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Stability of microbubbles prepared by co-axial electrohydrodynamic atomisation

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Abstract Previous studies have indicated that microbubbles prepared by co-axial electrohydrodynamic atomisation (CEHDA) are less stable than those prepared by other methods such as sonication and microfluidic techniques. The aim of this investigation was to determine the reasons for this observation and how this might be addressed in future work. Microbubbles were prepared by CEHDA using (i) a glycerol–air system, (ii) a glycerol–Tween 80–air system and (iii) a glycerol–zirconia–air system and also by simple agitation of (i) and (ii), in order to compare the effect upon the dissolution rate of microbubbles of different materials and processing methods. Both theoretical examination and the experimental results indicated that all three quantities were important in controlling the rate of microbubble dissolution, namely: surface tension at the gas/liquid interface, the effective diffusivity of gas through this interface and the initial concentration of gas dissolved in the surrounding liquid. However, it was the difference in gas concentration in the surrounding liquid that was indicated as the primary reason for the differences in stability observed with different processing methods. It was concluded, therefore, that improved stability could be achieved for microbubbles prepared using CEHDA by saturating the collecting fluid with gas and/or maintaining a high concentration of microbubbles during collection.

Keywords Microbubbles · Co-axial electrohydrodynamic atomisation · Diffusion · Surfactant · Particle stabilization

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Introduction

The preparation of microbubble suspensions is fundamental to a wide range of technological applications. In chemical engineering, for example, microbubble dispersions are used to enhance gas–liquid mass transfer (Bredwell and Worden 1998). In food technology, surfactant-stabilized microbubbles are used for protein recovery (Fuda et al. 2004). Specially designed pressure-resistant microbubbles are also used as aphron drilling fluids (Growcock et al. 2006). In biomedical applications, surfactant or polymer coated microbubbles are well established as contrast agents for diagnostic ultrasound imaging (Cosgrove 2006) and are under investigation in a range of therapeutic applications, including as vehicles for ultrasound mediated drug and gene delivery (Bekeredjian et al. 2005; Unger et al. 2002).

In all of these applications, microbubble stability is of great importance. With ultrasound contrast agents, for example, scattering efficiency is directly related to microbubble size, and thus any shrinkage due to gas diffusion from the bubbles will impair their effectiveness (Dijkmans et al. 2004; Stride et al. 2008b). Similarly, in therapeutic applications, deterioration of the microbubble coating may result in premature release of encapsulated material.

Conventionally, microbubbles have been prepared using techniques such as agitation/sonication and shear emulsification (Stride and Edirisinghe 2008). New methods have recently been developed, however, which provide a higher degree of control over microbubble characteristics (e.g. size, size distribution, coating uniformity). These include microfluidic techniques (Garstecki et al. 2004; Pancholi et al. 2008b) and co-axial electrohydrodynamic atomization (CEHDA) (Farook et al. 2007; 2008). In the latter, a liquid suspension and a gas are pumped through two concentric and coaxially aligned needles under the influence of an electrical potential difference to form a coaxial jet from which the microbubbles are obtained.

Although this method offers significant advantages in terms of production rate and control over microbubble uniformity, previous studies have indicated that microbubbles prepared using CEHDA are less stable in terms of dissolution time compared with those prepared by other methods such as sonication and microfluidic processing (Pancholi et al. 2008a). The aim of this investigation was to determine the reasons underlying this observation and methods for improving the stability of microbubbles prepared by CEHDA.

Experimental details

Microbubble preparation by CEHDA

Firstly, experiments were carried out on microbubbles prepared by electrohydrodynamic atomisation using glycerol, a glycerol–Tween 80 solution, and a glycerol–zirconia suspension as the liquid phase. To prepare the glycerol–Tween 80 solution, 2 wt% of Tween 80 (Lind-Chem Ltd. Norfolk, UK) was added to 245 g of glycerol and the mixture was left on a roller mixer for 12 h in order to prepare 250 g of homogeneous solution. The glycerol–zirconia suspension was prepared in a similar fashion but adding 2 wt% zirconia powder (Grade HSY3, mean particle size 0.5 μm, density 6,000 kg m^{-3}, Min-Chem Limited, Aldershot, UK) instead of Tween 80. The experimental set-up used for atomisation is shown in Fig. 1 and consisted of a pair of concentric stainless steel capillary needles (inner diameters 150 μm and 685 μm; outer diameters 300 μm and 1,100 μm). Both the needles were connected to the same DC power supply unit (Glassman Europe Limited, Bramley, UK) and earthed to a ring electrode (inner diameter 15 mm and outer diameter 20 mm) placed ~12 mm below the tip of the outer needle. The tip of the inner needle was raised ~2 mm inside the outer needle to ensure microbubble formation (Farook et al. 2007). The flow rates of the liquid and the air through the needles were controlled by high precision Harvard syringe pumps (Harvard Apparatus Ltd., Edenbridge, UK). The capacity of the syringes used for the air and liquid were 10 ml and 5 ml, respectively. The flow of material exiting the needles was observed and recorded using a LEICA S6D JVC-colour video camera attached to a zoom lens and a data DVD video recorder MP-600 using CDV Recorder/Editor DN-100 with a video screen for real time monitoring. For these experiments, the liquid and gas flow rates were 10 μl s^{-1} and 5 μl s^{-1}, respectively. The applied voltage was held constant at 8.7 kV. Microbubbles were collected for 300 s in a glass vial containing 6 ml of glycerol positioned 12 mm below the ring electrode.

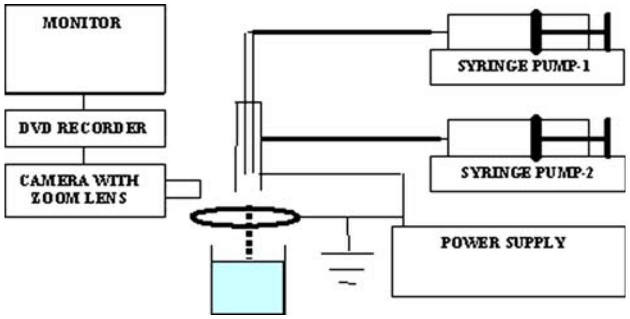

Fig. 1 Schematic of the experimental set-up for coaxial electrohydrodynamic atomisation

Microbubble preparation by agitation

Secondly, experiments were carried out on microbubbles prepared by simple manual shaking using firstly glycerol and then the glycerol–Tween 80 solution. 25 ml of glycerol or glycerol–Tween 80 was transferred to a cylindrical glass vial with a lid and shaken by hand for 900 s to prepare a microbubble suspension. This method was preferred to sonication because it is known that chemical modification of the coating material may occur (Suslick and Grinstaff 1990; Wang et al. 1996) during this type of processing due to inertial cavitation and this would increase the number of experimental variables. Chemical modification of the coating is not thought to be the main reason for the increased stability observed in previous studies, since microbubbles prepared by microfluidic processing, which does not involve inertial cavitation, were also seen to be more stable than those prepared by CEHDA.

Assessment of microbubble stability

Immediately following preparation, 0.05 ml of each microbubble suspension was transferred to a glass slide using a 1 ml syringe and examined via optical microscopy. A microbubble with diameter ~44 μm was selected and observed until the microbubble had dissolved to the extent that it was optically undetectable. The diameter of the microbubble was recorded at 120 s intervals. This process was repeated 3 times for each suspension.

Materials characterisation

The glycerol, glycerol–Tween 80 solution and glycerol–zirconia suspension were characterized at atmospheric pressure and ambient temperature (22°C) to determine their density, viscosity and surface tension values. Density was measured using a standard 25 ml specific gravity bottle. Viscosity was estimated using a VISCOEASY rotational viscometer (Camlab Limited, Cambridge, UK) and surface tension was measured using a Kruss Tensiometer (plate method).

Theory

The Laplace equation for the pressure difference across the surface of a bubble can be expressed as (Krasovitski and Kimmel 2006):

$$p_L = p_b - p_o = \frac{2\sigma}{R} \tag{1}$$

where, p_L is the Laplace or over-pressure, p_b is the pressure of the gas inside the bubble and p_o is the ambient pressure, R is the radius of the bubble and σ is the interfacial (surface) tension at the bubble surface. From Eq. 1 it is clear that a bubble subject to a higher surface tension will experience a higher over-pressure and so tend to dissolve more rapidly.

Even in the absence of surface tension, the presence of a gas concentration gradient between the bubble and the surrounding liquid will provide an additional driving force for microbubble dissolution. Clearly, this will depend upon the difference between the initial dissolved gas concentration (c_i) in the aqueous medium and the dissolved gas concentration at the bubble surface (c_s). The gas in the bubble is in equilibrium with the gas concentration at the bubble surface and is therefore equal to c_s (Duncan and Needham 2004). When $c_s > c_i$, the gas inside the bubble will diffuse outwards into the liquid causing a reduction in the bubble diameter ($D = 2R$).

The role of surface tension (σ) and the role of c_s and c_i in determining the stability of a bubble are well represented by the differential Eq. 2, developed by Epstein and Plesset (1950):

$$\dot{R} = \frac{k(c_i - c_s)BT}{M\left(p_o + \frac{4\sigma}{3R}\right)}\left(\frac{1}{R} + \frac{1}{\sqrt{\pi k t}}\right) \tag{2}$$

where $\dot{R}$ is the rate of change of bubble radius, k is the coefficient of diffusivity, B is the universal gas constant, T is the absolute temperature, M is the molecular weight of the gas and t is time.

It is clear from Eq. 2 that the rate of bubble diffusion will be higher for larger values of $(c_i - c_s)$. The third factor affecting bubble stability is the effective diffusivity of the interface, k. Both k and σ will depend upon the gas and liquid under investigation and also the presence of any coating material at the bubble surface which may reduce surface tension and significantly increase the resistance to gas diffusion (Borden and Longo 2002). These quantities will also be affected by the temperature and pressure at the bubble surface, as will the value of the dissolved gas concentration (c_s) there.

An approximate equation which accounts for the effect of surface tension upon c_s can also be derived following Epstein and Plesset as

$$\dot{R} \approx \frac{-kBTc_{sat}\left(1 + \frac{2\sigma}{R_o p_o} - \frac{c_i}{c_{sat}}\right)}{Mp_o\left(1 + \frac{4\sigma}{3Rp_o}\right)}\left(\frac{1}{R} + \frac{1}{\sqrt{\pi k t}}\right) \tag{3}$$

where c_{sat} is the saturation concentration for the gas in the surrounding liquid at a given temperature and pressure and R_o is the initial bubble radius. This equation neglects, however, any dependence of σ, k or c_s upon the bubble radius R.

Results and discussion

Effect of coating material upon microbubble stability

The material properties measured for the different liquid media are shown in Table 1.

The rates of dissolution for microbubbles prepared by electrohydrodynamic atomisation using the glycerol–air, glycerol–Tween–air and glycerol–zirconia–air systems are shown in Fig. 2. It is clear from this that the microbubbles prepared from the glycerol–air system dissolved at a greater rate than the microbubbles prepared from the other two systems. From Eq. 3, there are 3 factors which may potentially affect the stability of gas bubbles: (i) surface tension at the gas/liquid interface (ii) the gas concentration in the surrounding liquid and (iii) the diffusivity of the gas through the microbubble coating and/or in the surrounding liquid. Since the microbubbles from all three systems were collected in glycerol from the same source, the initial dissolved air concentration in the collecting medium (c_i) should have been the same for all three systems. As shown in Table 1, however, the surface tension values were different for each bubble (63 mN m^{-1}, 34 mN m^{-1}, 33 mN m^{-1}, for

Table 1 Measured material properties for the different liquid media used in the experiments

Material	Density kg m^{-3}	Surface tension mN m^{-1}	Viscosity mPa s
Glycerol	1217	63	740
Tween 80	1214	25	435
Glycerol with 2 wt% Tween 80	1214	33	690
Glycerol with 2 wt% zirconia	1240	34	760

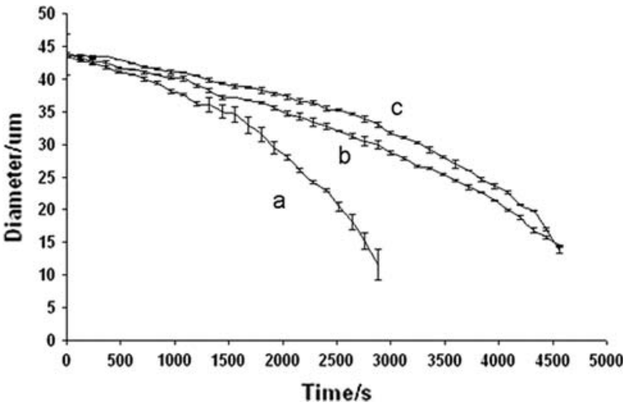

Fig. 2 Comparison of dissolution behaviour of microbubbles prepared by CEHDA using *a* glycerol–air–system *b* glycerol–2 wt% zirconia–air system and *c* glycerol–2 wt% Tween 80–air system

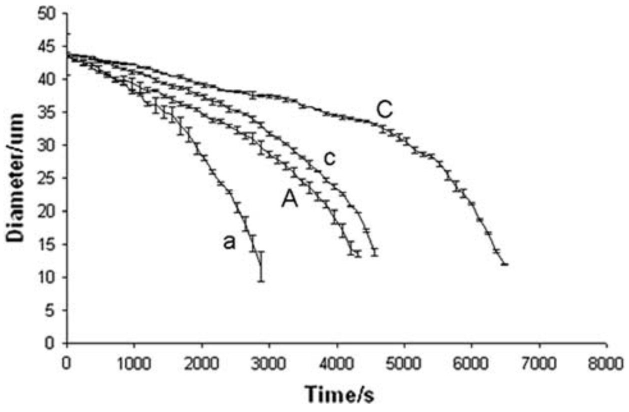

Fig. 3 Comparison of dissolution behaviour of microbubbles prepared by *a* CEHDA using glycerol–air system *A* simple agitation using glycerol–air system *c* CEHDA using glycerol–Tween 80–air system, and *C* simple agitation using glycerol–Tween 80–air system

glycerol, glycerol–zirconia suspension, and glycerol–Tween 80 solution, respectively)[1] and the observed behaviour (Fig. 2) of microbubbles with respect to the surface tension values is in qualitative agreement with Eq. 3. Similarly, the presence of the particles and/or surfactant at the bubble surface would also have modified the effective diffusivity at the bubble surface. In the absence of accurate measurements of this latter quantity, it is not possible to draw definite conclusions as to the relative influence of surface tension and diffusivity in this case, although it is clear from Eq. 3 that the rate of bubble dissolution has much stronger dependence upon k than σ.

Tween–80 is a well known surfactant and, as such, would be expected to reduce both surface tension and the effective diffusivity (Singhal et al. 1993). The stabilization of bubbles by solid particles is an interesting phenomenon, first observed by Pickering (1907) with the stabilization of emulsions by fine solid particles instead of surfactants and is widely known as Pickering stabilization. It is used in the recovery and separation of mineral ores, cleaning of crude oil, water treatment and in the food industry. However, since many of the underlying mechanisms were poorly understood, the topic has recently drawn the attention of many researchers once again (Dickinson et al. 2004; Binks and Lumsdon 2000; Du et al. 2003) and it has been demonstrated that stabilization is dependent on the hydrophobicity of the particles and hence their tendency to adsorb on to the bubble surface rather than disperse in the liquid medium. The results from this study (Fig. 2) show that microbubbles can similarly be successfully stabilized with solid particles which effect a reduction in surface tension (Table 1) and will reduce the surface area of the bubble available for diffusion (the size of the effect depending upon the size of the particles relative to that of the bubble and their concentration on the surface). Thus, it may be concluded that CEHDA does not affect the inherent ability of surfactants and/or particle suspensions to improve bubble stability.

Effect of the preparation method upon microbubble stability

In Fig. 3, the curves corresponding to the microbubbles prepared by CEHDA and agitation are denoted by lower and upper case letters, respectively (a/A refer to the glycerol air system and c/C to the glycerol–Tween80–air system). As may be seen, a similar stabilizing effect was observed in the presence of surfactant in both cases, but it is clear that the microbubbles prepared by agitation were more stable. This is in agreement with previous observations (Pancholi et al. 2008a) and there are a number of possible explanations.

Firstly, CEHDA may bring about an increase in surface tension and/or diffusivity which makes the bubbles less stable than those prepared by shaking (i.e. σ and k are larger for microbubbles prepared by CEHDA). Secondly, there may be insufficient time during atomisation for adsorption of a surfactant coating to take place, or the adsorption process is somehow inhibited (again leading to larger values of σ and k due to the lack of a complete coating). Thirdly, shaking may simply introduce more gas into the surrounding liquid so that the diffusion gradient is smaller, than for microbubbles prepared by CEHDA (i.e. the value of $c_i - c_s$ is lower).

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[1] Sato et al. (1998) reports that the surface tension of some liquids decreases under the influence of an electric field and thus there is a possibility that the surface tension values at the bubble surface were smaller than the values shown in Table 1. However, any reduction should have been proportional to the applied voltage which was the same for all three systems and it is not clear from the previous studies whether this effect would have persisted once the bubbles were removed from the electric field.

The results in the previous section indicate that CEHDA does not alter the qualitative effect of either surfactants or particles upon microbubble stability. Moreover, as mentioned above, it has previously been reported that the effect of an electric field is to reduce surface tension rather than increase it. Similarly, if CEHDA affected the process of surfactant absorption, then the curves for the glycerol–air system in the absence of surfactant (a and A) should still coincide, which they clearly do not. Thus the third hypothesis would seem to be the most likely explanation. Since there is a deliberate attempt to disperse air in the aqueous medium during shaking, the initial dissolved air concentrations for A and C will certainly have been higher than for a and c.

This would also account for the greater stability of the microbubbles prepared by microfluidic processing and sonication observed in the previous study (Pancholi et al. 2008a). On account of the nature of the microfluidic device and their monodispersity, the bubbles were much more closely packed in the collection vial in the case of the former and were also larger than those produced by CEHDA. Similarly, sonication involves both the entrainment of air in the solution being processed and produces much higher concentrations of microbubbles, so, again, stability would be expected to be higher on account of the higher gas concentration in the surrounding liquid.[2] A further characteristic of microbubble suspensions which is strongly affected by the preparation technique is the size distribution or polydispersivity. This is outside the scope of the present study but is discussed in detail in Stride and Edirisinghe (2008).

Given that it is the collection and storage of the microbubble suspensions which appears to be more important than the actual bubble formation process, improving the stability of microbubbles prepared by CEHDA should be relatively straightforward. Firstly, as shown in this study, both surfactants and particle suspensions can have a stabilizing effect and, thus, selection of the appropriate coating material is important. Particle stabilization may have other benefits in certain applications (Stride et al. 2008a), although of course bio-compatibility of the particles will be an essential requirement if these bubbles are to be used in biomedical applications. Collecting the microbubbles in high concentrations will also be beneficial, as will saturating the collecting liquid with the filling gas to reduce the concentration gradient.

Conclusions

The aim of this investigation was to determine the reasons underlying previous observations of reduced stability of microbubbles prepared by CEHDA as compared with those prepared by sonication or microfluidic techniques. Theoretical examination indicated that the three factors controlling microbubble dissolution were the surface tension at the gas/liquid interface, the effective diffusivity of gas through this interface and the initial concentration of gas dissolved in the surrounding liquid. The experiments indicated that all three of these quantities were important in controlling the rate of microbubble dissolution, but that it was the difference in gas concentration in the surrounding liquid that was the reason for the differences in stability observed with different processing methods. Consequently, it was concluded that the stability of microbubbles prepared using CEHDA could be improved by saturating the collecting fluid with gas and/or maintaining a high concentration of microbubbles during collection. Stabilization using both surfactants and solid particle suspensions was also found to be effective and to be unaffected by the CEHDA process.

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[2] As mentioned in Sect. Theory, chemical modification of the coating was not thought to be a significant factor for this study, although it should be noted that it can have a substantial effect upon microbubble stability with certain types of coating e.g. protein membranes prepared by sonication (Suslick and Grinstaff 1990).

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