

LASER DOPPLER VELOCIMETRY – NEW PALS TECHNIQUE

Laser Doppler electrophoresis is a technique used to measure the movement of charged particles in an electric field which utilizes the well-known Doppler effect. Light scattered from a moving particle experiences a frequency shift. Since the frequency of light is so high (10^{14}Hz), the shift in frequency can only be measured by an optical mixing or interferometric technique.

This is done in practise using a pair of mutually coherent laser beams derived from a single source and following similar path lengths. One of these beams must pass through the particle dispersion (this is called the scattering beam). The other beam (called the reference beam) can either pass through the sample or can be routed around the cell. The important point is that the two beams must be crossed at some point after the scattering beam has passed through the sample (although this could be at the crossing point of the two beams in the sample). By comparing the difference in frequency (i.e. the Doppler shift) between the scattered light and the incident light (the reference beam), the mobility of the particles under the influence of the applied electric field can be determined.

The optical configuration of the Zetasizer Nano ZS is shown in figure 10. It can be seen from this optical layout that only the scattering beam passes through the capillary cell and the reference beam is routed outside. This has a benefit in extending the concentration range over which measurements can be made as the reference beam is not attenuated by the sample.

A refinement of the system involves modulating one of the laser beams with an oscillating mirror at a known frequency. The mobility of the particles in an applied field will therefore produce a frequency shift away from that of the modulator frequency. This gives an unequivocal measure of the sign of the zeta potential. A second benefit of the modulator is that low or zero mobility particles give an equally good signal, so measurement is as accurate as for particles with a high mobility. This technique ensures an accurate result in a matter of seconds, with possibly millions of particles observed.

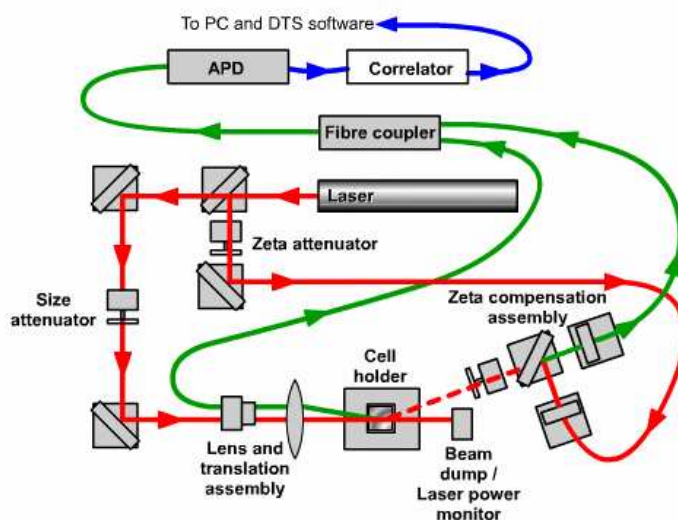
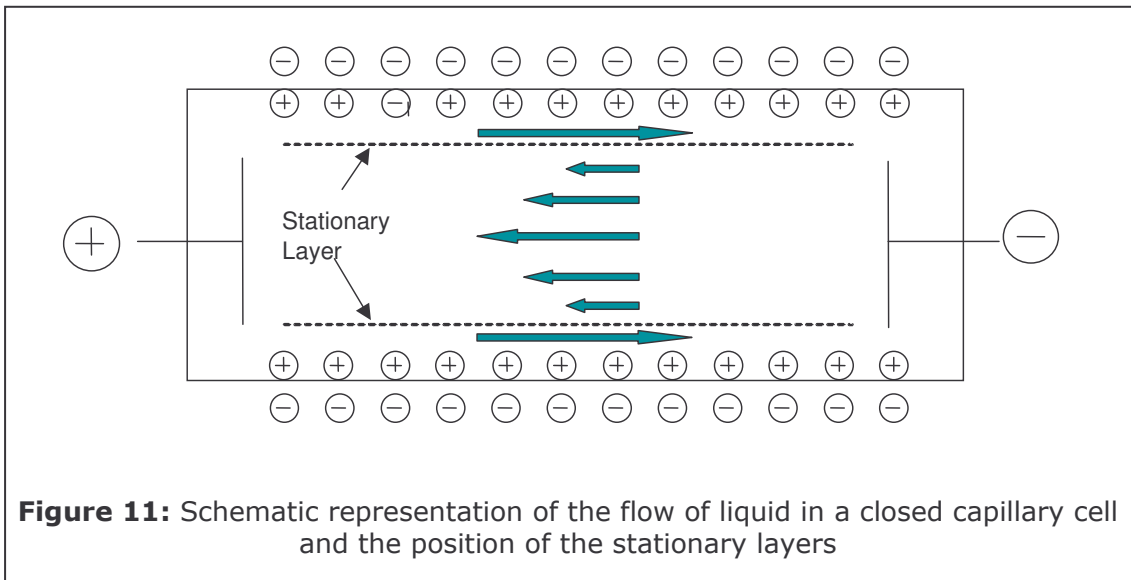


Figure 10: Optical configuration of the Zetasizer Nano ZS

5.5.1 Electroosmosis

The walls of the capillary cell carry a surface charge so the application of the electric field needed to observe electrophoresis causes the liquid adjacent to the walls to undergo electroosmotic flow. Colloidal particles will be subject to this flow superimposed on their electrophoretic mobility. However, in a closed system the flow along the walls must be compensated for by a reverse flow down the centre of the capillary in a parabolic profile.

There is a point in the cell at which the electroosmotic flow is zero and the measured particle velocity is the true electrophoretic velocity. This point is called the stationary layer and is where the measurement of the particle electrophoresis is normally done (the zeta potential measured is free of electroosmotic errors (figure 11)).



5.5.2 Avoiding Electroosmosis

The measurement of particle electrophoresis would be made much simpler if electroosmosis could be avoided altogether. The mobility of the particles at any point in the cell would then be the true mobility.

One approach is to neutralise or shield the charge on the cell walls with a coating. This can work well, but the coating is difficult to apply evenly and can wear off after a few measurements, especially if the sample pH is not close to 7.

Another method is the use of a 'dip' cell is to remove the effects of electroosmosis, but there are a number of disadvantages to this technique. With this type of cell a pair of electrodes are simply dipped into a cuvette containing the sample.

The Uzigris type 'dip' cell is the only real alternative that has been used in commercial systems. A universal dip cell is available on Malvern Nano Series as an optional accessory. As the electrodes have a large surface area very close to

the measurement zone, cleanliness of the electrodes is extremely important to avoid cross-contamination.

A theoretical electrokinetic analysis has shown that after the application of an electric field to a capillary cell, colloidal particles suspended in the liquid reach terminal velocity at least an order of magnitude more quickly than the establishment of electroosmosis (Minor et al (1997) J. Colloid and Interface Science 189, 370-375). Therefore, if an alternating electric field is applied with a sufficient high frequency then the liquid velocity due to electroosmosis becomes insignificant with respect to the electrophoretic mobility. This means that measurements do not have to be taken at the stationary layer in the capillary cell removing the need for alignment. This measurement strategy is called fast field reversal (FFR) and gives an accurate mean, but is lower resolution than the standard stationary layer technique.

5.5.3 The M3-PALS Technique

The Zetasizer Nano Series uses a combination of laser Doppler velocimetry and phase analysis light scattering (PALS) in a patented technique called M3-PALS to measure particle electrophoretic mobility. Implementation of M3-PALS enables even samples of very low mobility to be analysed and their mobility distributions calculated.

5.5.3.1 M3

As previously discussed, traditional electrophoretic measurements are performed by measurement of particles at the stationary layer, a precise position near the cell walls. With M3, the measurement can be performed anywhere in the cell, though with the Zetasizer Nano series, it is performed in the centre of the cell. M3 consists of both fast field reversal (FFR) and slow field reversal (SFR) measurements, hence the name mixed mode measurement (M3).

All systems that measure particle mobilities using laser Doppler velocimetry reverse the field periodically during the measurement. This is normally just the slow field reversal mentioned below. However, M3 consists of two measurements for each zeta potential measurement, one with the applied field being reversed rapidly – the FFR measurement, and a second with a slowly reversing applied field - the SFR measurement stage.

If the field is reversed very rapidly (FFR), it is possible to show that the particles reach terminal velocity, while the fluid flow due to electroosmosis is insignificant. This means that the mobility measured during this period is due to the electrophoresis of the particles only. The mean zeta potential value that is calculated by this technique is therefore very robust, as the measurement position in the cell is not critical.

However, as the velocity of the particles is sampled for such a short period of time, information about the distribution is degraded. Therefore, slow field reversal is applied to determine the distribution of mobilities present in the sample.

An M3 measurement sequence therefore consists of;

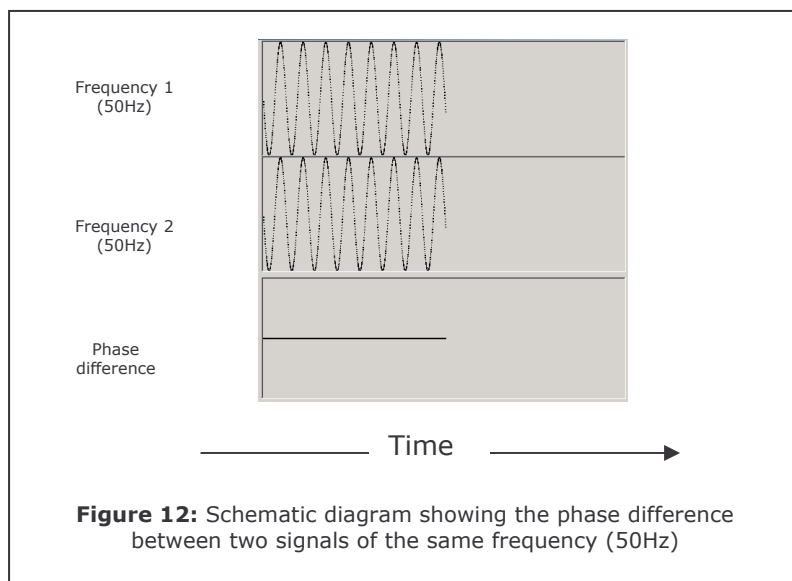
1. An FFR measurement is performed at the cell centre. This gives an accurate determination of the mean.
2. A slow field reversal measurement is made. This gives better resolution, however the mobility values are shifted by the effect of electroosmosis.
3. The mean zeta potentials calculated from the FFR and slow field reversal measurements are subtracted to determine the electroosmotic flow.
4. This value is used to normalise the slow field reversal distribution.
5. The value for electroosmosis is used to calculate the zeta potential of the cell wall

5.5.4 M3-PALS (Phase Analysis Light Scattering)

PALS is a further improvement on traditional laser Doppler velocimetry and the M3 implementation described above. Overall the application of PALS enables accuracy of the measurement of low particle mobilities. This can give an increase in performance of greater than 100 times than that associated with standard measurement techniques. This allows the measurement of high conductivity samples, plus the ability to accurately measure samples that have low particle mobilities. Low applied voltages can now be used to avoid any risk of sample effects due to Joule heating.

Particle mobility is determined using PALS by doing a phase comparison of the detected signal (scattered light) with that of a reference frequency during the FFR part of the measurement - the reference frequency being that of the optical modulator (320Hz).

Imagine two frequencies which are both 50Hz (figure 12). If the phase difference between these two signals is plotted over time, a flat line is obtained (i.e. there is no phase difference between the two signals). Now consider two signals which have different frequencies (in this example, they are 50 and 51Hz respectively). A plot of the phase difference between the two signals shows a gradient (figure 13). The phase difference will depend upon the velocity at which the particles are moving. Therefore the mean zeta potential value can be determined.



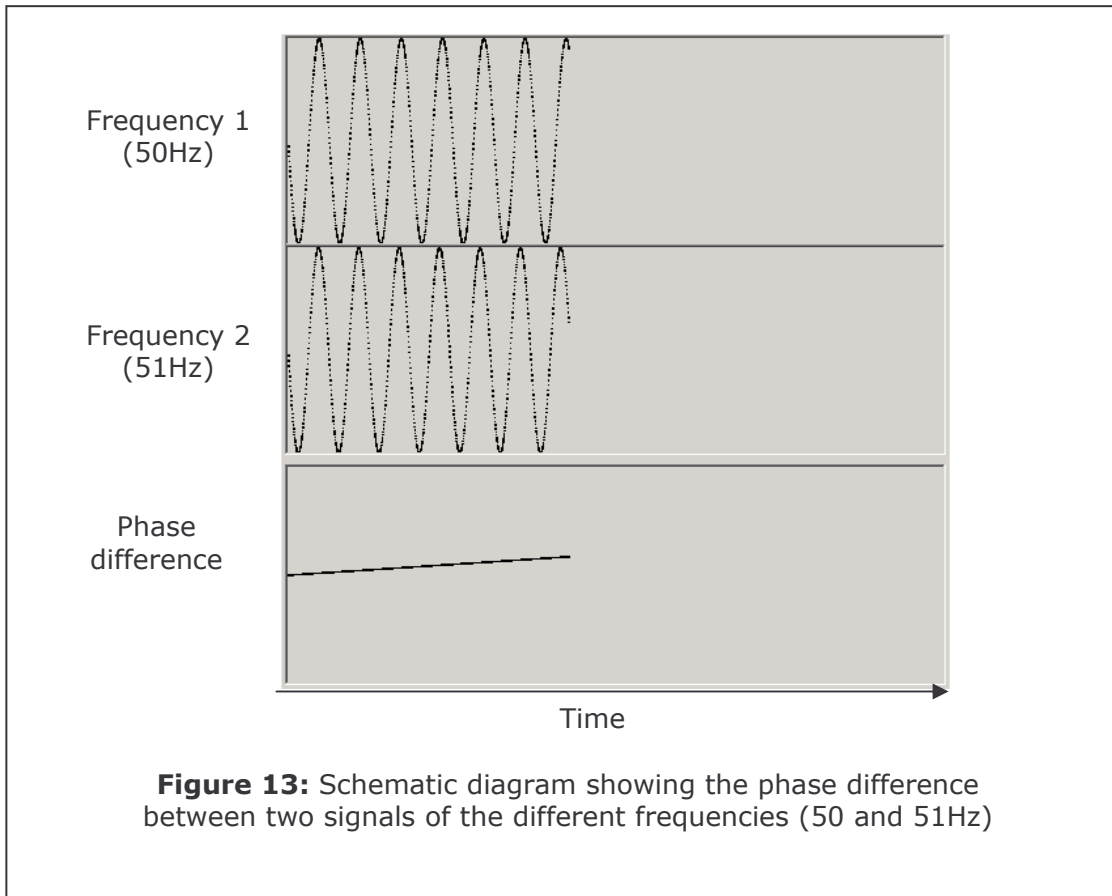


Figure 14 shows phase difference plots between 3 pairs of frequencies: (A) 320Hz and 320.01Hz, (B) 320 and 320.002 and (C) 320 and 320.005Hz respectively illustrating the resolution capability of PALS. These difference in frequencies could not be determined by using a conventional Fourier transformation analysis.

