Provided for non-commercial research and educational use only.
Not for reproduction or distribution or commercial use.


This article was originally published in a journal published by Elsevier, and the attached copy is provided by Elsevier for the author's benefit and for the benefit of the author's institution, for non-commercial research and educational use including without limitation use in instruction at your institution, sending it to specific colleagues that you know, and providing a copy to your institution's administrator.

All other uses, reproduction and distribution, including without limitation commercial reprints, selling or licensing copies or access, or posting on open internet sites, your personal or institution's website or repository, are prohibited. For exceptions, permission may be sought for such use through Elsevier's permissions site at:

http://www.elsevier.com/locate/permissionusematerial

Available online at www.sciencedirect.com
ScienceDirect
Colloids and Surfaces B: Biointerfaces 53 (2006) 121–126
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

# Ultrasonic characterization of proteins and blood cells

Andrei S. Dukhin [a,*], Philip J. Goetz [a], Theo G.M. van de Ven [b]

[a] *Dispersion Technology Inc., 364 Adams Street, Bedford Hills, NY 10507, USA*
[b] *Department of Chemistry, McGill University, 3420 University Street, Montreal, Que., Canada H3A 2A7*

Received 13 June 2006; accepted 10 August 2006
Available online 22 August 2006

**Abstract**

Ultrasound changes its intensity and speed when propagating through a liquid or a suspension containing particles. In addition it generates a weak electric signal by altering the motion of ions and charged particles. Hence acoustic and electroacoustic measurements provide information about the properties of suspended particles and molecules. Here we present both acoustic and electroacoustic results on blood suspensions and protein solutions, relevant to life sciences. For blood cells a strong increase in acoustic attenuation with volume fraction is found, from which the speed of sound in an erythrocyte is found to be about 1900 m/s, assuming the attenuation is due to scattering only. A similar value of 1700 m/s is found from the increase in sound speed of the dispersion with concentration. Electroacoustic measurements on bovine serum albumin (BSA) yield a charge of about seven elementary charges per BSA molecule. These results show the power and usefulness of acoustic and electroacoustic measurement techniques for biological systems.
© 2006 Elsevier B.V. All rights reserved.

*Keywords:* Acoustic attenuation; Sound speed; Colloid vibration current; Biological cells; Erythrocytes; Bovine serum albumin

## 1. Introduction

The roots of our current understanding of sound propagation through a heterogeneous system go back more than 100 years to the first experiments by Poisson on the scattering of sound by atmospheric disturbances [1]. Many hundreds of scientists have worked in these fields since then. A comprehensive review of their work, published 4 years ago [2], includes about 500 references. The most important steps include:

- Discovery by Stokes in 1845–1851 of the relationship between viscosity and sound attenuation [3].
- First ultrasound scattering theory by Lord Rayleigh, more known for his light scattering theory, who published his two volumes of "Theory of Sound" in 1875–1878 [4].
- Discovery of the electroacoustic effect by Debye in 1933 [5].
- Nobel price to Eigen et al. in 1967 for studying chemical kinetics using acoustics [6].

As a result of all these efforts we now have available a sophisticated and well-verified theory that describes the various effects that are induced by ultrasound in heterogeneous systems. We now also have a very good understanding of the measuring techniques that are employed in several available commercial instruments. Many details on theory, experimental verifications and experimental techniques can be found in the recent review mentioned above [2].

The main advantage of ultrasound over other techniques is that it allows characterization of intact concentrated systems, eliminating dilution. This makes this technique very attractive for many concentrated biological systems. This advantageous feature of ultrasonic techniques has been recognised long before. A number of papers dealing with various biological applications of these techniques were published, even before commercial instrument became available. Some of them are listed here [7–18,31], with applications to blood, haemoglobin, plankton, bovine serum albumin, polysaccharides, and other biological solutions. Introduction of commercial instruments in the mid 90th renewed interest in these techniques. Some of the new applications simply look for empirical correlations between acoustic properties of various bio-solutions and their biological functions (see e.g. [19]). One of the most promising developments of this kind is the use of acoustics for early diagnostics of Alzheimer

* Corresponding author.
*E-mail address:* adukhin@dispersion.com (A.S. Dukhin).

0927-7765/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2006.08.011

Authors personal copy

disease [20]. Another more sophisticated trend is the extraction of more detailed information on a particular system from measured raw data, using appropriate theories. Examples of this second approach are the characterization of intravenous fat emulsions [17], bio-polymer adsorption [21,22], magnetic fluids [23] and dairy products [24]. A more recent development is the characterization of single biological cells by acoustic imaging, using GHz frequencies [25].

Our main objective of this paper is attracting attention to these powerful techniques, by showing that they are suitable for biological systems. We present below several new experiments on proteins and red blood cells.

## 2. Methods

Two different characterization techniques based on ultrasound are acoustics and electroacoustics. Acoustic is simpler and older. Both types of measurement were performed with an Acoustic and Electroacoustic Spectrometer DT-1200 (Dispersion Technology, Bedford Hills, NY, USA).

### 2.1. Acoustics

Fig. 1 illustrates the main principles of the "transmission" measurements that are used. A piezo-electric transducer converts an input electrical tone burst to an ultrasound pulse of a certain frequency and intensity and launches it into the sample. The intensity of this pulse decays as it passes through the sample due to the interaction with the fluid. A second piezo-electric transducer converts this weakened acoustic pulse back to an electric pulse and sends it to the electronics for comparison with the initial input pulse. The total loss and time delay from the input to output transducer for each frequency and gap can be considered the "raw data" from which further interpretation is made. It is convenient to present these raw data in terms of an attenuation coefficient $\alpha$, defined as

$$\alpha = \frac{10}{f\,(\mathrm{MHz})L\,(\mathrm{cm})}\log\frac{I_{\mathrm{in}}}{I_{\mathrm{out}}} \quad (1)$$

where $f$ is the frequency of the pulse, $L$ the distance between transmitter and receiver, and $I_{\mathrm{in}}$ and $I_{\mathrm{out}}$ are the intensities of the emitted and received pulse, respectively. The sound speed $c$ is obtained from $c = L/t$, $t$ being the delay time between emitting and receiving the pulse. An attenuation frequency spectrum, typically in the range of 1–100 MHz, and sound speed are the usual experimental outputs of an acoustic spectrometer.

Such experimental data can be used either for empirical correlations with other properties of the system under investigation, or for further theoretical treatment. This second step is similar to light transmittance, from which e.g. the particle size distribution

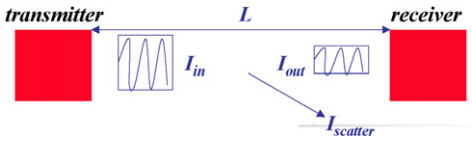


Fig. 1. General principles of acoustic measurement in "transmission" mode.

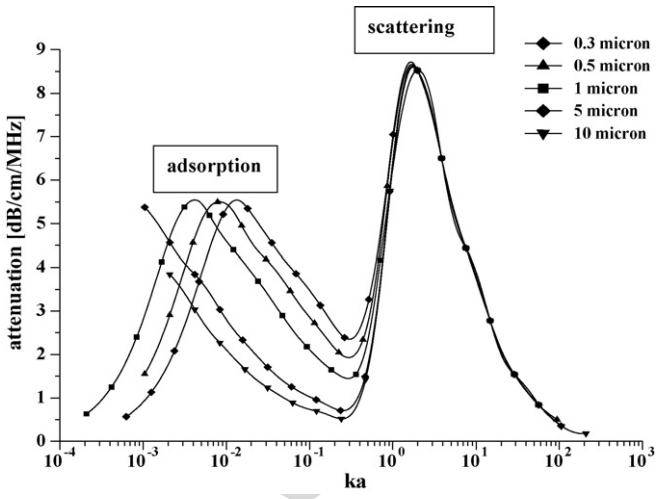


Fig. 2. Attenuation spectra calculated for monodisperse colloids with different sizes showing superposition of viscous adsorption and rigid particle scattering. Volume fraction is 10%, density contrast is 2, liquid is water.

can be extracted using well-known theory. Similarly the transmitted part of the total acoustic energy launched into the system also depends on particle size. This is illustrated by a graph in Fig. 2, which shows theoretical predictions of the dependence of ultrasound attenuation as a function of relative wavelength ka defined as $\mathrm{ka} = 2\pi a/\lambda$, where $a$ is the particle radius and $\lambda$ is the wavelength of ultrasound. These theoretical spectra are calculated from a general theory of sound propagation in heterogeneous systems, a general overview of which is given in Ref. [2]. Adequate theory does exist, even for very concentrated systems up to 50% by volume [26], and for systems that have some interconnecting structure or even more than one dispersed phase. It is seen that attenuation curve has two prominent ranges. The low frequency region corresponds to absorption; the higher frequency region corresponds to scattering.

Erythrocytes contribute to attenuation mostly through the scattering of ultrasound due to their relatively large size of around 7 μm. There is a simple expression for scattering attenuation $\alpha_{\mathrm{sc}}$ (in Np/cm) derived by Morse and Uno Ingard [27] for the Rayleigh long wavelength limit [4]:

$$\alpha_{\mathrm{sc}} = \frac{\varphi\omega^4 a^3}{2c_{\mathrm{m}}^4}\left[\frac{1}{3}\left(1-\frac{\rho_{\mathrm{m}}c_{\mathrm{m}}^2}{\rho_{\mathrm{p}}c_{\mathrm{p}}^2}\right)^2+\left(\frac{\rho_{\mathrm{p}}-\rho_{\mathrm{m}}}{2\rho_{\mathrm{p}}+\rho_{\mathrm{m}}}\right)^2\right] \quad (2)$$

where $\rho$ is the density, $\omega$ the frequency of the ultrasound, $c$ the speed of sound and the index m corresponds to the liquid medium, and p to that of the particles. This expression is valid for particles with diameters ($2a$) that are less than 1/6 of the wavelength. This restricts somewhat its applicability to the high frequency range of the DT-1200. Ultrasound wavelength at 100 MHz is about 15 μm, which is only two times larger than diameter of erythrocytes.

Eq. (2) shows that attenuation depends on the compressibility of erythrocytes through the value of its sound speed $c_{\mathrm{p}}$. We can determine the optimum value of this parameter that provides the best theoretical fit to the experimental attenuation frequency spectra. Alternatively we can also determine the compressibility

Author's personal

of erythrocytes from measurements of the speed of sound in a suspension.

Also for solutions of biomolecules the attenuation depends on the properties and concentrations of the biomolecules. Ultrasound applies longitudinal (perpendicular to shear) stress to the liquid. Consequently, propagation of ultrasound depends on the visco-elastic properties of the liquid. It is possible to introduce a "longitudinal complex modulus", $M$, for characterizing these visco-elastic properties [2]. The real ($M'$) and imaginary ($M''$) parts of this modulus are related to the sound speed $c_m$ and the attenuation $\alpha_m$ of the liquid, respectively:

$$M'_m = \rho_m c_m^2 \tag{3}$$

$$M''_m = \frac{2\rho_m c_m^3 \alpha_m}{\omega} \tag{4}$$

Biomolecules affect the visco-elastic properties of the liquid and this is reflected in the acoustic properties, according to Eqs. (3) and (4). This means that acoustics gives us an opportunity to characterize high frequency visco-elastic properties of biomolecules.

For suspensions of particles, including biological cells, also the sound speed contains contributions from the suspended particles. We can apply the theory of ultrasound propagation through heterogeneous systems, developed by many scientists in 20th century and discussed in Ref. [2]. The speed of sound is given by Wood's [28]:

$$\frac{c_m^2}{c_s^2} = \frac{\rho_s}{\rho_m}\left(1 - \varphi + \varphi\frac{\rho_m c_m^2}{\rho_p c_p^2}\right) \tag{5}$$

where $\varphi$ is volume fraction and $\rho_s = \varphi\rho_p + (1-\varphi)\rho_m$ is the density of the colloid. The index s refers to the solution as a whole. For low volume fractions this relation reduces to

$$\frac{c_s}{c_m} = 1 + \left(1 - \frac{\rho_p^2 + B\rho_m^2}{2\rho_m\rho_p}\right)\varphi \quad \text{with } B = \left(\frac{c_m}{c_p}\right)^2 \tag{6}$$

This equation shows that for low volume fractions the speed of sound varies linearly with volume fraction, the slope determined by the densities of the particles and the medium ($\rho_p$ and $\rho_m$) and the ratio of the sound speeds in the medium and the particle.

### 2.2. Electroacoustic measurements

Debye [5] first predicted an electroacoustic effect 70 years ago. In either electrolyte solutions or dispersions, the effect is related to a coupling between electrodynamic and mechanical phenomena. For instance, the transmission of ultrasound through an electrolyte solution or dispersion generates a current, which is usually referred to as an ion/colloid vibration current (CVI). Alternatively one can apply an oscillating electric field with creates a sound wave, which can be measured as an electrosonic amplitude (ESA). Commercial instruments for measuring these effects are available with the purpose of determining the $\zeta$-potential of dispersed particles in liquids. The CVI is measured with an electroacoustic zeta potential probe, inside which is a piezo-electric transducer, which generates a sound pulse that creates an electroacoustic effect on the front phase of the probe. The colloid vibration current (CVI) between a central gold electrode and a stainless steel cover is measured electronically. This signal can be converted into a $\zeta$-potential of the particles using the following equations:

$$\mathrm{CVI} = C\frac{\rho_p - \rho_m}{\rho_m}\varphi\mu_d\nabla P \tag{7}$$

where $C$ is an instrumental constant that can be found from calibration, $\nabla P$ the pressure gradient and $\mu_d$ is the dynamic mobility, given by

$$\mu_d = \frac{\varepsilon_m\varepsilon_o\zeta}{\eta}\frac{(\rho_p - \rho_s)\rho_m K_s}{(\rho_p - \rho_m)\rho_s K_m} \tag{8}$$

where $\varepsilon_m$ and $\varepsilon_o$ are the dielectric constant of the medium and the permeability of free space, $\eta$ the viscosity of the medium and $K_s$ and $K_m$ are the conductivities of the suspension and suspending medium, respectively. For spherical particles (with radius $a$ and a double layer thickness $1/\kappa$) of low charge, the zeta potential $\zeta$ is related to the charge, $q$, of the particles by

$$q = 4\pi\varepsilon_m\varepsilon_o(1 + \kappa a)a\zeta \tag{9}$$

A detailed description of the electroacoustic spectrometer can be found elsewhere [2]. This device can characterize electric surface properties of micro- and nano-objects in liquids with no dilution. This is an enormous advantage over traditional electrophoretic techniques.

## 3. Results and discussion

We present here results for the characterization of suspensions of blood cells and protein solutions using ultrasound.

### 3.1. Acoustic properties of blood

The main purpose of these tests was to verify the possibility of measuring acoustic properties of blood with high precision, which should be more than adequate for characterizing properties of blood components. From initial blood samples of a human donor, we collected plasma and erythrocytes. We performed multiple measurements of the plasma, followed by an erythrocyte sample at 95% weight fraction. After this we made sets of dilution by plasma, measuring erythrocyte dispersions at different concentrations.

Attenuation spectra of the blood plasma are shown in Fig. 3. It can be seen that the attenuation exceeds substantially the attenuation by water. The precision increases with frequency, reaching about 0.002 dB/cm MHz at 100 MHz. The difference between water and plasma is 150 times larger than the measurement precision. This opens the possibility to characterize plasma proteins with ultrasound attenuation.

Attenuation spectra of various erythrocyte samples are shown in Fig. 4. Attenuation increases with increasing erythrocyte concentration, which indicates that observed differences can be attributed to the erythrocytes. These differences exceed the measurement precision a hundred times, showing that this measurement can be used for erythrocyte characterization. Differences

Author's personal copy

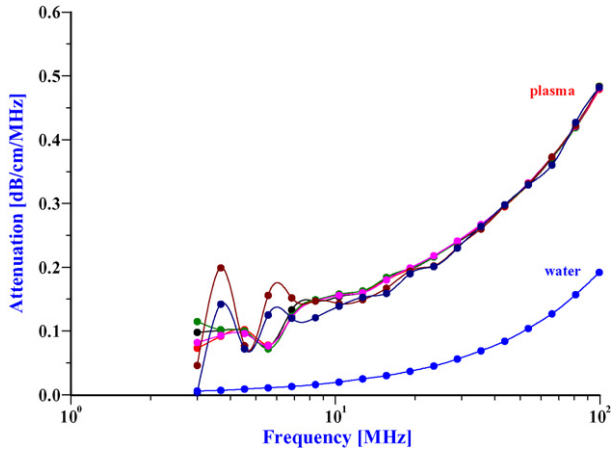


Fig. 3. Attenuation spectra of water and repeated measurements of blood plasma.

are most pronounced at high frequency. The results clearly indicate that such measurements must be done at frequencies above 10 MHz, preferably at 100 MHz and higher. Fitting the data for the 9.5% erythrocyte suspension to the theory (Eq. (2)), using the speed of sound in an erythrocyte, as an adjustable parameter, yields a speed of 1900 m/s. Results of the best fit are shown in Fig. 5.

This analysis leads us to the conclusion that acoustic measurement of blood could reveal variations in erythrocyte compressibility, which might be used for diagnostic purposes.

The sound speed in an erythrocyte dispersion shows a progressive change with concentration of erythrocytes, as shown in Fig. 6. It can be seen that the speed of sound increases linearly with volume fraction for low concentrations. Comparing the initial slope with Eq. (6), using $\rho_p = 1.08$ g/cm$^3$ for the density of an erythrocyte, yields $c_p = 1700$ m/s. This value is close to the value obtained from attenuation data, the difference likely due to some effects other than scattering contributing to the attenuation, and the approximate nature of Eq. (2) for large particles. Additional fits for the speed of sound in an erythrocyte, obtained from Eq. (5) and Fig. 6 are shown in Table 1. Values for the speed of sound in biological cells can vary from 1600 to 1850 m/s [25]. The sound speed of 1700 m/s for erythrocytes falls within this range.

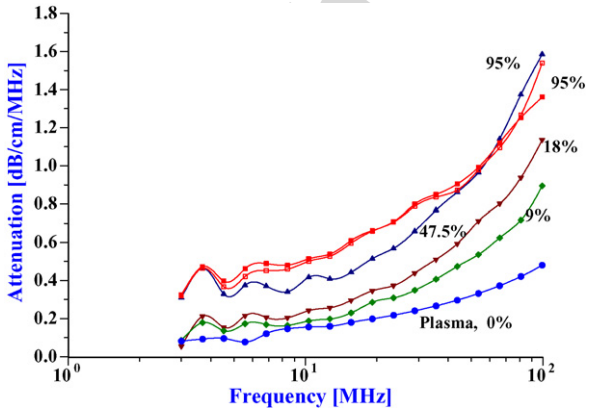


Fig. 4. Attenuation spectra of the samples with various erythrocyte concentrations in blood plasma.

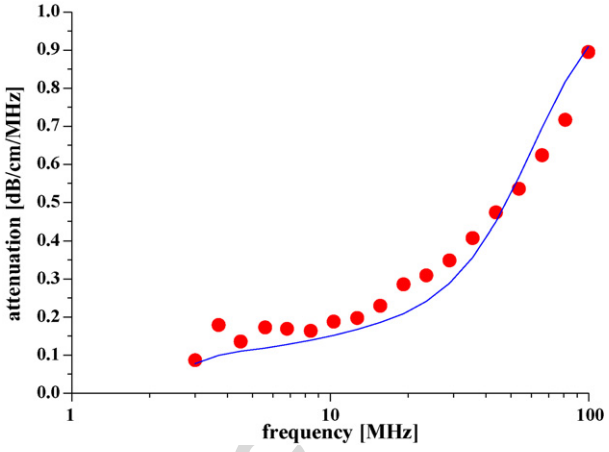


Fig. 5. Experimental attenuation of the sample with 9.5% erythrocytes and theoretical fit assuming compressibility of erythrocytes that corresponds to a sound speed of 1900 m/s.

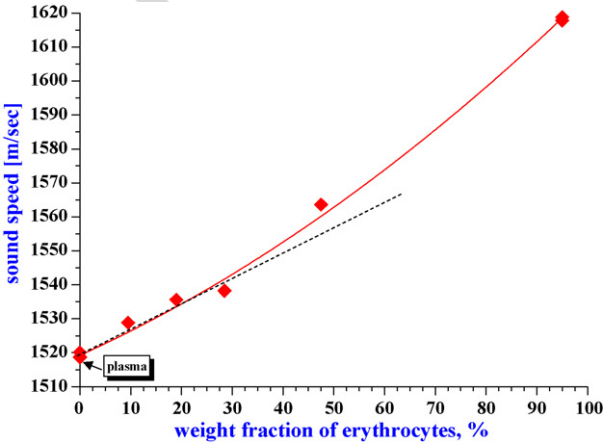


Fig. 6. Experimental sound speed of erythrocytes solutions versus weight fraction of erythrocytes.

Applying the Wood equation (Eq. (5)) to a single erythrocyte, neglecting scattering from the membrane and assuming a protein concentration of 32% [29], yields a sound speed of 2500 m/s in the protein fraction, a value typical of proteins [25].

### 3.2. Electric charges of proteins by electroacoustics

Ultrasound offers a simple way of measuring electric charges of proteins. We have illustrated this by measurements on bovine serum albumin. Fig. 7 shows the electroacoustic signal (CVI) measured for several different volume fractions of protein. Eq. (7) indicates that the CVI is proportional to the volume fraction.

Table 1
Sound speed of erythrocytes solution at 9.5 wt%, experiment and theory assuming various values of the sound speed in erythrocytes, which corresponds to different compressibility of erythrocytes

| | |
|---|---|
| Experiment sound speed (m/s) | 1528.8 |
| Theory, $C_p = 1700$ (m/s) | 1529 |
| Theory, $C_p = 1900$ (m/s) | 1539 |
| Theory, $C_p = 5000$ (m/s) | 1576 |

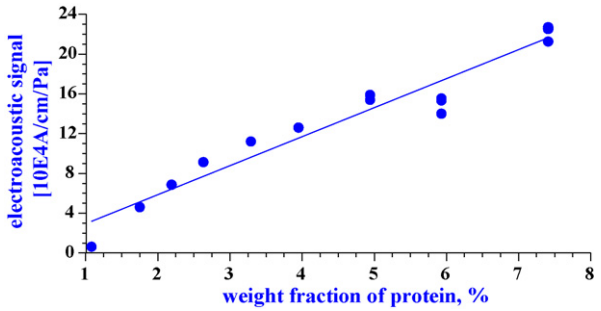


Fig. 7. Colloid vibration current of bovine serum albumin at various concentrations.

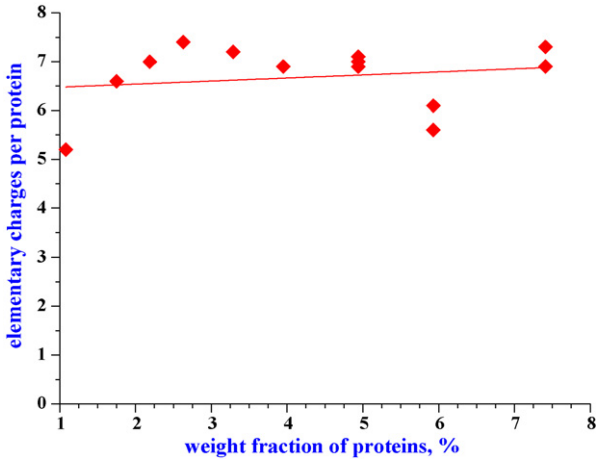


Fig. 8. Electric charge of bovine serum albumin in elementary charges per particle at various concentrations.

This is indeed observed experimentally as shown in Fig. 7. Eqs. (7)–(9) allow us to calculate the electric charge of the particles from the CVI. This surface charge should be independent of the volume fraction of particles, because it is related to the electrochemical equilibrium between protein and the bulk liquid. Fig. 8 shows that this is indeed true. At neutral pH the zeta potential of BSA has been reported as −23 mV [30]. Calculating the charge, using Eq. (7) yields a net charge of 6, in close agreement with the value found here from the CVI. These experiments prove that electroacoustics is a suitable tool for the measurement of electric charges of proteins and other bio-macromolecules.

## 4. Conclusions

We have shown that existing ultrasound-based instruments can measure acoustic and electroacoustic properties of biomolecular solutions and dispersions of biological cells. The experimental data can be used for characterizing mechanical and electrical properties of biomolecules. It was found that each BSA molecule carries a (negative) charge equivalent to seven monovalent charge groups. With regard to dispersions of biological cells, these ultrasound-based techniques can yield information on the rheological properties of cells, such as their compressibility, reflected in the speed of sound. This might be used for diagnostic purposes.

Author's personal copy

## Acknowledgments

We would like to express our gratitude to Professor Harry Goldsmith, Department of Experimental Medicine, McGill University, and his group for providing us with blood samples and valuable advice. The Canadian Foundation of Innovation (CFI) is acknowledged for proving funding for the purchase of an electroacoustic spectrometer.

## References

[1] Poisson, Sur l'integration de quelques equations lineaires aux differnces prtielles, et particulierement de l'equation generalie du mouvement des fluides elastiques, Mem., de l'Institut, t.III, 1820, p. 121.
[2] A.S. Dukhin, P.J. Goetz, Ultrasound for Characterizing Colloids, Elsevier, 2002.
[3] Stokes, On a difficulty in the theory of sound, Phil. Mag. (1848).
[4] J.W. Rayleigh, The Theory of Sound, vol. 1, Macmillan and Co., London, 1926;
J.W. Rayleigh, The Theory of Sound, vol. 2, 2nd ed., Macmillan and Co., NY, 1896;
J.W. Rayleigh, The Theory of Sound, 1st ed., Macmillan and Co., 1878.
[5] P. Debye, A method for the determination of the mass of electrolyte ions, J. Chem. Phys. 1 (1933) 13–16.
[6] Eigen, deMaeyer, in: Weissberger (Ed.), Techniques of Organic Chemistry, vol. VIII, Wiley, 1963, p. 895, Part 2.
[7] E.L. Carstensen, Kam Li, H.P. Schwan, Determination of acoustic properties of blood and its components, J. Acoust. Soc. Am. 25 (2) (1953) 286–289.
[8] T.F. Hueter, H. Morgan, M.S. Cohen, Ultrasonic attenuation in biological suspensions, J. Acoust. Soc. Am. (1953) 1200–1201.
[9] V.J. Stakutis, R.W. Morse, M. Dill, R.T. Beyer, Attenuation of Ultrasound in Aqueous Suspensions, J. Acoust. Soc. Am. 27 (3) (1955).
[10] G.J. Gruber, R. Meister, Ultrasonic attenuation in water containing brine shrimp in suspension, J. Acoust. Soc. Am. 33 (6) (1961) 733–740.
[11] E.L. Carstensen, H.P. Schwan, Acoustic properties of hemoglobin solutions, J. Acoust. Soc. Am. 31 (3) (1959) 305–311.
[12] J. Watson, R. Meister, Ultrasonic absorption in water containing plankton in suspension, J. Acoust. Soc. Am. 35 (10) (1963) 1584–1589.
[13] P.D. Edmonds, Ultrasonic absorption of haemoglobin solutions, J. Acoust. Soc. Am. 63 (1962) 216–219.
[14] L.W. Kessler, F. Dunn, Ultrasonic investigation of the conformal changes of bovine serum albumin in aqueous solution, J. Phys. Chem. 73 (12) (1969) 4256–4262.
[15] S.A. Hawley, L.W. Kessler, F. Dunn, Ultrasonic absorption in aqueous solutions of high molecular weight polysaccharides, J. Acoust. Soc. Am. (1965) 521–523.
[16] P. Hauptmann, R. Sauberlich, S. Wartewig, Ultrasonic attenuation and mobility in polymer solutions and dispersions, Polym. Bull. 8 (1982) 269–274.
[17] M.L. Carasso, W.N. Rowlands, R.A. Kennedy, Electroacoustic determination of droplet size and zeta potential in concentrated intravenous fat emulsions, JCIS 174 (1995) 405–413.
[18] R.W. O'Brien, T.A. Wade, M.L. Carasso, R.J. Hunter, W.N. Rowlands, J.K. Beattie, Electroacoustic determination of droplet size and zeta potential in concentrated emulsions, JCIS (1996).
[19] V. Buckin, B. O'Driscoll, Ultrasonic waves and material analysis: recent advances and future trends, Lab Plus Int. 16 (3) (2002) 17–21.
[20] http://www.tf-instruments.com.
[21] K. Rezwan, L.P. Meier, M. Rezwan, J. Voros, M. Textor, L.J. Gauckler, Bovine serum albumin adsorption onto colloidal $Al_2O_3$ particles: a new model based on zeta potential UV–vis measurements, Langmuir (2004).
[22] K. Rezwan, L.P. Meier, L.J. Gauckler, Lyzozyme and bovine serum albumin adsorption on uncoated silica and AlOOH-coated silica particles: the influence of positively and negatively charged oxide surface coatings, Biomaterials 26 (2005) 4351–4357.

[23] T. Rheinlander, T. Priester, M. Thommes, Novel physicochemical characterization of magnetic fluids, J. Magn. Magn. Mater. 256 (2003) 252–261.
[24] A.S. Dukhin, P.J. Goetz, Use of ultrasound for characterizing dairy products, J. Dairy Sci. 88 (2005) 1–15, JDS4463 Take B098.
[25] J. Bereiter-Hahn, C. Blase, Ultrasonic characterization of biological cells, in: T. Kundu (Ed.), Ultrasonic Non-Destructive Evaluation, CRC Press, Boca Raton, FL, 2004, pp. 725–760, Chapter 12.
[26] V.N. Shilov, Yu.B. Borkovskaja, A.S. Dukhin, Electroacoustic theory for concentrated colloids with arbitrary $\kappa a$ nano-colloids. Non-aqueous colloids, JCIS 277 (2004) 347–358.
[27] P.M. Morse, K. Uno Ingard, Theoretical Acoustics, McGraw-Hill, NY, 1968;
P.M. Morse, K. Uno Ingard, Theoretical Acoustics, Princeton University Press, NJ, 1986.
[28] A.B. Wood, A Textbook of Sound, Bell, London, 1940.
[29] M. Toubal, M. Asmani, E. Radziszewski, B. Nongaillard, Acoustic measurement of compressibility and thermal expansion coefficient of erythrocytes, Phys. Med. Biol. 44 (1999) 1277–1287.
[30] J.S. Kang, J.K. Shim, H. Huh, Y.M. Lee, Colloidal adsorption of bovine serum albumen on porous polypropylene-*g*-poly(2-hydroxyethyl methacrylate) membrane, Langmuir 17 (2001) 4352–4359.
[31] R.J. Urick, A sound velocity method for determining the compressibility of finely divided substances, J. Appl. Phys. 18 (1947) 983–987.

Author's personal copy