

LASER DOPPLER ELECTROPHORESIS APPLIED TO COLLOIDAL SYSTEMS

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LASER Doppler Electrophoresis (LDE) has appeared recently as an alternative to classical methods of electrophoresis in the study of colloid systems.^(1, 2) In microelectrophoresis, individual particles are tracked under a microscope, while in LDE the counting of particles of various mobilities is performed automatically by means of optical processes. LDE provides a less time consuming and inherently more objective method than microelectrophoresis for analysing the mobility distribution of a system of particles.

LDE may also have advantages over other classical electrophoretic methods in many cases. Moving boundary electrophoresis is useful for small particles or macromolecules, whereas mass transport electrophoresis is more suited to concentrated suspensions. LDE is applicable to reasonably dilute systems of particles which range in size from molecular dimensions to micrometers. The LDE system described here is designed to study particles in the upper part of this range.

Basic laser Doppler electrophoresis system^(3,4)

THE LDE system consists of a coherent light source (laser), a transparent cell, which contains the suspension and which incorporates electrodes for applying the electric field, and a photodetector. Associated equipment includes optical components, electronics for shaping the electric field, and electronics for processing the signal developed by the detector. Fig. 1 may be consulted for the arrangement of these components. Light scattered from particles in the sample cell is intercepted at a fixed angle from the incident beam. Information relating to the velocity of the particles drifting in the electric field is carried by the scattered light in the form of minute frequency (doppler) shifts. These frequency shifts, which are proportional to the mobilities of the

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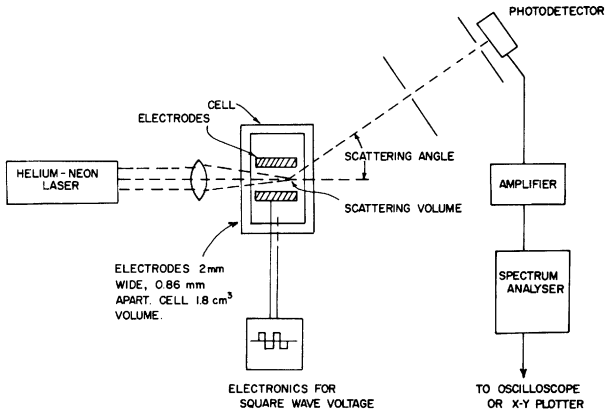


Fig. 1—Arrangement of experimental components in laser doppler electrophoresis

particles in the cell, are extracted from the scattered beam at the detector by several methods, which are noted below.

In a typical experimental situation, an electric field is applied to the suspension in the cell, and a frequency spectrum is acquired from a spectrum analyser following a two to thirty-two-minute integration. This spectrum is taken as a direct representation of the mobility distribution of the particles, with a scale relationship as indicated in equation (1),

$$u = \frac{d\lambda f}{\sin \theta v}, \quad \dots \dots \dots (1)$$

where u = mobility

d = electrode spacing

λ = λ_0/n = laser wavelength in medium

θ = scattering angle

f = observed frequency shift

V = electrode voltage drop

System design criteria^(1,3)

THERE is substantial flexibility in approach towards the design of an LDE system. Four criteria on which a decision must be made relating to the design will be discussed here briefly.

First there is the option of employing heterodyne or homodyne spectroscopy. In the first case, the desired spectral components of the scattered light are extracted by mixing the scattered light with light having no frequency shift, obtained directly from the laser. Mixing takes place at the photo-

detector surface. The heterodyne approach is usually advantageous for studies of relatively large particles and where mobility is of greater interest than particle size determination. In the homodyne case, the shifted light beats or mixes with itself. This approach is usually preferable in cases where the diffusion coefficient of relatively small particles is of interest. The system described in this paper employs the heterodyne approach.

The second option relates to the way in which the unshifted light is recombined with the frequency-shifted beam at the detector, in the event that the heterodyne approach has been chosen. In our experiment, we have chosen to take light scattered from the (stationary) walls of the cell as the unshifted beam. Other methods may give better results but are more costly and entail critical alignment procedures.

The third option concerns the spacing of the electrodes. A major limitation of classical microelectrophoresis is that the field must remain on for a period of time long enough for an observation to be made, and therefore the electrodes must be far enough apart to make the error due to polarisation negligible. In LDE, the field may be pulsed or alternated positive and negative. Polarisation may then be ignored no matter what the electrode spacing. The approach we have chosen is to move the electrodes to within a millimeter of each other and to calculate the electric field directly from the voltage and spacing, i.e., V/d in equation (1). The advantage of having the electrodes so close is that electro-osmosis due to nearby cell walls may be neglected and therefore parabolic profile effects are absent.⁽⁵⁾ It is not necessarily beneficial to have them so close. In the experiments of Flygare and Ware,^(1, 6) a different configuration has been chosen.

Finally, there is the option of how to shape the electric field. In our experiment, a square wave (alternating polarity, single voltage value) is employed and the spectrum analyser integrates continuously. The alternating voltage waveform will complicate the analysis of the resulting spectrum,⁽⁵⁾ but as we shall see, this situation can be turned to advantage. An approach which is preferable in some instances is to pulse alternatively positive and negative and gate the analyser to accept data only during pulses of one polarity.

The upper limit on the size of particle amenable to LDE is determined by the tendency of large particles to sediment and by the requirement that the particle be much smaller than the dimensions of the focused laser beam (about 100 micrometers). On the low side, there must be sufficient scattering at the laser wavelength (632.8 nm for HeNe) to keep the signal-to-noise ratio high. Consequently, one may go to lower sizes with a particle of refractive index high with respect to that of the medium (e.g., TiO₂) than with a particle of refractive index near that of the medium. It also follows that with an argon

ion laser, smaller particles may be investigated than with a helium-neon laser because of the higher power in the beam and the more intense scattering in the blue region.

There are also practical limitations on the ionic strength of the systems to be investigated. The upper limit is determined by the amount of current that can be supplied to the electrodes, and the lower limit arises when extraneous ions or parallel conducting paths within the cell are responsible for conductance comparable with that of the added electrolyte. In our system $0.016 \text{ mol dm}^{-3}$ of NaCl is an upper limit. The lower limit of $0.0002 \text{ mol dm}^{-3}$ is imposed by a combination of the small cell volume (*ca.* 2 cm^3) and electrode cleaning problems.

The limitations on concentration of particles are related to the scattering coefficient (low for particles of low relative index of refraction in the $0.1 \text{ }\mu\text{m}$ to $1 \text{ }\mu\text{m}$ size range) and the onset of multiple scattering. For the latter reason, the concentration of $0.2 \text{ }\mu\text{m}$ diameter latex particles should be below 30 ppm ($7 \cdot 10^9 \text{ particles/cm}^3$) and for $0.1 \text{ }\mu\text{m}$ TiO_2 particles below 5 ppm ($10^{10} \text{ particles/cm}^3$). In our system, the lower limit on concentration is not much below this because the scattering intensity is needed to keep the signal-to-noise ratio high.

Examples of experimental results

At this point it is appropriate to present examples of mobility distributions obtained with LDE in order to give concrete visualisation of its operation. Fig. 2 shows the spectrum of a well dispersed rutile suspension. The mobility peak is easily identifiable, resolvable to within several per cent along the frequency axis. The vertical axis represents in arbitrary units the number of particles within a small interval of mobility. The location of the peak in hertz converts to a mobility of $2.85 \cdot 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ sec}^{-1}$. The smaller hump near zero frequency is mainly an artifact of non-equilibrium fluid motion in the cell, which can interfere with the measurement at low mobilities.

Fig. 3 shows the spectrum of a partially coagulated rutile suspension. In this case, the structuring of the mobility peak into harmonic components, each separated by 5 Hz from its neighbours, is conspicuous. The harmonics are narrow and distinct for the coagulated particles but wide and poorly resolved for the dispersed particles. In both cases, the centre mobility is taken from the envelope. The comparison here illustrates two points: (a) that the square wave design option results in structured peaks and (b) that the size of the particle is inversely proportional to the width of the harmonic peaks. It should be pointed out that the spectrum of Fig. 2 is consistent with a single-valued mobility.

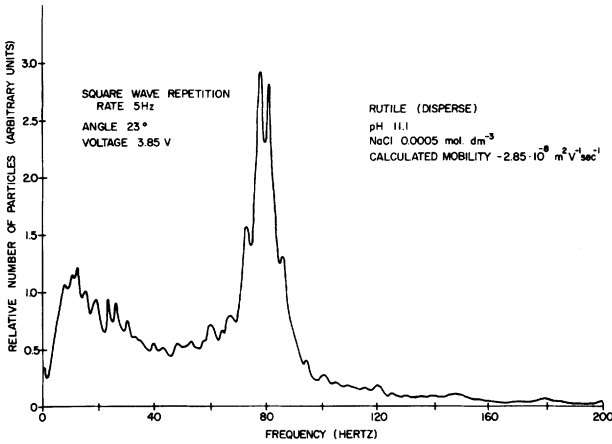


Fig. 2—Mobility spectrum for dispersed rutile particles

The full width half maximum (FWHM) of the harmonic peaks, as for example in Fig. 3, is related to the diffusion coefficient of the particles. If the Stokes relation between radius and diffusion coefficient is valid, the particle radius may then be obtained from the width of the harmonic peaks. For instance, at 23° C the following relation for the radius r holds:

$$r = \frac{1.139}{FWHM} \text{ in } \mu\text{m}, \quad (2)$$

FWHM (Full width half maximum) in Hz.

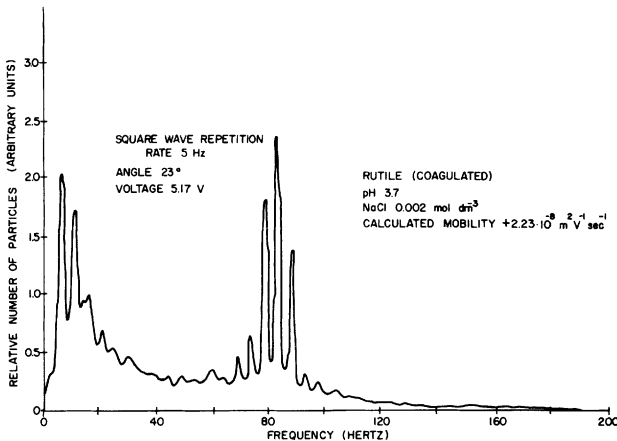


Fig. 3—Mobility spectrum for coagulated rutile particles

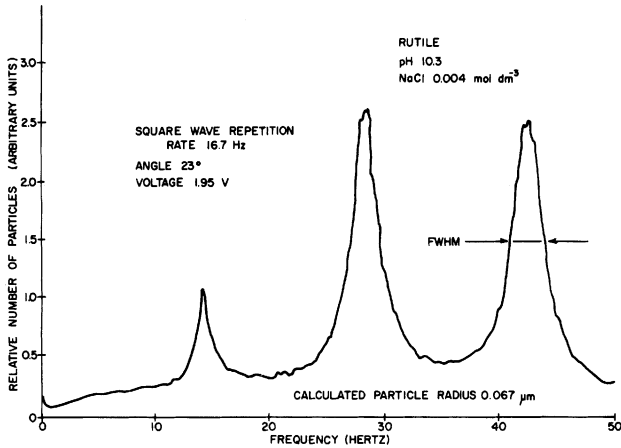


Fig. 4—Mobility spectrum for rutile when square-wave repetition rate is increased in order to measure harmonic peak widths

The square-wave voltage approach, while complicating the spectrum, does allow the effect of particle size on the spectrum to be isolated from the effect of 'polydispersity' in mobility. It might be thought that for well dispersed samples, as in Fig. 2, it would not be possible to measure the width of such closely spaced harmonic peaks. However, such measurement is possible if the square wave repetition rate is increased, thus enhancing the resolution of the harmonics. Decreasing the scattering angle also increases the resolution.

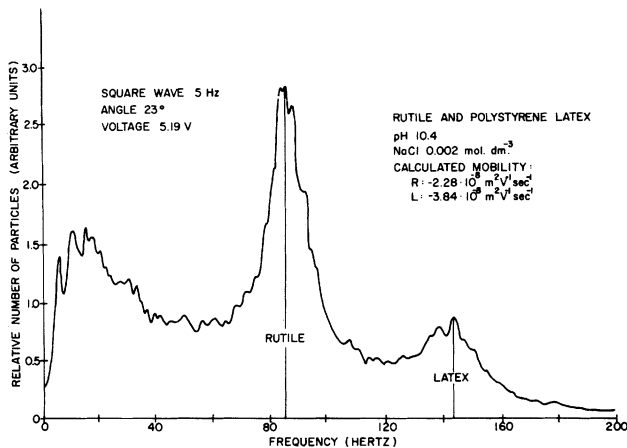


Fig. 5—Mobility spectrum for non-interacting mixed suspension of rutile and polystyrene latex

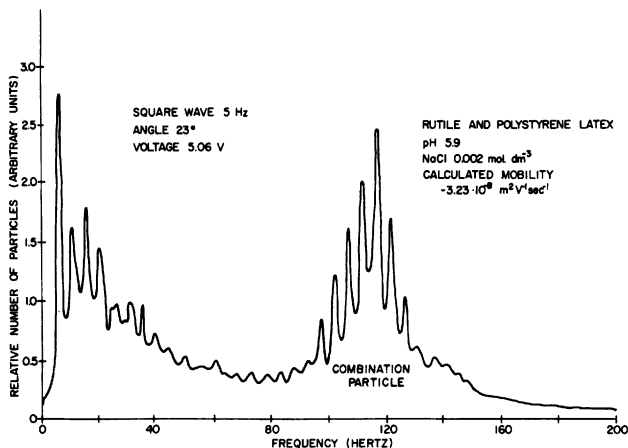


Fig. 6—Mobility spectrum for mixed rutile-latex suspension which has undergone heterocoagulation at the rutile isoelectric point

With repetition rates much higher than 8 Hz, the mobility cannot be determined simultaneously with the particle size.

Fig. 4 shows the spectrum obtained with a 16.7 Hz square wave instead of the 5 Hz wave. The harmonics of this spectrum are far enough apart to facilitate the measurement of the FWHM for this well dispersed, submicron-sized sample of rutile. Since the peaks theoretically have an approximately Lorentzian shape, a computer-assisted fit to a sum of several Lorentzians for each harmonic can be useful in estimating the range of particle sizes in disperse systems. However, a *precise* analysis of a wide size distribution would prove difficult.

Many spectra could be shown to demonstrate various interesting aspects of LDE. As one example, we indicate the result of mixing rutile and polystyrene latex in the same suspension. In Fig. 5, the spectrum clearly contains two peaks in addition to that near zero frequency. The higher peak is known to represent the latex and the lower peak the rutile, in view of the fact that the positions of these peaks are identical to the positions of the latex and rutile examined separately. As the pH is lowered to the i.e.p. of rutile, heterocoagulation occurs. The resulting spectrum, shown in Fig. 6, exhibits a single-mobility peak slightly lower in mobility than that of the dispersed latex. Moreover, an analysis of the width of the harmonics leads to the conclusion that an aggregate of at least a thousand particles of rutile and latex has formed, constituting a unique combination particle with its own characteristic mobility.

References

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Transcription of Discussion

Discussion

Mr B. A. Nazir Could I ask the size of the rutile particles, please?

Goff The size was 0.1 μm in length, rod like and very homogeneous in size. They tend to form clusters, however, this was the primary length.

Nazir What I was thinking about was that the length of the microcrystalline cellulose that you used was 0.35 μm and it is probably difficult for this cellulose to coat the particles of rutile. I wonder if you used a smaller size of microcrystalline cellulose and if the coating of the cellulose on the rutile affected the electrokinetic properties.

Goff If you go much smaller in size then it will be virtually dissolved cellulose; for example if one uses the supernatant from a centrifuged suspension of cellulose then a similar effect will be observed, but it is not the same as the case which I described which is heterocoagulation. It is in the same direction; it tends to move the mobility of the rutile towards that of cellulose. The spectrum is not as nice as some of the ones that I have shown but that is the effect. That is why I say that I think that there are soluble species playing a part too. I'm not sure because the experiment was not done that carefully.

Dr U. Beck I have a difficult question. Laser electrophoresis may be of great interest for systems which contain dyestuffs, say for suspensions of dispersed dyes and there you add dispersing agents. Do you have, to date, any knowledge of how your spectrum is disturbed by micelles which are surrounding particles or dispersed dyestuff or something like that?

Goff I haven't really looked at micelles at all and I don't think that I can answer that question.

Beck Perhaps the slopes become more diffuse?

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Goff Yes, if you have a spread in mobility then the peak becomes wider.

Prof. J. T. G. Overbeek It may help that as a rule the total scattering by the micelles is much smaller than by pigment particles so the signal from the particles may be more important than the signal from the micelles.

Goff In the set-up that we have there is certainly a lower limit to the particle size that you can measure. It is not a fundamental limit and other designs would be sensitive to smaller sizes. The lower limit appears to be around $0.1 \mu\text{m}$. Of course, the more the particle scatters, for example rutile, the more successfully one can investigate smaller particles.

Dr M. B. Lyne With your system, if you use a mixture of particles of different sizes the laser beam will be diffracted at different angles depending on the size of the particle, smaller particles causing diffraction at larger angles. Since you only use one angle, would this not produce a bias in the distribution that is recorded?

Goff That is certainly true and it means that the larger the particle, the more the scattering is directed into forward angles and there is a limit to how high an angle you can use. I don't want to go into this; there are advantages in going to higher angles but a compromise must be reached. An angle has to be chosen which proves sufficient scattering and still allows you to get far enough away from the forward position.

Dr H. G. Higgins I am gratified to see that someone at last has used the equation that we introduced in the Third Symposium. But I would like to point out that it assumes a linear pressure drop across the pad. This is a restriction on its general applicability. We tried to go further but the mathematics became too involved. Our apparatus was later fitted with manometers so that we could measure the pressure drop at different points across the pad, and we have a great deal of unprocessed data, but the theory becomes extremely difficult when you try to allow for a non-linear distribution of pressure across the pad.