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International Journal of Pharmaceutics 341 (2007) 189–194

INTERNATIONAL JOURNAL OF PHARMACEUTICS

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Quality by design: Characterization of self-nano-emulsified drug delivery systems (SNEDDs) using ultrasonic resonator technology☆

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Received 27 October 2006; received in revised form 16 March 2007; accepted 10 April 2007

Available online 21 April 2007

Abstract

In the present work, a novel application of ultrasonic measurements is detailed to characterize nano-emulsion formulations as a part of the overall Quality by Design (QbD) goal. Ultrasonic resonator technology (URT) was utilized to measure sound velocity and absorption of self-nanoemulsified drug delivery systems (SNEDDs) consisting of various ratios of oil:surfactant:co-surfactant. A QbD concept was used to create different SNEDDs formulations utilizing sweet orange oil (oil), Emulphor-620 (surfactant), and Capmul (co-surfactant) by dissolving Cyclosporine A in oil. The mixture was emulsified in water and ultrasonic measurements were carried out in an ultrasonic resonator system isothermally for a period of about 15–20 min. Compressibility of the individual components in the droplets, hydration of the droplets and the influence of the composition on droplet stability were studied by systematic ultrasonic measurements at a single resonator frequency. The adiabetic compressibilities for the oil, aqueous and interfacial components were 68, 44.6, and 53 [10^{-11} Pa^{-1}], respectively as calculated using Urick's equation. Also the ultrasonic absorption correlated droplet size of nano-emulsions linearly with R^2 of 0.84 indicating this can be used as an additional technique to measure the droplet size of nano-emulsions. Correlation of ultrasonic data with formulation components indicated that the ultrasonic velocity correlated negatively with increasing oil amount in the formulation as well as surfactant-to-cosurfactant ratios where as droplet diameter correlated positively with these formulation factors. It can be envisioned from the results that the compressibility of the media increases with the addition of the oily component and thus reducing the sound velocity. Thus URT enabled direct and convenient analysis of the physical properties as well as influence of formulation factors of nano-emulsions which is an important indication of stability of these nano-emulsions.

Keywords: Self-nano-emulsified drug delivery systems; Ultrasonic absorption; Ultrasonic velocity; Characterization

1. Introduction

Self-nanoemulsified drug delivery systems (SNEDDs) are becoming an attractive alternative to overcome poor aqueous solubility of new and existing drug candidates (Pouton, 2000; Nazzal et al., 2002). Self-emulsifying drug delivery systems (SEDDs) are isotropic mixtures of oil, surfactant, co-surfactant and drug that form fine oil-in-water emulsions when introduced into aqueous medium under gentle agitation. Recently, application of process analytical technology and quality by design to the preparation and characterization of SNEDDs for Cyclosporine A was reported (Zidan et al., 2007). In SNEDDs, oil was mixed with surfactant-cosurfactant mixture, with a systematic study of formulation factors effect by a series of experiments by utilizing QbD approach. In the new QbD paradigm of drug regulation, it is important that a product and process is understood well to overcome future product failures.

Complete characterization of emulsions is important to assess quality of innovative and pre-approved products. Extensive characterization of their stability and physico-chemical properties are prerequisites to ensure their efficacy throughout their shelf-life. Among the important characteristics are the structure, the hydration and the size of the nano-emulsion droplets. Various spectroscopic techniques have been reported for their characterization which include light scattering (Berne, 1978; Goddeeris et al., 2006), neutron scattering (Kotlarchik et al.,

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0378-5173/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2007.04.009

1984), NMR spectroscopy (Kreilgaard et al., 2000), electric and electroacoustic techniques (Jian et al., 2001; Dukhin et al., 2000). However, measurements by these techniques sometimes require dilution of the sample which might lead to structural alterations of these nano-droplets (Hou et al., 1988; Coupland and McClements, 2001). Thus, lack of sensitive and fast analytical technology might hinder the efficient development of nano-emulsions. Ultrasonic Resonator Technology (URT) is an enabling "platform" since the ultrasonic properties measured can be indicative of structural integrity of nano-emulsions. The structure of the water surrounding and binding to the substances present can be envisioned using Ultrasonic Resonator Technology (URT). It can give rise to prediction models for such emulsions at early stages of development. Ultrasonic waves as they transverse through the sample lead to a compression and decompression in the medium. This changes the distance between the particles and molecules in the sample, which in turn evaluates intermolecular attractions and repulsions. In this way the ultrasonic wave permits access to the molecular levels of organization in the sample (Sarvazyan, 1991). Sound scattering properties of dispersed particles may be used to determine the size and the distribution of particle sizes in particle dispersions (Povey, 1997). The theory of scattering of sound by inhomogeneities in fluids has been developed (McClements, 1991) and approaches for calculating the scattering coefficients of both fluid and solid particles are available in the literature (Epstein and Carhart, 1953; Allegra and Hawley, 1971). Thus, acoustical methods can be used for characterizing particles and size distribution of particles in sample preparations by broad band measurements. In most cases the attenuation spectra of ultrasound are measured in a wide frequency range and from these spectra, particle sizes and distributions of particle sizes are derived under certain assumptions. Very recently ultrasonic measurements on concentrated microemulsions preparations were reported where the influences of the increase of water concentrations up to 50% in the phase diagram were studied (Hickey et al., 2006). The technique was primarily utilized to study microstructural transitions in the phospholipid phase of the water-in-oil emulsion systems.

The objective of the present work was to quickly and efficiently perform the characterization of nano-emulsion systems using sound properties of these droplets as an aided technique to those currently being utilized such as measurement of their physical properties including droplet size and surface tension of these nano-emulsions.

2. Materials and methods

2.1. Materials

Cyclosporine (Purity 99%) (Cas No.: CYC140175) was obtained from Poli Industria Chemica S.P.A, Rozzano, Milano, Italy. Mono and diglycerides of caprylic acid (Capmul MCM-C8) (Lot No.: 50130-6) was supplied by Abitec Corp., Janesville, WI, USA. Emulphor:Ethoxylated castor oil (Alkamuls El-620) (Lot No.: 347653) was a gift from Rhodia Inc., Cranbury, NJ, USA. Sweet orange oil (Cas No.: 8016-38-4) was obtained from Sciencelab Inc., Houston, TX, USA.

2.2. Preparation of SNEDDs

SNEDDs for Cyclosporine A were prepared according to the method described by Zidan et al. (2007). A Box-Behnken DOE was used to create formulations containing different ratios of componets of the SNEDDs system. Briefly, Cyclosporine A was dissolved in sweet orange oil and then Emulphor EL 620 and capmul were added to it in various ratios (Table 1). The mixture

Table 1
Composition of various SNEDDs formulations and their acoustic and physical properties

Oil parts	Surfactant parts	Co-surfactant parts	Delta U[a] [m/s]	Delta A[b] [10–14 s^2/m]	Number weighted avg droplet[c]	Volume weighted avg droplet[c] diameter	Surface tension [N/m]
10	20	50	0.855	0.236	44.5	47.9	0.030
10	50	30	0.945	0.068	10.3	11.3	0.035
10	50	70	0.898	0.096	34.2	37.5	0.031
10	80	50	0.891	0.041	7.2	6.8	0.033
30	20	30	0.632	0.130	40.5	40.0	0.034
30	20	70	0.730	0.338	87.0	92.6	0.031
30	50	50	0.821	0.091	32.6	33.8	0.034
30	50	50	0.755	0.070	35.1	33.2	0.034
30	50	50	0.751	0.061	37.8	35.9	0.033
30	80	30	0.825	0.113	16.8	15.2	0.035
30	80	70	0.837	0.049	11.3	9.2	0.033
50	20	50	0.538	0.340	88.3	93.4	0.034
50	50	30	0.583	0.087	9.8	8.7	0.036
50	50	70	0.635	0.063	49.2	50.3	0.033
50	80	50	0.759	0.064	14.5	11.3	0.035

[a] Delta U values are the differences between the ultrasonic velocities U in samples and the ultrasonic velocity U_0 in water as reference. Delta $U = U - U_0$ where $U_0 = 1496.70$ [m/s].

[b] Delta A values are the differences between the ultrasonic absorption A in samples and the ultrasonic absorption A_0 in water as reference. Delta $A = A - A_0$ where $A_0 = 1.445\,[10^{-14}\mathrm{s}^2/\mathrm{m}]$.

[c] Droplet diameters were measured using dynamic light scattering using Nicomp particle sizer.

was mixed and slightly heated below 40 °C to ensure mixing of all the components. A sample of 60 mg of the SNEDDs was carefully weighed in 10 mL volumetric flask and was then emulsified with water by vortexing. The samples were kept at room temperature until measurement for about 20 min so as to remove all the entrapped air.

2.3. Ultrasonic measurements

The ResoScan® System (TF Instruments Inc., Monmouth Junction, NJ) was used for measuring the ultrasonic velocity U and ultrasonic absorption. A special procedure was utilized for evaluating the energy losses in the resonator cavity and correcting for losses which were not due to the sample (scattering, refraction and reflection in the cavity). The frequency of the ultrasonic waves used for the measurements was about 10 MHz, their intensity was about 5×10^{-3} W/cm^2.

The ResoScan® System contains two closed resonator cavities with a path length of 7.0 mm. Evaporation of the sample is prevented by gas tight lids. In the instrument, the samples can be heated or cooled via Peltier elements at rates between 0.001 and up to 0.35 °C/min. The absolute accuracy of the thermostat temperature is ± 0.01 K. The resolution of the ultrasonic velocity is 0.001 m/s. Repeatability of absolute velocity after automatic re-initialization is 0.01 m/s. The resolution of the ultrasonic absorption is 1–3%, depending on the level of attenuation.

A sample (170 microliters) was injected into the cell with a syringe, measurements were performed, and the cells were cleaned after removing the sample. A blank reference media was always introduced in the second cell to compare the ultrasonic measurements of the sample. All operations except sample loading and unloading were under computer control.

2.3.1. Isothermal measurements

After equilibration of temperature and initialization of the ultrasonic measurements, the ultrasonic velocity U and the ultrasonic absorption A were continuously measured at 25.0 °C for at least 10 min using the ultrasonic resonator technology (URT) of the ResoScan® instrument.

2.4. Droplet size measurements

The size measurements were performed on nanosizer from Nicomp (Particle Sizing Systems, Santa Barbara, CA). The measurements were performed by entering the viscosity (0.933 cps) and refractive index (1.335) values for water. Each sample was measured for 10 min and Gaussian distribution for number weighted particle size was used. The results were expressed as number weighted average ± S.D.

2.5. Surface tension measurements

The air–liquid surface tension of all the formulations were measured on surface tensiomat (Fisher Sci., Pittsburgh, PA) by ring du nouy detachment method principle at 25.0 °C. The surface tension was calculated using the formula, $S = P \times F$, where S is the actual surface or interfacial tension, P is an apparent surface or interfacial tension which was recorded from the dial reading on the instrument and F is a correction factor calculated as

$$F = 0.7250 + \sqrt{\frac{0.01452P}{C^2(D-d)} + 0.04534 - \frac{1.679r}{R}}$$

where R is the radius of the ring, r the radius of the wire of the ring, D the density of the lower phase, d the density of the upper phase, C is the circumference of the ring.

3. Theory

The ultrasonic absorption, $A = \alpha/f^2$ where α is the attenuation coefficient and f is the frequency. The attenuation coefficient α of the sound wave contains all the energy losses of sound propagation, and f is the frequency of the ultrasonic wave. α is defined as

$$\alpha = -\frac{1}{x}\ln\frac{W_x}{W_0} \tag{1}$$

where W_0 is the initial amplitude of the ultrasonic wave ($x = 0$) and W_x the amplitude after travelling a distance x. The sample cells are designed as ultrasonic resonator cavities in which a standing wave is generated. To initialize the liquid resonator the system scans the resonance frequencies in the range from 7 to 9 MHz. It evaluates the order of the resonances and selects an optimal peak (master peak) in this range for the measurements. The ultrasonic velocity and the ultrasonic absorption are calculated from the frequency and broadness of this peak.

The special intrinsic routines which are used by the ResoScan® System to calculate the ultrasonic velocity U are based on the relationship of the fundamental resonance frequency f_1 and the path length of the cavity d:

$$U = 2\mathrm{d}f_1 \tag{2}$$

Since the higher orders of the resonance frequencies f_n are in reality non-exact multiples of f_1, an appropriate mathematical routine is used to evaluate f_1 from the resonance frequency f_n and the order n of the master peak (Funck and DeMaeyer, 2001).

3.1. Compressibility of nano-emulsion particles

The ultrasonic velocity U in a pure homogeneous fluid is determined by its density ρ and the compressibility κ of the fluid by Eq. (3) (Wood, 1964)

$$U = \frac{1}{\sqrt{\kappa\rho}} \tag{3}$$

The sound velocities in mixtures can be described by the Urick equation Eq. (4a) and 4 (Ulrick, 1947).

$$\frac{1}{U_{12}^2\rho_{12}} = \frac{\phi_1}{U_1^2\rho_1} + \frac{\phi_2}{U_2^2\rho_2}, \tag{4a}$$

where U_1, U_2 and U_{12} are the ultrasonic velocities in component 1, component 2 and in the mixture, respectively, ρ_1, ρ_2 and ρ_{12} are the densities of component 1, component 2 and of the mixture, respectively, and ϕ_1 and ϕ_2 the volume fractions of the components 1 and 2 in the mixture.

$$\kappa_{12} = \phi_1\kappa_1 + \phi_2\kappa_2 \tag{4b}$$

where κ_1, κ_2 and κ_{12} are the adiabatic compressibilities in component 1 and 2 and in the mixture, respectively. If the densities in components 1 and 2 and also in the mixture are very similar, Eq. (4a) and 4 may be simplified:

$$\frac{1}{U_{12}^2} \approx \phi_1\kappa_1 + \phi_2\kappa_2 \tag{5}$$

The data measured for U_{12} are not consistent with Eq. (5), if for the aqueous phase the known value k_1 for the compressibility of water is used. Eq. (5) may be extended also for a mixture of three components, which corresponds closer to the situation in the nano-emulsions of the SNEDDS.

$$U^2 \approx \frac{1}{\phi_0\kappa_0 + \phi_1\kappa_1 + \phi_2\kappa_2} \tag{6}$$

In Eq. (6) subscript 0 is used for the aqueous component. The central oil component in the droplets is represented by the second term with subscript 1. In the quantitative evaluations, the hydrated mixed phase of surfactant and co-surfactant can be treated by an additional single term (subscript 2).

4. Results and discussion

4.1. Adiabatic compressibility of individual components in nano-emulsion droplets

The various ratios used for oil:surfactant:co-surfacant to create all different SNEDDs formulations are listed in Table 1. The amount of Cyclosporine A was kept constant in all these formulations. The ultrasonic velocity and absorption data measured at 25 °C are also given in Table 1 for all formulations. Also the droplet diameters for all the formulations measured at 25 °C are listed in the Table 1. No satisfactory fit of the data was achieved to simple Urick equation Eq. (4a) and 4. However, if the extended Urick equation Eq. (6) as described in the theory section was applied for the determination of the adiabatic compressibilities of the oil and the combined surfactant and co-surfactant layer, a satisfactory interpretation of the experimental data was possible. Table 2 shows the compressibility values evaluated for three sets of SNEDDs formulations. The correlation analysis of all formulations using the extended Urick equation Eq. (6) is shown in Fig. 1.

Table 2
Adiabatic compressibilities determined in different phases of particles in individual nano-emulsion formulations

	Adiabatic compressibility $[10^{-11}\ \text{Pa}^{-1}]^{a}$		
Ratio of oil:surfactant: co-surfactant	Aqueous component	Oil component	Interfacial phase
10:20:50	44.6	65	55
30:20:70	44.6	68	53
50:80:50	44.6	64	51

[a] The values of the adiabatic compressibilities were calculated by iterative least square treatment of $1/U^2_{\text{measured}}$ and relation to $1/U^2_{\text{Calculated}}$ according to Urick's equation Eq. (6).

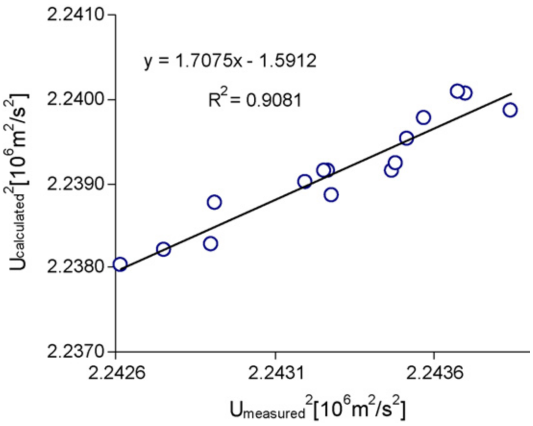

Fig. 1. Correlation between U^2_{measured} and $U^2_{\text{Calculated}}$ in 15 SNEDDS formulations. The calculated value was determined using Eq. (6) in iterative optimizing the correlation coefficient R^2 by variation of κ_1 and κ_2. For κ_0. the adiabatic compressibility of the aqueous phase. The value of water at 25 °C was used $\kappa_0 = 44.6\ [10^{-11}\ \text{Pa}^{-1}]$. The obtained values for the oil phase and the surfactant phase are $\kappa_1 = 70\ [10^{-11}\ \text{Pa}^{-1}]$ and $\kappa_2 = 50\ [10^{-11}\ \text{Pa}^{-1}]$, respectively.

These experimental results are consistent with the following interpretation of the ultrasonic parameters measured in the formulations. The droplets of SNEDDs prepared in the study have a central oily component as they form oil-in-water (O/W) type of emulsions. The compressibility of this oil was determined by the application of the extended Urick model Eq. (6). The compressibility value evaluated for the central component of about 70 $[10^{-11}\ \text{Pa}^{-1}]$ correlates well with the value of 69 $[10^{-11}\ \text{Pa}^{-1}]$ measured in a sample of the isolated oil phase.

4.2. Correlation of droplet size and ultrasonic measurements for nano-emulsions

The increase in ultrasonic absorption (A) due to the increase of the droplet diameter showed a correlation of (r^2 ~0.84) with the size of the droplets measured by dynamic light scattering (Fig. 2). This direct correlation of the geometry of the droplets with the observed ultrasonic absorption is consistent with the explanation of the scattering of the sound waves by the droplets

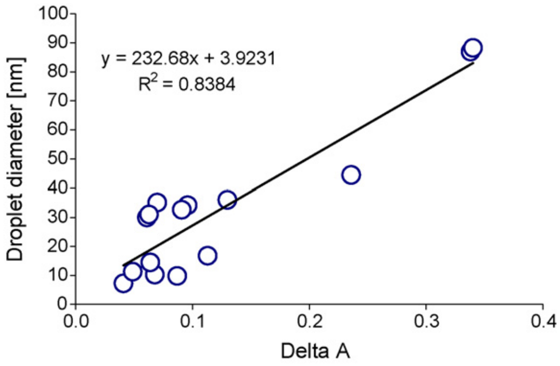

Fig. 2. Correlation of droplet diameter with Delta A values measured in SNEDDS Formulations. Delta A was calculated as described in Table 1.

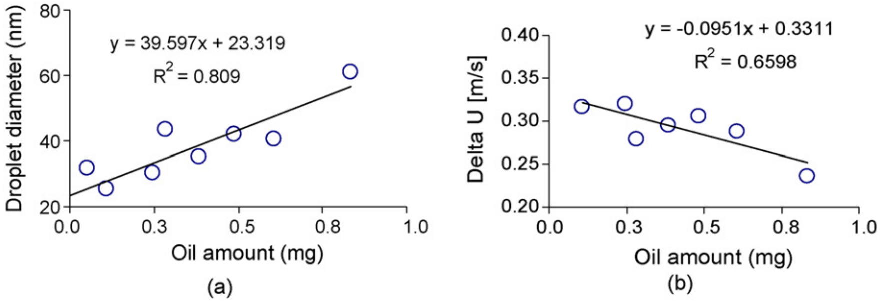

Fig. 3. Correlation of droplet diameter (a) and delta U (b) with amounts of oil for SNEDDs in which oil amount was increased keeping surfactant and co-surfactant amounts constant.

of the emulsion. This moderate correlation of the ultrasonic absorption data with the droplet size of these nano-emulsions might be due to the structuring influences of the non-pola part of the surfactant which prevents a significant arching of the surface protuding polar groups.

The droplets of SNEDDs prepared in the study have a central oily component as they form oil-in-water (O/W) type of emulsions. The compressibility of this oil was determined by the application of a modified Urick model Eq. (6). The compressibility value of about 70 [10^{-11} Pa^{-1}] correlates well with the value 69 [10^{-11} Pa^{-1}] measured in a sample of the oil phase. In the nano-emulsions, the central oil component is surrounded by an aqueous component consisting of surfactant and co-surfactant. In principle, both these surfactant components are miscible and should form a homogeneous layer surrounding the oil and are surrounded with the hydration sphere. It was observed that the measured compressibility for this is considerably lower than expected for material of this composition (Table 2). As the system was always above critical micellar concentration (CMC) as their surface tension remained constant for all the formulations (Table 1), the orientation of surfactant and co-surfactant molecules in the layer enabled formation of micelles. Above CMC, the polar parts of the molecules in the surfactant layer are oriented towards the outside surface of the particle. The non-polar aliphatic parts of the molecules are either in contact with the central oil component containing the drug or with their aliphatic end groups embedded into the surface of the oil phase.

A correlation of the sound absorption and velocity data with the droplet size of these nano-emulsions exhibited a moderate correlation with r^2 of ~0.84 (Fig. 2). This might be due to structuring influences of the non-polar part of the surfactant which prevents a significant arching of the surface with protruding polar groups. Thus crowded situation in the range of non-polar parts would prevent the formation of a closed surface. This could also explain the use of co-surfactant as surfactant alone might not produce smaller droplet size with arched surfaces. The addition of molecules with smaller polar groups and shorter aliphatic chains opens up the possibility of arching the surface. Thus co-surfactant molecules can slip in between the surfactant molecules and enable the formation of curved surface due to existence of small non-polar part in co-surfactant molecules. Thus, the presence of the co-surfactant enables the formation of smaller droplet size. From this view it is consistent that the ratio of surfactant and co-surfactant in the outer phase determines the extent of curvature and consequently the size of the oil droplets. Considering the size and ultrasonic data correlation (Fig. 2), it should be noted that the droplet size is influenced by surfactant-to-cosurfactant ratio. Therefore, while developing a linear relationship with the components of SNEDDs systems, the ratio of surfactant-to-cosurfactant should be utilized as opposed to correlating them individually. For this, a different set of formulations was created by keeping surfactant and co-surfactant amounts constant but just increasing the oil amount. Fig. 3a shows the relation between particle diameter with amount of oil in the formulation. As expected, the droplet size goes up as oil amount in the formulation increases. Sound velocity reduced as a function of oil amount in the formulation as evidenced by Fig. 3b. This in turn explains higher compressibility of oily phase as depicted in Table 2. Also as evidenced by Fig. 4a and b, the droplet diameter increases as surfactant to co-surfactant ratio increases and ultrasonic velocity goes down considerably with increasing ratio. This can support the ongoing discussion about need of co-surfactant to stabilize the curvature and reduce the droplet size. And this in turn reduces the ultrasonic veloc-

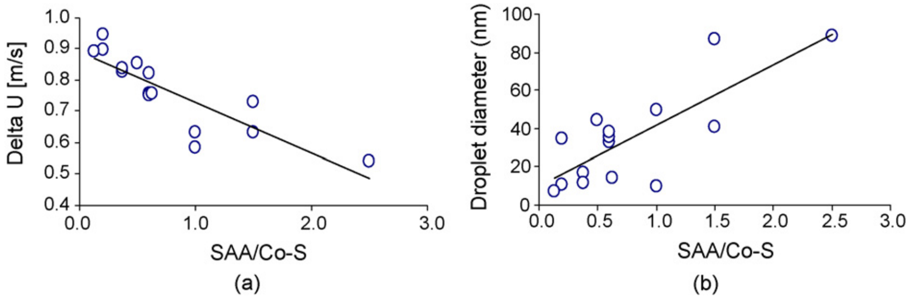

Fig. 4. Correlation of Delta U (a) and droplet diameter (b) with surfactant-to-cosurfactant ratio.

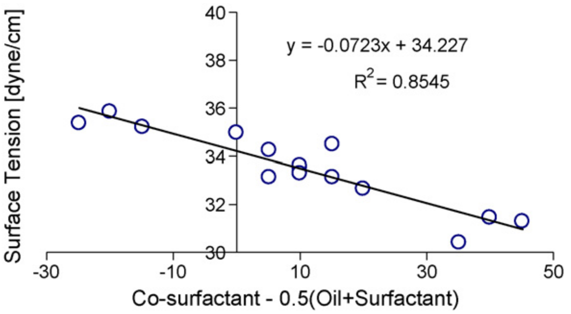

Fig. 5. Correlation of surface tension of SNEDDs with formulation components (oil, surfactant and co-surfactant).

ity and thus indirectly gives an indication of reducing droplet size.

The important influence of the co-surfactant on the droplet size which is closely related to the surface tension of the emulsified phase is illustrated in Fig. 5. The measured surface tensions in the various formulations are practically constant. The key component for the surface tension is obviously the co-surfactant which occupies the water-air interface in all formulations and reduces the surface tension of the water-air interface from 0.073 to practically 0.035 N/m.

If the penetration of the nano-emulsion droplets by ultrasonic waves is considered, it becomes clear that the sound waves encounter only the volumes (concentrations) of the components and not the forces which effect the formation of small particles.

One could compare the investigations with sound waves to the observation of the particles in a “supermicroscope” which will detect and quantify only the volume contributions of components to the particles. Therefore, it is consistent that the ultrasonic velocity is dependent on the sum of the components in the emulsion particles, whereas the particle size is determined by the ratio of the significant components.

Thus attenuation data measured at a single frequency contain information on the droplet size as indicated in Fig. 2. A refinement of this information by the application of the scattering theory and the combination with accurate data on the ultrasonic velocity could be promising for a determination of droplet sizes by ultrasonic resonator measurements at a single frequency, if the distribution of droplet sizes is pre-defined.

5. Conclusions

The oil component in the droplets and hydration of the droplets around their surface and the properties of the mixed phase of surfactant and co-surfactant can be characterized and quantified by measuring the ultrasonic velocity. The droplet size can be also measured considerably with ultrasonic methods and it might provide an alternative for the determination of the physical properties of nano-emulsions and for an estimation of the diameter of the nano-droplets. The size of the nano-droplets is an important indication of stability of these types of systems. As formulation factors determine the final droplet size and quality of these nano-emulsions which could be measured with higher correlation utilizing novel ultrasonic measurements. Thus the ultrasonic technique provides a promising tool to assess product quality of these pharmaceutical nano-emulsions and thus enabling with a new characterization tool.

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