SIGNIFICANCE AND METHOD OF DETERMINING POSITION OF STATIONARY LEVELS DURING MICROELECTROPHORETIC MEASUREMENTS

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When measuring electrophoretic movement of particles in suspension of γ-Al_2O_3 placed in a microelectrophoretic cell it has been found that charge of the cell wall changes in the course of the measurements. This effect is due to sedimentation and sorption of the particles on both walls, whereby changes in electroosmotic flow take place. For that reason, total velocity of particles in the cell must be taken into account to calculate electrophoretic movement of particles.

In the microelectrophoresis of suspensions, movement of individual suspension particles is directly observed by means of a microscope[1-3]. Electroosmotic flow of a liquid appears near to the wall of the cell[1-3]; however, because of the cell being closed, the liquid comes back through its centre. Distribution of velocities of the liquid movement is illustrated by curve 1 in Fig. 1. Velocity of the particles movement is considered to be the same with respect to the liquid movemenover the whole cross-sectional area of the cell. The observed velocity of the particles movement, V_p, (curve 2, Fig. 1, the so-called velocity profile of particles in suspension) is then given by sum of the liquid movement and that of the particles. According to this assumption it is then of importance to find such levels in the cell, where the liquid movement equals zero and actual particles movement is observed. To determine these so-called stationary levels, calculations may be used that take into account shape of the measuring cell[4,5]. For a flat electrophoretic cell, where width highly predominates over thickness of the working space, relation $x_{1,2} = (1/2 + 1/\sqrt{12})\, x_0$ has been derived, where $x_{1,2}$ is the distance between stationary levels and bottom of the cell and x_0 is the thickness of the latter.

More general is the Komagata relationship which has the form $x_{1,2} = [1/2 \pm \sqrt{(1/12 + 32x_0/\pi^3 d)}]\, x_0$, where d is the cell width. Position of the stationary levels can be also determined from the velocity profile of particles in the cell, taking into account the fact that mean velocity of particles (V_s, see Fig. 1), obtained by integrating the velocity profile of particles and dividing this value by height of the cell working space, is equal to their actual velocity with respect to the liquid (V). The position of the stationary levels is then given by point of intersection of the value of actual particles velocity with curve of the velocity profile[6].

EXPERIMENTAL AND RESULTS

Considerations about the stationary levels are valid only, if the suspension observed is measured in a perfectly clean cell whose both walls have consistent charges. In this case only, electroosmotic flow in the cell is symmetric and the velocity profile of particles a symmetric parabola.

Common measurements, however, cannot practically meet this requirement. The gravity, namely, causes sedimentation of larger particles onto the lower wall, its charge being changed gradually. As has been further established, even the upper wall on which fine particles are likewise adsorbed is subject to changes, provided they have a charge opposite to that of the wall. As an example, velocity profiles of the particles of the γ-Al_2O_3 suspension in redistilled water, measured at different time periods after filling the cell, are presented. Velocity of the particles movement was studied for nine different levels within a time period from filling the clean cell up to the stationary state.

Result of the measurements is graphically presented in Fig. 2, where nine curves provide for connection of values of the observed velocity of the particles movement of the same level in the cell within the whole time interval involved. When reading out from this figure values of velocities in individual levels after certain time intervals, we obtain curves of the velocity profiles corresponding to the state of suspension and

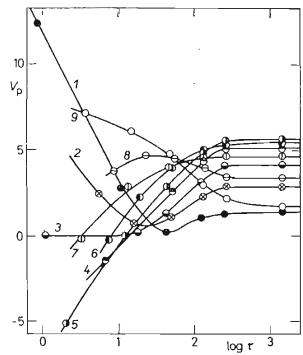

FIG. 1

Illustration of Movement of Particles and Liquid within Cross-Sectional Area of the Electrophoretic Cell

1 Curve of the liquid movement, *2* curve of the observed movement of particles ("velocity profile"); x_0 is the thickness of the cell, x_1, x_2 distance of the stationary levels from bottom of the cell, V velocity of the particles movement with respect to liquid, V_p observed velocity of the particles movement, V_k velocity of the liquid movement, V_s mean velocity of the particles movement.

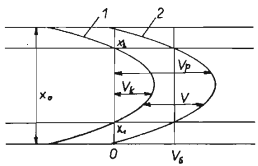

FIG. 2

Dependence of the Observed Velocity of the Particles Movement, (V_p, μm/s) upon Time (τ, min) after Filling the Cell with Suspension, for Different Levels

Distance of levels from bottom of the cell: *1* 50, *2* 100, *3* 150, *4* 200, *5* 266, *6* 332, *7* 382, *8* 432, *9* 482 m.

cell walls at an appropriate time after filling the cell with the suspension. These curves are shown in Fig. 3. It can be seen that after the first ten minutes, shape of the profile curve corresponds to the negatively charged glass surface, since liquid near the cell walls is, due to electroosmosis, moved to the right. In the course of time, however, a reverse of the curve takes place (at the lower cell wall due to sedimentation considerably earlier than in the upper part), only after 24 hours the curve has a quite reversed course and is nearly symmetric.

DISCUSSION

The effect described may be explained by sedimentation and sorption of particles of the suspension on glass walls of the cell, whereby their charge is influenced. During the experiment, gradual occupation of the surface of both cell walls by microscopic γ-Al_2O_3 particles takes place, due to the opposite charges of glass and these particles so that their character of movement corresponds to the velocity particles profile measured in the cell whose walls are made of aluminium oxide. We used electron microscope to prove sorption of fine γ-Al_2O_3 particles onto the upper wall of the cell. Latex emulsion was dropped on a microscope slide with adsorbed particles to estimate their size, carbon was in an oblique way vapour-deposited on the surface after drying, and replicas were taken to produce photographs. Fig.4* shows (microscoppic enlargement 5000) aggregates of very fine particles, of irregular shapes, among which white latex globules of diameter about 0·4 μm are present. The shadow gives us an idea of the space arrangement of aggregates in the area.

Light little cones were obviously produced during vapour-deposition of carbon and prevent us to determine areal occupation of the surface, since the particles in fact seem to us to be enlarged. Nevertheless, it may be pointed out that occupation of the surface is not greater than 10%. It is of interest that this density of particles is already

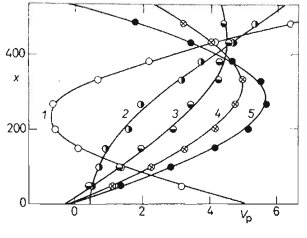

FIG. 3

Dependence of the Observed Velocity of the Particles Movement (V_p, μm/s) upon the Observed Level (x, μm) at Different Times after Filling the Cell with Suspension: *1* 10, *2* 30 min, *3* 1, *4* 2, *5* 24 h.

* See insert facing p. 2666

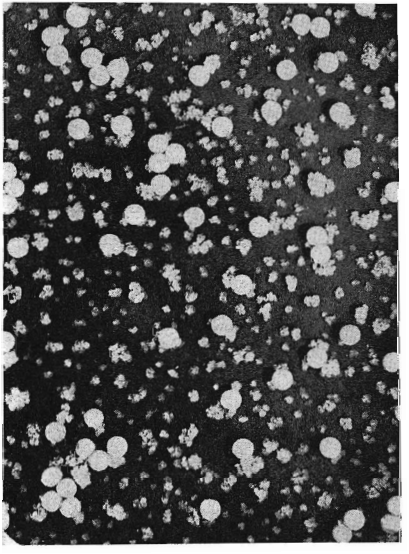

Fig. 4

Electron-Microscope Photograph of Glass Surface with Adsorbed Particles of Suspension, Enlargement 5000

White latex globules of diameter about 0·4 μm serve to estimate size of the particles.

under given experimental conditions sufficient to change the charge of the glass cell wall.

It is evident that with the enlargement employed in a microelectrophoretic apparatus, great majority of particles are no more visible. However, bigger aggregates of particles, which can be observed in the electrophoretic measurements, are subject to sedimentation and are captured on the upper cell wall only quite exceptionally.

It appears that rate and magnitude of the surface occupation of the cell walls are dependent on density of the suspension, size of particles, and their charge. This finding results in the fact that reverse of the profile does not occur at equal speed and often does not take place at all. In such cases we cannot, of course, succeed in obtaining the velocity profile that would have a shape of a symmetric parabola, and measurement of the mobility of particles in the stationary levels is therefore of no value. It still holds, however, that average mobility of particles throughout the cell is equal to their actual mobility with respect to the liquid. If we then measure mobility of particles in a flat horizontal cell, we must measure the whole velocity profile of the particles, construct appropriate graph and establish area above the curve. Division of the area by height of the cell provides actual mobility of the particles.

In special cases, if a suspension fine enough is employed, charges of both cell walls may be balanced. The profile of particles is then represented by a symmetric parabola that may be expressed by equation $V_p = V_0 + K(x_0 x - x^2)$, where V_p is the velocity of particles observed in any place of the cell, V_0 the observed velocity of particles at the walls, K the constant defining shape of the parabola, x the distance of the level, in which velocity V_p is measured, from bottom of the cell. Constant K and value V_0 which is difficult to measure, can be with advantage found using calculus of observations. From the K and V_0 value thus obtained, we can then determine mean velocity of particles according to $V_s = V_0 + 1/6Kx_0^2$, which is, according to considerations mentioned above, likewise equal to the studied actual velocity V with respect to the liquid.

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