAGENTS

Glibenclamide pharmaceutical grade (a gift from S.C. Laur Med S.R.L), metformin pharmaceutical grade (a gift from Chemicals Pharmaceuticals Manufacturing Impex S.R.L.), sodium tetraborate (Fluka), n-butanol (Chimactiv S.R.L.), n-heptane (Rotisolv, Carl Roth GmbH), natrium dodecyl sulphate (Merck) were used without any previous preparation.

Borate buffer solution (BBS) 10 mM, pH = 9, was prepared in volumetric flasks by weighing the appropriate amounts of chemicals and dissolved in ultrapure water; pH was measured by a Metrohm 716 DMS Titrino pH-meter.

Stock solutions of glibenclamide 5.5×10^{-4} M in BBS and metformin hydrochloride 1.5×10^{-3} M in water were used.

The microemulsion (MEM) was prepared according to the literature data [16]. In order to obtain a composition of 3.3% w/v SDS, 6.6% w/v n-butanol and 0.8% w/v heptane, in 100 mL volumetric flask the appropriate amounts of SDS, butanol and heptane were weighed and brought to volume with BBS; the mixture was sonicated for 30 min. Blank solution was prepared using the same procedure without heptane.

Deionized water was used all over experiments.

METHODS

Instrumentation

Conductivity (k) of the microemulsions was measured using a Corning 441 conductometer at room temperature (reference temperature 25 °C). Input operation conditions fitted: impedance greater than 10^{12} ohms, operating ranges: conductivity –2.00 to 199.9 μS/cm.

Relative permittivity (ε_r) was measured using a 60 GK dielcometer, at room temperature, with a calibrating scale. The standard for the evaluations was the air with a dielectric constant value near to unit ($\varepsilon_a = 1.0006$).

Viscosity measurements were conducted with a Multi-Visc, Fungilab rotational viscosimeter, using the LCP adaptor (for low viscosities).

Resonance light scattering spectra were registered using a LS 50B (Perkin Elmer Inc.) spectrofluorimeter. Synchronous scanning was performed with $\lambda_{excitation} = \lambda_{emission}$, with 1% attenuation filter and 8 nm excitation-emission slits. The RLS intensity (I_{RLS}) for microemulsion systems and for the reagent blank (I_0) was measured. The corrected scattered intensity, $\Delta I = I_{RLS} - I_0$, was computed and used for further evaluation.

MEEKC assays were carried on an Agilent Technologies G1600A system, a 48 cm (40 cm effective length), 50 μm internal diameter fused-silica capillary, on-column diode-array detector and Peltier controlled temperature of the cassette. Data were acquired using Agilent Chemstation version B.0 2.0 software. Electrophoretic procedure: at the beginning of the working day the capillary was rinsed with 1 M NaOH for 10 min, then with water for 15 min, and conditioned with the working microemulsion for 10 min. In between runs the capillary was flushed for 6 min with the microemulsion. At the end of the working day, the capillary was washed with water for 10 min, 1 M NaOH for 5 min, water for 5 min, and a 2 min air flush.

All solutions were degassed and filtered through a 0.45 μm syringe micro filter before use.

Measurements were performed at 25 °C, hydrodynamic injection (5 sec at 50 mbar), 20 kV the applied voltage. Detection was performed at 208 nm and 233 nm, with full spectra recording between 200 and 600 nm.

Molecular modeling

All calculations based on semi-empirical molecular orbital theory have been carried using HyperChem 7.5 molecular modeling software [17]. The structures of the investigated molecules (glibenclamide, metformin) in ground state were optimized using AM1 semi-empirical method with restricted Hartree-Fock (RHF) basis and AMBER (Assisted Model Building and Energy Refinement) force field. All calculations referred to isolated molecule (gas phase). The conjugate gradients algorithm (Polak-Ribier) was employed for the geometry optimization using a convergence set to the value of 0.01 kcal/(Å·mol). The geometry optimization was done by minimization of the binding energy of the molecule. During energy minimization, we searched for a molecular structure for which the energy did not change with infinitesimal changes in geometry and, respectively, the total root-mean-squared gradient (RMS-gradient) was close to zero.

RESULTS AND DISCUSSION

PHYSICAL-CHEMICAL PROPERTIES OF MICROEMULSIONS

Viscosity, relative permittivity and conductivity were determined for the microemulsion studied. In Table 1 the results are presented comparatively with the same physical-chemical properties of the borate buffer (pH = 9) and blank solution (micellar SDS with n-butanol solution).

One can observe that the viscosity slightly increases and conductivity significantly decreases when microemulsion is formed.

The relative permittivity of the microemulsion is lower than in pure water, indicating a possible change of pKa values of metformin and glibenclamide when dissolved in these media. Therefore ionization constant values cannot be used to predict their interactions with the components of the microemulsion system.

Table 1

Physical-chemical constants of the microemulsions
(each value is mean of 5 determinations)

Solution	ε_r	k (μS/cm)	Viscosity (cP)
Borate buffer (pH = 9)	26.5	1452	1.76
SDS in BBS with n-butanol	27.3	7.04	2.08
Microemulsion	26.85	6.77	2.24

Conductometry is a reliable and well established method used to evaluate the presence of ionic species in solution, even in low millimolar concentration range. Electroneutral species (unionized molecules, ion-pairs) do not contribute to solution conductivity [4]. Conductometric determinations were performed on a microemulsion system containing the substances investigated at 6×10^{-5} M concentration. For the microemulsion system containing metformin, conductivity increases at 7.058 μS/cm, indicating the existence of ionized form or as ion pairs with low association constant, while for glibenclamide the conductivity value remains almost unchanged (6.848 μS/cm), indicating its existence in molecular form.

RESONANCE LIGHT SCATTERING EXPERIMENTS

In microemulsions, solutes are partitioned between the charged oil droplets and the aqueous buffer phase. Inclusion into the oil droplet or adsorption at its surface is generally favored for the hydrophobic compounds. An ionized compound is present mostly in the aqueous phase. The hydrophobicity can be

increased, thus its interactions with the oil droplet, if the possibility to form ion pairs exists.

A maximum at 373 nm appears in the microemulsion RLS spectra; the slight bathochromic and hyperchromic shift for RLS spectra of SDS – butanol-borate buffer solution compared with SDS-borate buffer (without n-butanol), correlated with the physical-chemical characteristics indicates that n-butanol addition results in micelles formation (Fig. 1). Also, RLS spectra of microemulsions (spectrum No. 2 compared with 3 and 4) show an intensity decrease of the signal, suggesting that a compact structuration of SDS molecules around oil droplets takes place (charged oil droplets are smaller than SDS micelles).

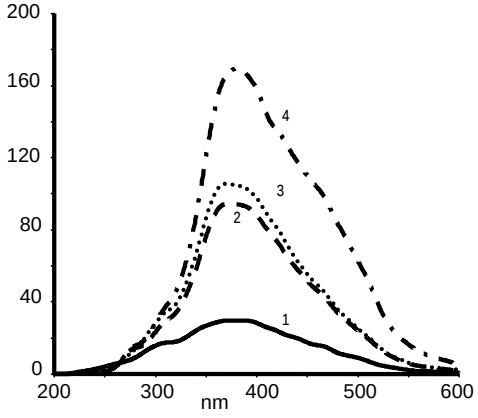

Fig. 1. RLS spectra of microemulsions and components
(1 – borate buffer, pH = 9; 2 – microemulsion; 3 – 0.1 M SDS in borate buffer without n-butanol;
4 – 0.1 M SDS in borate buffer with n-butanol).

In order to evaluate the distribution of analytes (glibenclamide, metformin) in microemulsion, the changes in the RLS spectra (Figs. 2 and 3) were studied. Synchronous scan was performed on microemulsion with 0.01 mg/mL analytes and without analytes.

Glibenclamide and metformin in 40 mM borate buffer solution present a maximum at 390 nm and 381 nm, respectively (Table 2).

When glibenclamide and metformin were dissolved in microemulsion significant changes in RLS spectra appeared: a blue shift and a decrease of maximum compared with microemulsion scattering spectra takes place (Fig. 3). If the absorption part of the RLS spectrum is considered, electrostatic and charge transfer interactions between analytes and components of microemulsion system are indicated [8].

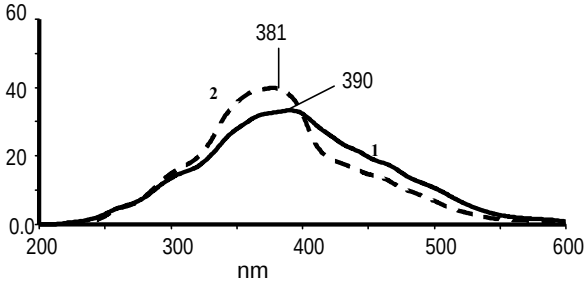

Fig. 2. RLS spectra of Glibenclamide (1) and Metformin (2) in BBS.

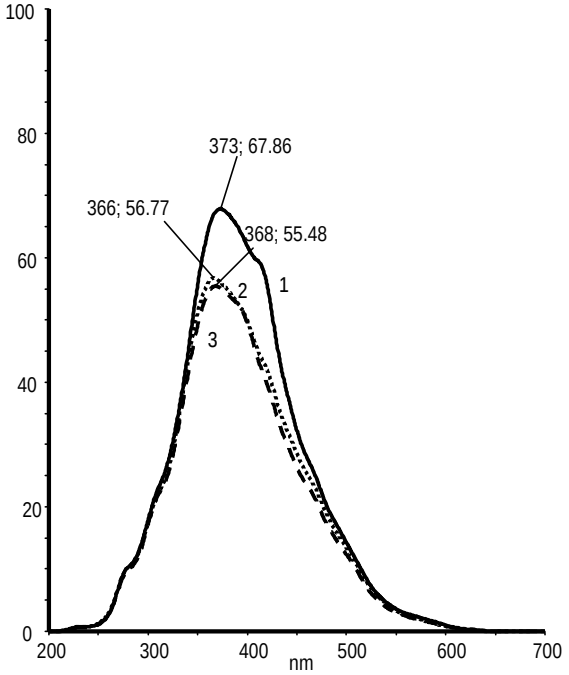

Fig. 3. RLS spectra of MEM (1), glibenclamide-MEM (2), and metformin – MEM (3).

Table 2

Changes in the RLS spectra of analytes in MEM systems (SDS-heptane-BBS)

Analyzed solution	**λ_{max} (nm)**	**$\Delta\lambda$ (nm)**	**I_{RLS} (r.u.)**	**ΔI (r.u.)**
MEM	373		67.86	
Glibenclamide/MEM	366	7	56.77	11.09
Metformin/MEM	368	5	55.48	12.38

MOLECULAR MODELING

Molecular modeling performed using HyperChem 7.5 software shows for glibenclamide, in molecular form, a two-dimensional geometry and a positive electronic charge density area on the sulfonylurea group (Fig. 4). These features facilitate charge transfer interactions with negatively charged oil droplet. If the hydrophobic character of glibenclamide is also taken into account, it can be assumed that, in the studied microemulsion system, this analyte is strongly retained by the hydrophobic pseudostationary phase.

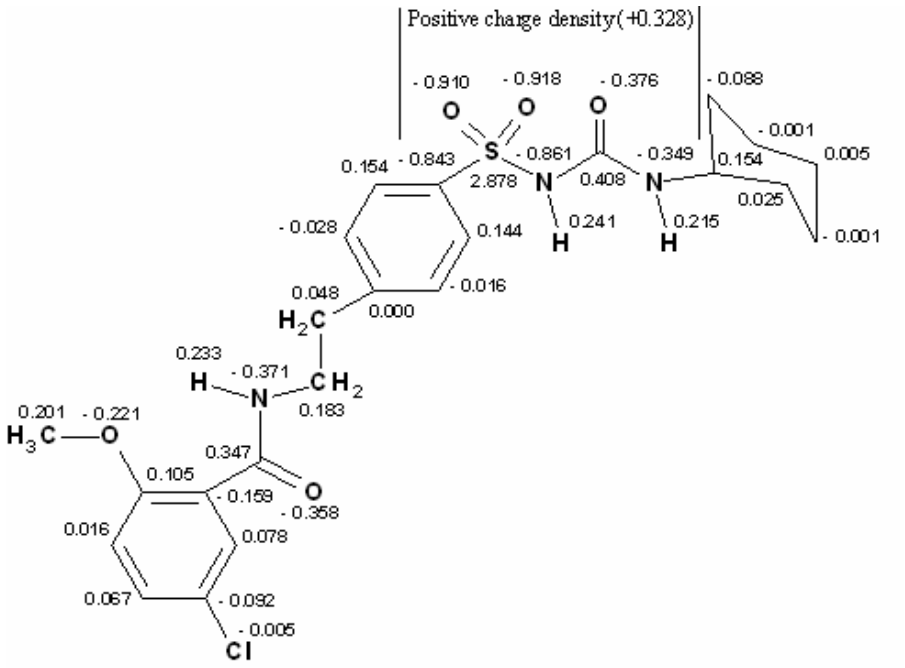

Fig. 4. Charge distribution on glibenclamide molecule in ground state.

Charge distribution calculated on molecular metformin (Fig. 5) indicates internal charge transfer between hydrogen in position 15 and nitrogen in position 9, and negative electronic charge density (–0.135) on guanidinic group. Thus guanidinic group can be easily protonated gaining positive charge. Ion pairing interactions driving by electrostatic forces with negative charged oil droplet are favored. Correlation of these data with the increased conductivity value (7.058 μS/cm) suggests that ion pairs with low association constant are formed between ionized metformin and anionic SDS molecules [5, 11]. Therefore, in microemulsion system metformin is located mostly in the aqueous phase.

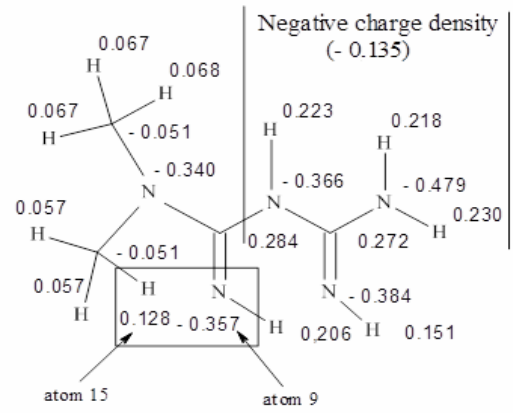

Fig. 5. Charge distribution on metformin molecule in ground state.

CAPILLARY ELECTROPHORESIS

MEEKC proved for the two studied analytes an electrophoretic behavior in accordance with the mechanisms proposed. Glibenclamide, due to the retention on the pseudostationary phase surface, has a longer migration time (22.2 minutes) and a lower effective electrophoretic mobility (μ = 4.33 $cm^2/s \cdot V$) than metformin with a migration time of 4.1 minutes and electrophoretic mobility of 23.20 $cm^2/s \cdot V$, which forms ion pairs with low stability with SDS molecules (Fig. 6).

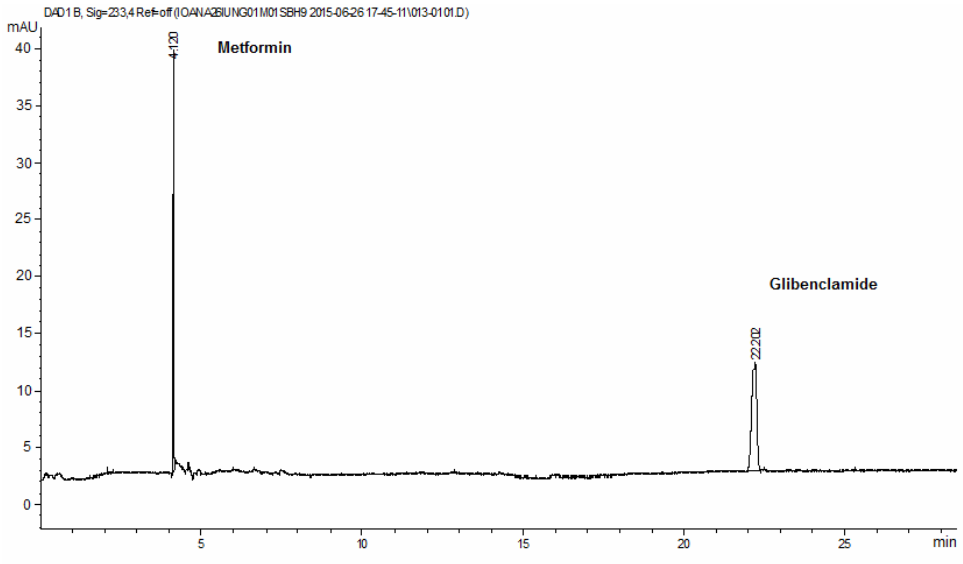

Fig. 6. Electrophoregram on 0.01 mg/mL of metformin and glibenclamide in MEM.

CONCLUSIONS

The paper surveys the possibility to include resonance light scattering (RLS) spectrometry as a preliminary step in the development of microemulsion electrokinetic chromatography method. Thus the scope of the RLS technique is extended. Studies indicated that RLS spectra of microemulsions show significant changes when substances with different lipophilic/hydrophilic character were added. These changes are strongly correlated with their physical-chemical characteristics (dielectric constant, viscosity, relative permittivity). Data obtained allow evaluating the interaction mechanisms between analytes and the components of the microemulsion system and thus can be used to predict their electrophoretic behavior.

Antidiabetic drugs metformin and glibenclamide were studied in MEM of SDS-butanol-borate buffer using RLS. Based on the correlations with physical-chemical parameters and molecular modeling, mechanisms of interaction are proposed. Thus glibenclamide in molecular form is adsorbed at the oil droplet surface and metformin, in cationic form, participates at ion pair equilibrium with anionic SDS molecules. Electrophoretic behavior for the two analytes was consistent with the mechanisms proposed.

REFERENCES

1. BERA, A., A. MANDAL, T. KUMAR, Physicochemical characterization of anionic and cationic microemulsions: water solubilization, particle size distribution, surface tension and structural parameters, *J. Chem. Eng. Data*, 2014, **59**, 2490–2498, dx.doi.org/10.1021/je500274r.
2. BRETNALL, A.E., G.S. CLARKE, Metformin hydrochloride, in *Analytical Profiles of Drug Substances and Excipients*, vol. 25, H.G. Britain ed., Academic Press, Inc., London, United Kingdom, 1998, pp. 243–284.
3. CAZABAT, A.M., D. LANGEVIN, J. MEUNIER, A. POUCHELON, Critical behavior in microemulsions, *Adv. Colloid Interface Sci.*, 1982, **16**, 175–199.
4. CECHI, T., *Ion-pair Chromatography and Related Techniques*, CRC Press, Taylor & Francis Group, LLC, 2010.
5. DAVID, V., A. MEDVEDOVICI, F. ALBU, Retention behavior of metformin and related impurities in ion-pairing liquid chromatography, *J. Liq. Chromatog. R. T.*, 2005, **28**(1), 81–95.
6. DE PAULA, J.C., J.H. ROBBLEE, R.F. PASTERNACK, Aggregation of chlorophyll a probed by resonance light scattering spectroscopy, *Biophys. J.,* 1995, **68**, 335–341.
7. FLOREA, M., C.M. MONCIU, M. ILIE, Resonance Rayleigh Scattering study of streptomycin – Congo Red ionic association in view of analytical application, *Farmacia*, 2014, **62**, 318–328.
8. KARUKSTIS, K.K., D.A. SAVIN, C.T. LOFTUS, N.D. D'ANGELO, Spectroscopic studies of the interaction of methyl orange with cationic alkyltrimethylammonium bromide surfactants, *J. Colloid Interface Sci.*, 1998, **203**, 157–163.
9. LI, N., S. LIU, H. LUO, A new method for the determination of the first and second CMC in CTAB solution by resonance Rayleigh scattering technology, *Anal. Lett*., 2002, **35**, 1229–1238.
10. LIU, S.P., H.Q. LUO, H. XU, N.B. LI, Resonance Rayleigh Scattering study of interaction of heparin with some cationic surfactants and their analytical application, *Spectrochim. Acta A*, 2005, **61**, 861–867.

11. RADULESCU, M., V. DAVID, Partition versus electrostatic model applied to the ion-pairing retention process of some guanidine based compounds, *J. Liq. Chromatog. R. T.*, 2012, **35**(14), 2042–2053.
12. RYAN, R., K. ALTRIA, E. McEVOY, S. DONEGAN, J. POWER, A review of developments in the methodology and application of microemulsion electrokinetic chromatography, *Electrophoresis*, 2013, **34**, 159–177.
13. TAKLA, P.G., Glibenclamide, in *Analytical Profiles of Drug Substances and Excipients*, vol. 10, K. Florey ed., Academic Press, Inc., London, United Kingdom, 1981, pp 338–355.
14. WEISS, J., E.A. DECKER, J.D. MCCLEMENTS, K. KRISTBERGSSON, T. HELGASON, T. AWAD, Solid lipid nanoparticles as delivery systems for bioactive food components, *Food Biophys*., 2008, **3**, 146–154.
15. YGUERABIDE, J., E.E. YGUERABIDE, Light-scattering submicroscopic particles as highly fluorescent analogs and their use as tracer labels in clinical and biological applications, *Anal. Biochem.*, 1998, **262**, 137–156.
16. YUHUA, C., S. JIANWEI, Microstructure of microemulsion in MEEKC, *Electrophoresis*, 2010, **31**, 672–678.
17. * * *, HyperChem 7.5 Release for Windows, HyperCube, Inc., Gainsville, Florida, USA.