Surface Charge Measurement of SonoVue™, Definity™ and Optison™: a comparison of Laser Doppler Electrophoresis and Micro-Electrophoresis

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Abstract

Microbubble (MB) contrast-enhanced ultrasonography is a promising tool for targeted molecular imaging. It is important to determine the MB surface charge accurately as it affects the MB interactions with cell membranes. In this paper, we report the surface charge measurement of SonoVue™, Definity™ and Optison™. We compare the performance of the widely used laser Doppler electrophoresis (LDE) with an in-house micro-electrophoresis system. By optically tracking MB electrophoretic velocity in a microchannel, we determined the zeta potentials (ZP) of MB samples. Using micro-electrophoresis, we found ZP values of SonoVue™, Definity™ and Optison™ to be -28.3, -4.2 and -9.5 mV with relative standard deviations (RSD) of 5%, 48% and 8% respectively. In comparison, LDE gave -8.7, +0.7 and +15.8 mV with RSD of 330%, 29,000% and 130% respectively. We found that the reliability of LDE is compromised by MB buoyancy. Micro-electrophoresis determined ZP values with a 10-fold improvement in RSD.

Keywords: Microbubbles (MB), ultrasound contrast agents, SonoVue™, Definity™, Optison™, surface charge, zeta potential, micro-electrophoresis, laser Doppler electrophoresis (LDE), particle tracking

Introduction

Medical ultrasound is a widely established, powerful tool for diagnostic imaging applications. Combined with the use of microbubble contrast agents, it has been shown to be a promising tool for drug delivery and therapeutic applications (Kang and Yeh, 2011; Villanueva, 2012). Microbubbles (MB) are small (typically 2-3 μm in diameter) gas cores stabilised by a biocompatible shell and suspended in an aqueous dispersion. For lipid-based MB shells, changing the lipid type to be cationic, anionic or neutral can affect the bulk MB-cell interactions (Fisher et al., 2002). This in turn can have an overall effect on the MBs' suitability as a therapeutic agent (Xie et al., 2012) and provide another factor for improving targeted drug delivery. It is therefore important to be able to characterise the surface charge of the MB shell accurately. Current laser Doppler electrophoresis (LDE) methods for determining MB surface charge rely on measuring the MB electrophoretic mobility in an electric field by light scattering and calculating its zeta potential (Malvern Instruments Ltd., 2004). The main limitation of this method is that the buoyancy force acting on the bubbles significantly affects the measurement, because of the vertical configuration of an LDE cell, often leading to inaccurate results.

An alternative configuration to measure the zeta potential of charged microparticles is micro-electrophoresis, where optical microscopy is used to directly image the particles and characterize their electrophoretic mobility. Here the microchannel where the particles flow is horizontal and it is positioned on a microscope stage. This method has been used to characterize the surface charge of bubbles, primarily in the context of mineral floatation, to measure the zeta potential that bubbles acquire upon adsorption from solution of surfactants (Collins et al., 1978; Yoon and Yordan, 1986) or polymers (Oliveira and Rubio, 2011) and as a function of pH (Han and Dockko, 1998).

In this study, we report the surface charge of the clinical MBs: SonoVue™, Definity™ and Optison™ using both LDE (Malvern Zetasizer Nano, Malvern Instruments Ltd., UK) and an in-house micro-electrophoresis setup. Due to MB buoyancy, we found the LDE measurements to be

unsatisfactory. Our measurements by micro-electrophoresis are the first systematic characterisation of the surface charge of SonoVue™, Definity™ and Optison™.

Theory

Ultrasound contrast agents are typically prepared in saline solution (0.15M NaCl). At the boundary between the aqueous solution and the phospholipid layer, an electrical double layer (EDL) forms, because ions (Na^+ and Cl^-) from the surrounding solution are attracted to the charge on the surface (see Fig. 1). It is, in general, not possible to determine the actual surface potential (ψ) as it is always shielded by the diffuse electric double-layer. The quantity that is typically measured is the zeta potential (ζ), i.e., the potential at the plane where this layer of ions is able to slip past the bulk electrolyte. The distance from the zeta potential plane to the surface potential plane is the thickness of the diffuse double-layer, called Debye length, κ^{-1}, and is determined using the following equation (Berg, 2010):

$$\boldsymbol{\kappa^{-1}} = \sqrt{\frac{\varepsilon_r \varepsilon_0 k_b T}{2 N_a e^2 I}} \qquad (1)$$

where ε_r is the dielectric constant of the dispersion medium, ε_0 is the relative permittivity of free space, k_b is the Boltzmann constant, T is the absolute temperature, N_a is Avogadro's constant, e is the elementary charge and I is the ionic strength of the solution (in a 1:1 electrolyte such as sodium chloride, I is equal to the concentration). Therefore, in 0.15 M NaCl, I = 0.15 mol/dm^3.

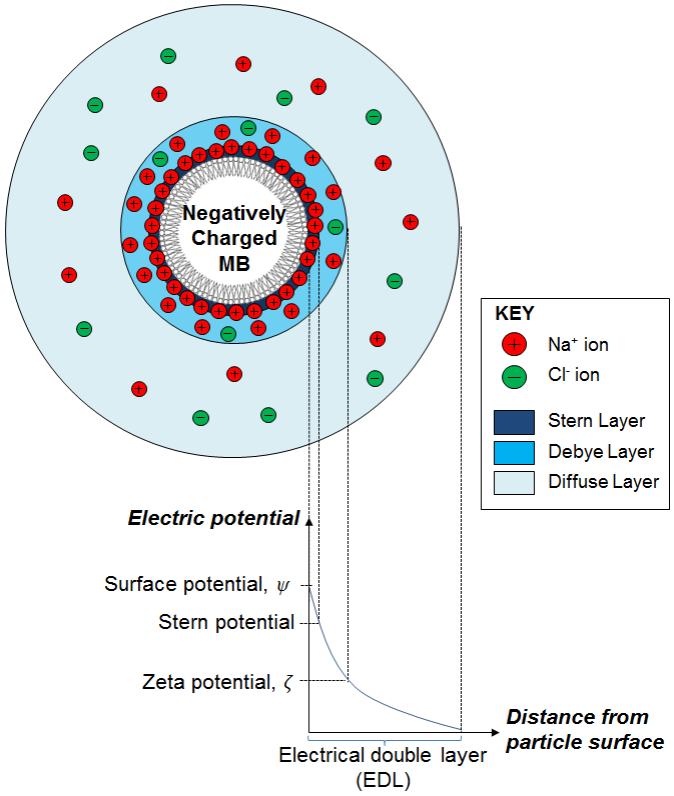


Figure 1 *– Schematics of the electrical double layer (EDL) formed when a negatively charged microbubble (MB) is in 0.15 M NaCl solution. Diagram adapted from (Hunter, 2001).*

One way to determine zeta potential of charged microparticles is by measuring the electrophoretic mobility of the particle in an electric field. At low Reynolds numbers, the particle velocity is proportional to the applied field (Hunter, 2001) and electrophoretic mobility, U_e, can be determined by measuring the particle velocity in the electric field using the following equation:

$$U_e = \frac{v}{E} \qquad (2)$$

where v is the measured velocity of the particle and E is the electric field strength. The zeta potential can then be calculated using Smoluchowski's mobility equation (Smoluchowski, 1903):

$$U_e = \frac{\varepsilon_r \varepsilon_0 \zeta}{\eta} \qquad (3)$$

where ε_r is the dielectric constant of the dispersion medium, ε_0 is the relative permittivity of the free space and η is the dynamic viscosity of the dispersion medium. The electrophoretic mobility is

independent of the particle radius. This equation is valid only in the limit of a thin double layer, $\kappa a \gg 1$, where κ is the reciprocal thickness of the double layer and a is the particle radius (Booth, 1948; Overbeek, 1950). For our MBs, using I = 0.15 mol/dm^3 and T = 298 K, we have calculated κ = 0.959 nm, therefore, for particles with sizes in the 2-3 μm, κa = 2086, thus making this equation suitable.

Laser Doppler Electrophoresis (LDE)

The most widely used method for determining particle surface charge is laser Doppler electrophoresis (LDE). In this setup, a coherent laser source is split and the two beams are made to intersect in the zeta cell which contains the sample dispersion. The two intersecting beams form interference patterns. As a particle moves through this interference patterns, it causes fluctuations which can then be related to the speed of the particles. This method effectively measures the frequency of particles of a certain mobility, thereby giving a distribution of mobilities rather than a single average value. However, considering the orientation of the zeta cell, the obtained electrophoretic mobility of the charged particles is strongly affected by MB buoyancy. In the standard design of this instrument, the electrophoretic mobility is determined from particle displacements in the vertical direction, which is also the direction for MB buoyancy (see Fig. 2).

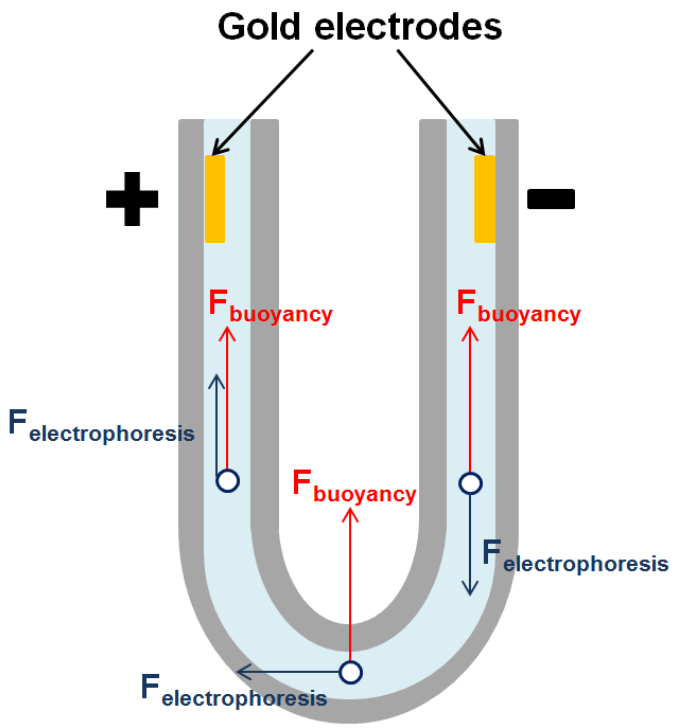


Figure 2 *– Schematic representation showing forces acting on a buoyant, negatively charged microbubble (MB) in a disposable folded capillary cell (Malvern Instruments, Worcestershire, UK). Depending on the location of MB, its true electrophoretic mobility can be affected by buoyancy. Mean terminal velocities for 2-5µm MB is in between 2-14µm/s which is comparable to the measured electrophoretic velocities obtained by laser Doppler electrophoresis (LDE).*

For typical colloidal particles, the density difference between the particles and the fluid is not sufficiently large that sedimentation or buoyancy affect the measurements. However, for gas MB, the density difference is approximately 1000 kg/m^3 and the buoyancy force significantly affects the vertical translation of bubbles. Therefore, the speed of bubbles towards the electrode does not give the true electrophoretic mobility of the charged MB.

Micro-electrophoresis

Micro-electrophoresis is a simpler approach for determining particle surface charge and is based on the same mechanisms as LDE. In this method, particle movement under electric field is directly observed under bright-field microscopy. Particle velocity is determined by measuring particle lateral displacement within a certain period of time. Using equations 2 and 3 the zeta

potential of the charged microparticle can be obtained. To obtain reliable measurements, it is important to take into account that the velocity profile in such a capillary is affected by electro-osmotic flow generated if the surface of the capillary walls is charged (see Fig. 3). This electro-osmotic flow causes a back flow, which can cause the particles to move in the reverse direction. The points in the cell where the osmotic flow balances the forward flow are known as the stationary planes. It is therefore crucial to identify the position of these stationary planes in order to accurately determine the particle's true electrophoretic mobility.

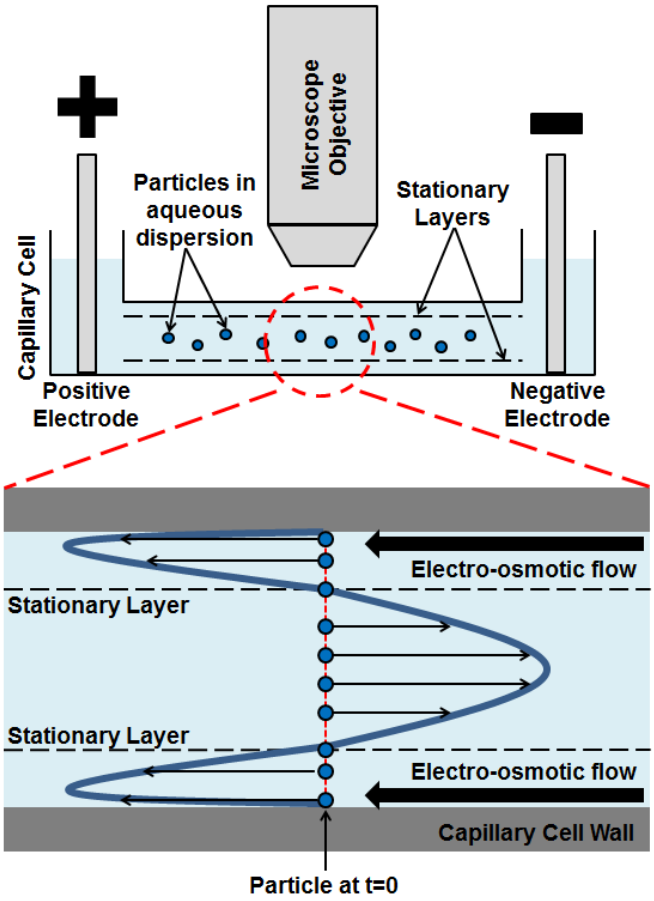


Figure 3 *– Schematics of a micro-electrophoresis setup. Particle movement was observed under bright-field microscopy and particle zeta potential was determined by measuring particle displacement under electric field. Part of the capillary was magnified to show flow velocity profile in an electric field (side view). Electro-osmotic flow generated by charged cell wall causes a back flow, affecting particle velocity. Image adapted from (Hunter, 2001).*

In LDE, particle mobilities are determined at the middle of the cell, away from the cell walls. In micro-electrophoresis setups, the particle's true electrophoretic mobility can be determined by focusing on the plane in the middle of cell, but often this is difficult to determine in a narrow channel. Instead, electro-osmotic effects can be minimised by ensuring that the electrodes do not come into contact with the cell walls and by using a neutral material or coating.

Experimental

Preparation of microbubble (MB) suspensions

In this study, the following clinically-approved ultrasound MB contrast agents were used: SonoVue™ from Bracco Diagnostics Incorporated (Milano, Italy), Definity™/Luminity™ from Bristol-Myers Squibb Medical Imaging Incorporated (New York, USA) and Optison™ from GE Healthcare Medical Diagnostics Division (Princeton, NJ, USA). All MB samples were prepared according to the manufacturer's instructions, then diluted to approximately 10^6 MB/ml with saline (0.15 M NaCl). MB size distributions and concentrations were determined optically following protocols laid out by (Sennoga et al., 2012). Table 1 lists all of the MBs used, the gas core, shell compositions and reported charge (Miller and Nanda, 2004).

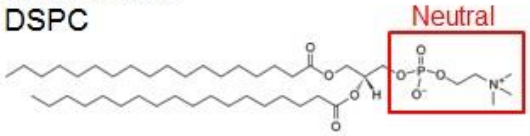
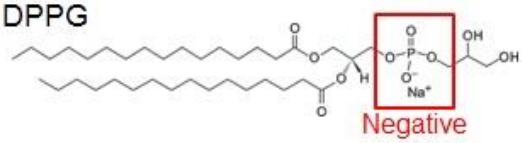
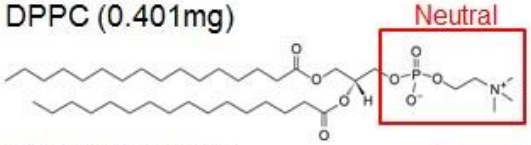
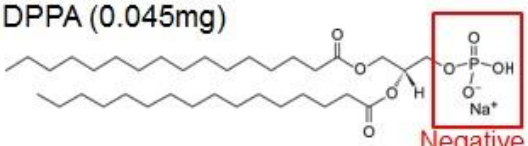
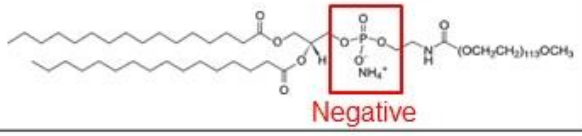
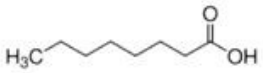
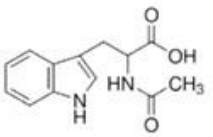
Microbubble Agent	Gas*	Shell Type*	Shell/Lipid Composition*	Reported Charge†
SonoVue™	SF_6	Lipid	• Macrogol 4000 (PEG) • Palmitic acid • DSPC  • DPPG 	Negative
Definity™	SF_6	Lipid	• DPPC (0.401mg)  • DPPA (0.045mg)  • MPEG5000-DPPE (0.304mg) 	Negative
Optison™	C_3F_8	Albumin	• Human serum albumin (amino acid dominated) • Caprylic acid  • N-acetyltryptophan 	Slight Negative

Table 1 *– Microbubble (MB) contrast agents used and their gas cores, shell compositions (with charged groups highlighted) and reported effective charge of shells. *Gas cores and shell compositions obtained from manufacturers' leaflets. †MB effective charge were reported by* (Miller and Nanda, 2004)*, no experimental data provided.*

Zeta potential measurement using Laser Doppler Electrophoresis (LDE)

Zeta potentials of MB dispersions were determined by LDE, using a Zetasizer Nano Z (Malvern Instruments Ltd., Worcestershire, UK). Diluted (to approximately 10^6 MB/ml) MB dispersions of each MB agent were inserted into 0.75 ml cuvettes (disposable folded capillary cells,

Malvern Instruments Ltd.), whilst avoiding the introduction of air bubbles into the zeta cell. The cuvette was then loaded into the machine and measurements were run as controlled by the Zetasizer Software (Version 7.10, Malvern Instruments Ltd.). Measurements were repeated three times on three independent samples to ensure reproducibility of results. The cuvette was re-agitated between repeat measurements to ensure thorough mixing of MB, calibration time was set at 10 seconds and internal runs reduced to 3, in order to minimise particle aggregation due to MB buoyancy. The applied voltage was reduced to 30 V to ensure MB integrity in the electric field and to ensure results were comparable to micro-electrophoresis experiments.

Preparation of microchannel

Ibidi™ µ-slide I (0.2) LUER uncoated microchannels (Thistle Scientific, UK) with 50 µl channel volumes were used. Since the hydrophobic channel is charged, microchannels were coated with Lipidure™-CM5206 (NOF America) in order to reduce electro-osmotic flow on the walls as well as to avoid MB sticking to channel walls (due to hydrophobic-hydrophobic interaction). The microchannels were flooded with a mixture of 0.5% w/v Lipidure™ dissolved in 96% w/v ethanol and left overnight to evaporate the ethanol. The channels were then flushed with distilled water then sodium chloride solution (0.15 M) before use.

Micro-electrophoresis set-up

Approximately 50 µl of MB suspensions (diluted to approximately 10^6 MB/ml) flowed through the Lipidure™-CM5206 coated ibidi™ microchannel and an electric potential difference of 29.8 V was applied across the channel via stainless steel electrodes (see Fig. 4). The set-up was mounted onto an inverted, bright-field microscope with a motorised stage. MBs, due to their gas core, have a very different refractive index compared to the liquid surroundings, so they can easily be observed under normal light, without the need for fluorescence tagging.

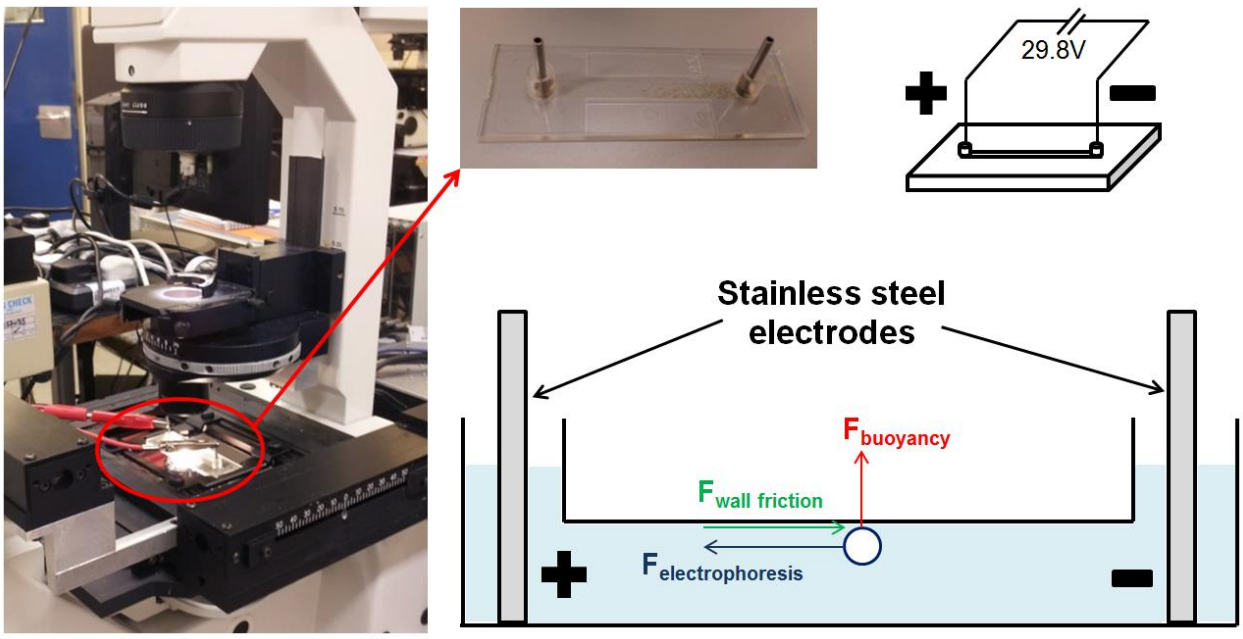


Figure 4 *– (Left) Experimental set-up showing an ibidi™ μ-slide I (0.2) luer (channel dimensions: L = 5 cm, W = 0.5 cm, H = 200 μm, V = 50 μl) and (Right) schematic representation of microchannel showing forces acting on a buoyant, negatively charged MB in an ibidi™ microchannel (side view). Electrophoretic mobility in the lateral direction is measured, minimising the effect of buoyancy.*

MB single particle tracking

An in-house MATLAB particle tracking software was developed and used to measure the average MB electrophoretic mobility over time. By setting threshold values, this particle tracking algorithm identifies MBs based on their roundness, sizes and displacements between frames. Therefore, only MBs of a certain size, roundness and velocity were tracked (see Video 1). The algorithm rejected bubbles which form aggregates, those that were stationary, not perfectly round and outside a certain size range (smaller than 1 μm and larger than 7 μm). From the measured MB velocity under electric field, the zeta potential was then determined using equation 2. Optical tracking enabled us to simultaneously determine the electrophoretic velocity and particle size for each MB detected by the algorithm. With this information we experimentally tested the relationship between electrophoretic velocity and particle size to confirm that no other forces affect MB motion.

Results and Discussion

Zeta potential measurements by LDE

In a prior study, MB concentrations in the zeta cell were determined optically before and after zeta potential measurements with LDE (Sennoga et al., 2010) and via the instrument's built-in sizing function. The MB concentration was found to have decreased after the measurement, which suggested that MBs were destroyed during the measurement. Further interrogation of the machine specification showed that, due to the low conductivity of MB particles, the Zetasizer automatically increases the voltage applied during the measurement to up to 150 V. In order to minimise destruction at such high voltages, in this study we set the applied voltage to 30 V. Zeta potentials reported here are at 30 V, which is still high enough to cause significant electrophoretic movement.

Zeta potential distribution graphs, obtained using the LDE method, were reported in Figure 5. The mean zeta potential values for SonoVue™, Definity™ and Optison™ were -9mV, +0.69mV and +15.78mV respectively. (Miller and Nanda, 2004) reported the surface charges of SonoVue™, Definity™ and Optison™ to be negative. The results we obtained with LDE were not in agreement with the values reported by (Miller and Nanda, 2004). We found a wide distribution of values in each sample (see Fig. 5). The relative standard deviations (RSD) for each sample ranged from 130% to 30,000%, which indicated poor reproducibility of the results. In some cases, it was not possible to determine whether the zeta potential was positive or negative.

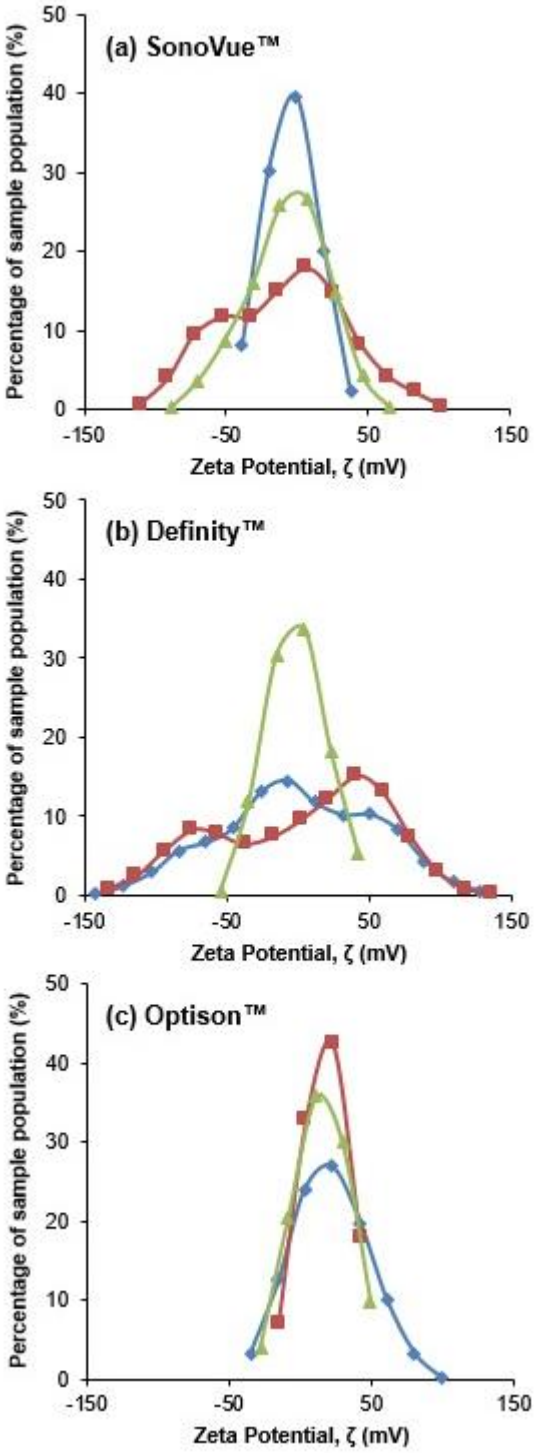


Figure 5 *– Zeta potential distribution graphs obtained with laser Doppler electrophoresis (LDE) for (a) SonoVue™, (b) Definity™ and (c) Optison™ respectively. Different coloured lines show repeats (n=3). The mean zeta potential values for SonoVue™, Definity™ and Optison™ were -9mV, +0.69mV and +15.78mV respectively, which, due to wide distribution of values obtained is not representative of the sample. The relative standard deviations (RSD) for each sample ranged from 130% to 30,000%, making it difficult to determine whether the zeta potential was positive or negative.*

For the range of zeta potential values found here (-10 mV to 20 mV), the electrophoretic mobilities were in the range of (-1 to 1.5 X 10^{-8} m^2 V^{-1} s^{-1}) which corresponded to electrophoretic velocities in the range of -3 µm/s to 5 µm/s. We compared this range of velocities with the mean terminal velocity of buoyant bubbles to assess the magnitude of the effect on the LDE measurement. The mean terminal velocity of a buoyant, coated bubble in the range of 2-5 µm can be calculated by balancing, at steady state, the viscous drag force, F_d, given by Stokes' equation:

$$F_d = -6\pi r v_t \eta \qquad (4)$$

with the buoyancy force, F_b:

$$F_b = \frac{4}{3}\pi r^3 g \Delta\rho \qquad (5)$$

where, r is the bubble radius (m), v_t is the mean terminal velocity, η is the dynamic viscosity of water, g is the the acceleration due to gravity and $\Delta\rho$ is the difference in density between the two mediums (in this case, between water and air) (Clift et al., 1978).

At steady state, $F_b + F_d = 0$, and therefore we obtained the mean terminal velocity of a buoyant coated bubble of diameter 2-5 µm to be in the range of 2 µm/s to 14 µm/s, comparable to the electrophoretic velocity detected by the LDE, which is in the range of 0.01µm/s to 5µm/s. Note that steady state is reached in a few microseconds, and therefore the initial transient behaviour does not affect this analysis. The effect of buoyancy is reflected in the results obtained, which show both positive and negative zeta potential values for each sample. The disposable zeta cell design and orientation causes the electrophoretic velocity to be affected by bubble buoyancy, as the electrophoretic velocity is measured in the same direction as bubble buoyancy. If the mean terminal velocity exceeds the electrophoretic velocity, which is the case for either the larger MBs in the sample, or for MBs with low zeta potential, the bubbles move upwards irrespective of the direction of the electric field and are detected as being attracted to the opposite electrode (see Fig 2).

Zeta potential measurements by micro-electrophoresis

By tracking single MB movement under electric field, particle sizes and electrophoretic velocities were measured. The zeta potential of MB was determined using equations 2 and 3.

As our MB samples were polydisperse, we have presented our micro-electrophoresis data in the form of distribution graphs for better representation of the variation in values (see Fig. 6). Our results confirmed that SonoVue™, Definity™ and Optison™ are negatively charged, with average zeta potentials of -28.3, -4.2 and -9.5 mV with relative standard deviations (RSD) of 5%, 48% and 8% respectively. All three MBs agree with the reported values by (Miller and Nanda, 2004), which showed all three MBs to be negatively charged. We have presented here a systematic study of MB surface charge.

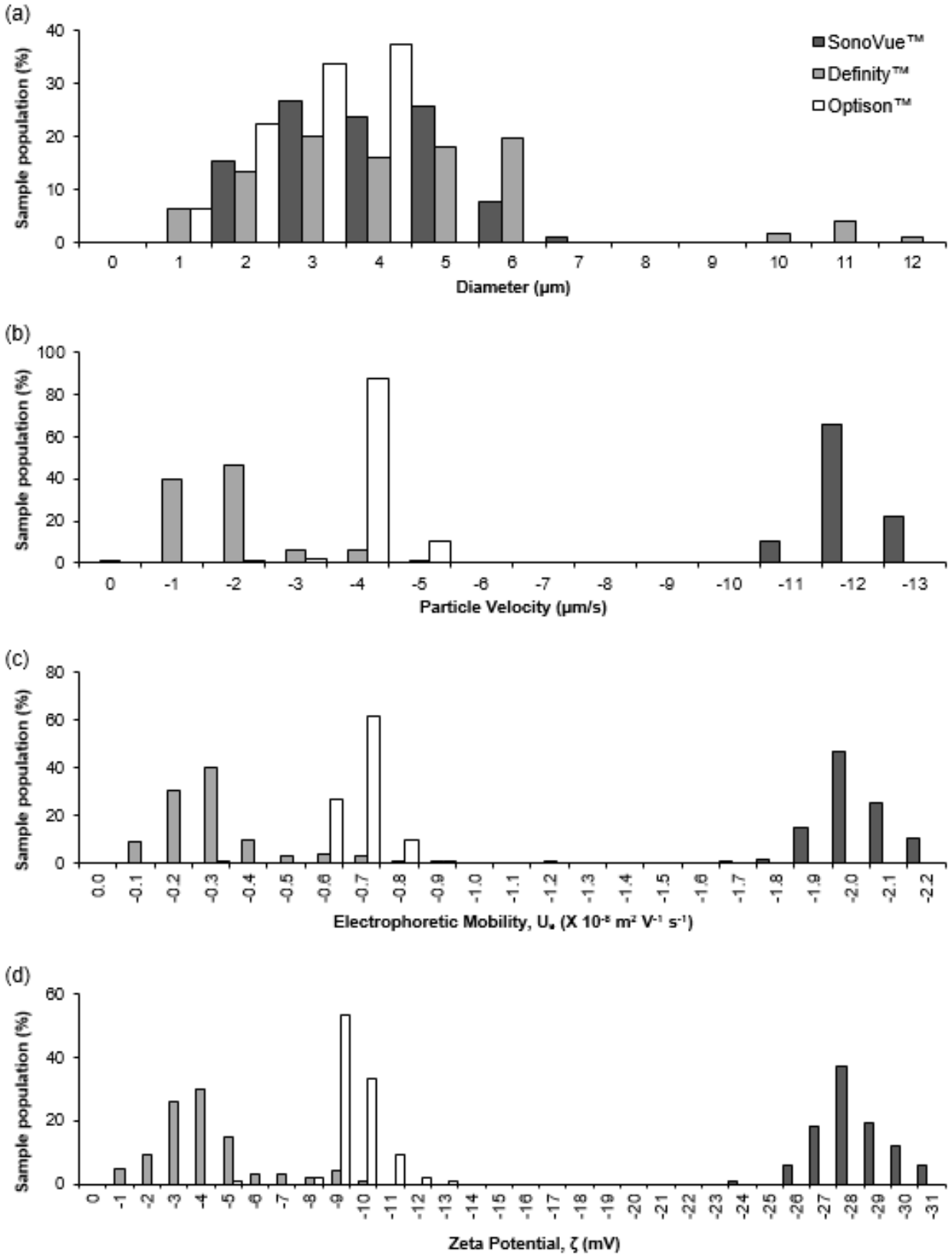


Figure 6 *– Histograms showing (a) size distributions, (b) measured particle velocities, (c) electrophoretic mobilities and (d) zeta potentials of SonoVue™, Definity™ and Optison™ respectively.*

Our method showed a 10-fold improvement in RSD values. The magnitude of the surface charge of lipid MBs depends on the amount of charged lipid groups in the MB shell. The amount of charged DPPG available in SonoVue™ is not known as the exact formulations were not given by the manufacturers. We expected Definity™ to have a low negative zeta potential value, as the shell is made up of only a small amount of negatively charged lipid, DPPA (0.07 µmol) and MPEG5000-DPPE (0.05 µmol). The majority of the shell is made up of the neutral lipid, DPPC, 0.55 µmol (see Table 1).

For Optison™, it is difficult to quantify the charged groups present on the MB shell. Optison™ consists of denatured human serum albumin, a protein dominated by amino acid groups which has a positive charge (Fogh-Andersen et al., 1993). However, the overall charge for Optison™ has been reported to be slightly negative (Miller and Nanda, 2004). We found Optison™ to have a zeta potential of -9.5 mV, which is in agreement with the reported negative charge. There may have been positively-charged groups present on Optison™'s shell surface, but the overall negative charge suggest that there are more negatively-charged groups contributing to the overall negative charge.

Our method proved successful for measuring the surface charge of Optison™. The hydrophobic-hydrophobic interaction between the hydrophobic pockets on the proteins that make up the Optison™ shell and the uncoated hydrophobic microchannel is sufficiently energetically favourable as to stop the Optison™ MBs from electrophoretic movement. Coating the hydrophobic microchannel with Lipidure™-CM5206 has allowed successful measurement of Optison™ surface charge.

In order to confirm that no other forces act on the MBs in the micro-electrophoresis setup, we plotted the zeta potential values as a function of MB diameter (see Fig. 7). We found limited correlation ($R^2 << 1$) between particle size and speed, confirming that other forces in this configuration do not significantly affect the measurement. For instance, because the bubbles are in contact with the top wall of the microchannel, sliding friction or rolling of the bubbles could affect

the measurement. These effects depend on the size of the bubbles. Since the observed dependence of the velocity on the bubble size is very weak, we conclude that, even though the bubbles are in contact with the wall, the measurement of the zeta-potential can be considered satisfactory.

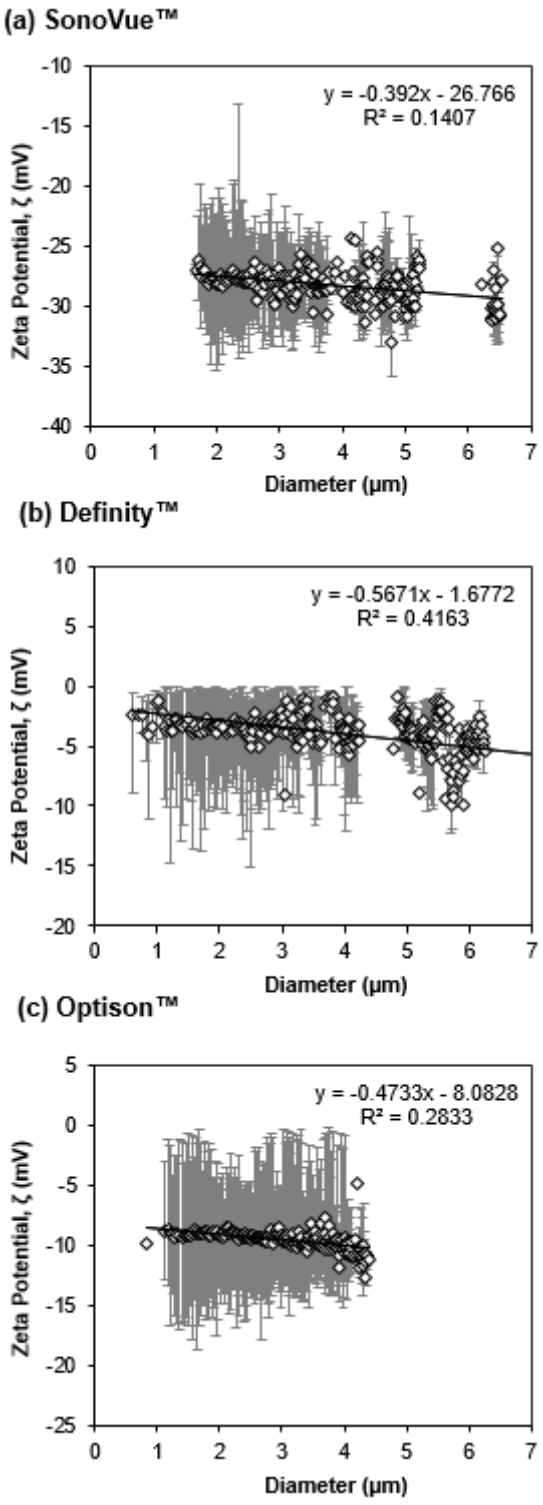


Figure 7 *– Graphs of zeta potentials versus particle diameter for (a) SonoVue™, (b) Definity™ and (c) Optison™. R^2 values show minimal correlation suggesting minimal wall effects as there is limited dependency between MB velocity and size.*

This observation is consistent with a study conducted by (Agnihotri et al., 2009) where values of electrophoretic mobilities of different-sized spherical gold particles were found to be dependent on electrolyte concentration and independent of sphere size.

The biggest possible effect on the measured particle velocity is electro-osmotic effects at the channel walls. We minimised this effect by coating the microchannel walls to make them neutral. Another factor that can affect the measured particle velocity in the micro-electrophoresis setup is the contact interaction between the buoyant MB and the top surface of the microchannel. This contact may introduce a sliding or rolling friction force, which could affect our measured particle velocity. The results of Figure 7 confirm that if contact forces are present, they did not significantly affect the measurement of electrophoretic mobility.

When comparing our method to the widely-used LDE (see Table 2), we found that our method show a lower relative standard deviation of values (at least a 10-fold improvement) and because we are visualising MB movement under bright-field microscope, we can unambiguously determine whether MBs are positively or negatively charged. In addition, the volume required in order to carry out zetasizing (0.75 ml) is large when compared to our microchannel approach where the channel volume is only 50 µl. The volume required is merely one-fifteenth of the LDE approach. This method is easily replicable with reproducible results.

	Laser Doppler Electrophoresis (LDE)				*Micro-electrophoresis*			
	Mean	**±S.D.**	**RSD (%)**	**Range (Min ~ Max)**	**Mean**	**±S.D.**	**RSD (%)**	**Range (Min ~ Max)**
SonoVue™								
Size, D (μm)	0.50	0.10	21	0.37 ~ 0.67	3.88	1.26	32	1.67 ~ 6.53
P.V. (μm/s)	-2.55	8.00	328	-0.23 ~ 0.17	-12.08	0.56	5	-14.07 ~ -10.39
E.M. ($X10^{-8}$ m^2 V^{-1} s^{-1})	-0.67	2.09	328	-5.97 ~ 4.49	-2.03	0.09	5	-2.36 ~ -1.74
Z.P. (mV)	-8.66	27.16	328	-77.50 ~ 58.14	-28.29	1.31	5	-32.96 ~ -24.33
Definity™								
Size, D (μm)	0.71	0.67	95	0.23 ~ 1.98	4.37	2.29	52	0.62 ~ 11.57
P.V. (μm/s)	0.02	12.75	29216	-21.43 ~ 28.64	-1.78	0.86	48	-6.93 ~ -0.38
E.M. ($X10^{-8}$ m^2 V^{-1} s^{-1})	0.05	3.31	29216	-7.71 ~ 7.44	-0.30	0.14	48	-1.16 ~ -0.06
Z.P. (mV)	0.69	42.97	29170	-99.94 ~ 96.38	-4.20	2.01	48	-16.24 ~ -0.89
Optison™								
Size, D (μm)	0.91	0.33	36	0.43 ~ 1.27	3.04	0.90	30	0.82 ~ 4.40
P.V. (μm/s)	4.69	6.15	134	-8.19 ~ 18.44	-4.07	0.37	8	-5.39 ~ -2.05
E.M. ($X10^{-8}$ m^2 V^{-1} s^{-1})	1.22	1.60	134	-2.62 ~ 4.79	-0.68	0.06	8	-0.90 ~ -0.34
Z.P. (mV)	15.78	20.71	134	-33.99 ~ 62.08	-9.50	0.80	8	-12.62 ~ -4.80

Table 2 *– Tabulated mean, standard deviation (±S.D.), relative standard deviation (RSD, %) and range (minimum to maximum) in values of MB diameters, D (μm), particle velocities, P.V. (μm/s), electrophoretic mobilities, E.M. ($X10^{-8}$ m^2 V^{-1} s^{-1}) and zeta potentials, Z.P. (mV) for SonoVue™, Definity™ and Optison™ obtained by laser Doppler electrophoresis (LDE) using the Malvern Zetasizer Nano (Malvern Instruments, UK) and micro-electrophoresis. Zeta potential values obtained by micro-electrophoresis show at least a 10-fold improvement in RSD.*

Conclusion

In this study, we used LDE to determine the surface charge of charged MBs and found zeta potential values of SonoVue™, Definity™ and Optison™ to be -8.7mV, +0.7mV and +15.8mV with relative standard deviations (RSD) of 330%, 29,000% and 130% respectively. We found the

results through LDE to be unsatisfactory as MB electrophoretic mobility was compromised by MB buoyancy. Using micro-electrophoresis, we demonstrated a relatively cheaper and easily replicable way to determine MB surface charge with a 10-fold improvement in measurement precision. MB movement was directly observed under bright-field microscopy in real time. MB electrophoretic mobility was determined in the lateral direction, thus excluding the effects of MB buoyancy on the translation velocity. Using this method, we found zeta potential values of SonoVue™, Definity™ and Optison™ to be -28.3mV, -4.2mV and -9.5mV with relative standard deviations (RSD) of 5%, 48% and 8% respectively.

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