THE INFLUENCE OF SIZE, SHAPE AND CONDUCTIVITY OF MICROSCOPICALLY VISIBLE PARTICLES ON CATAPHORETIC MOBILITY.

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INTRODUCTION.

Although the cataphoresis of microscopically and submicroscopically visible particles has been very frequently investigated, the influence of size, shape and conductivity of the particle has not been completely determined. Solution of this problem is of the utmost importance for all applications of the method of cataphoresis. Before differences in mobility in an electric field may be ascribed to variations in the nature of the electric conditions at the phase boundary of the particle, the influence of variation of size, shape and conductivity of particles equal in other respects must be recognized. The theories of Helmholtz, Lamb, and von Smoluchowski (1) lead to the formula for cataphoresis.

$$V = \frac{1}{4\pi} \frac{X D \zeta}{\eta},$$

(V = cataphoretic velocity. X = field strength. D = dielectric constant of the medium. ζ = electrokinetic potential. η = viscosity of the medium. All units c.g.s. electrostatic.) According to this formula neither size nor shape should influence the migration velocity. Debye and Hückel (2), making certain assumptions, came recently to the conclusion that the factor $\frac{1}{4\pi}$ was valid only for cylindrical particles whereas the factor $\frac{1}{6\pi}$ should be substituted in the case of spherical particles. This difference is great enough to be determined experimentally.

Previous experiments dealing with the effect of size, shape and conductivity may be briefly summarized as follows. Hardy (3) found that various solutions of submicroscopic particles of protein prepared by adding different amounts of acetic acid to the protein solutions had identical cataphoretic mobilities. As the different solutions varied in diffraction color, it was believed that the mobility observed was independent of particle size. Burton (4) found that Bredig silver solutions presumably of different particle size had equal cataphoretic velocities. Ellis (5), however, referring to a statement of Cotton and Mouton that the velocity varies with size, compared droplets of the same size only. McTaggart (6) noted that gas bubbles 60μ to 160μ in diameter migrated independently of the diameter. In contrast to these findings are the experiments of Mooney (7). He found that for droplets of Nujol* 0.5μ to 40μ in diameter, there was a considerable increase in migration velocity with increasing diameters of the droplets. Thus for a 22 fold increase in diameter, from 0.5μ to 11μ, the velocity was doubled. Mooney obtained similar results for ten other kinds of emulsions made up in water and in dilute electrolytes. In $CuSO_4$ solutions these differences supposed by Mooney to depend upon particle size disappeared. The technic employed by Mooney was different from that used by most recent authors. He worked with a vertical, cylindrical, capillary glass tube with sealed in electrodes. The effect of gravity on the droplets, although presumably accounted for in estimation of velocity, was considerable. These observations of Mooney are unique in that such marked differences in velocity had never previously been described. Freundlich and Abramson (8) and Abramson (9) found that (1) single red blood cells and their aggregates of different sizes and shapes, (2) irregularly shaped polymorphonuclear leucocytes, (3) quartz particles of various sizes (0.5μ to 15μ) and shapes in water, dilute electrolytes and non-electrolytes, (4) glass and clay particles suspended in similar solutions, all migrated independently of size and shape. McTaggart's experiments with air bubbles were also confirmed by studying air bubbles in gelatin gels.

Although there is no extensive theoretical treatment of the influence of particle conductivity on cataphoretic mobility, the theories have always been derived for insulators.

* A paraffin oil.

In connection with the influence of the conductivity of the particles two problems arise for discussion. First, the whole particle may be homogeneous. In this case the influence of the conductivity of the particle cannot be investigated experimentally as the conductivity of the particle cannot be varied without varying the composition of the whole particle including its phase boundary. Secondly, the particle may be heterogeneous when the surface film is composed of material entirely different from that of the bulk. Particles having identical surface films, but varying in the conductivity and chemical make-up of the enclosed bulk of the particles may be then compared. Such particles can be obtained by suspending particles of varied chemical nature and electric conductivity in dilute protein solutions (10). The adsorbed film of protein determines the properties of the surface which is responsible for the mobility of the particle, while the chief material is excluded from direct contact with the medium. If the material making up the bulk of the particle were to influence the velocity of migration, then this influence could only be due to the varying conductivity if the influence of size and shape of particle can be excluded.

Methods.

Apparatus.—A horizontally fixed microscopic cataphoresis cell similar in arrangement to that described by Northrop and Kunitz (11) was employed. Certain modifications were found useful which have been described in detail elsewhere (12). The cell was of Pyrex glass, about 0.8 mm. in depth, 9 mm. wide, and the hydrodynamic conditions followed the principles laid down for such systems by von Smoluchowski (1). Non-polarizable electrodes were used. The applied E.M.F.'s were great enough to give easily measurable velocities with no heating effects.

Materials.—Droplets of Nujol, benzyl alcohol, castor oil, a paraffin oil, and coca butter were studied in media (alcohol-water, cane sugar-water mixtures containing traces of electrolytes) of approximately the same specific gravity as the oils themselves. Certain droplets were studied in systems containing sufficient protein to give the droplets at least a complete surface of protein. This procedure gives very uniform surfaces because of the similarly adsorbed protein films, and was

TABLE I.

Nujol in Water-Ethyl Alcohol Mixture Having Same Density as the Oil. A Few Drops of pH 7.4 Phosphate Buffer Added to About 50 cc. of Emlsion. The Same Result Was Obtained for Emulsions in Methyl Alcohol Mixtures.

Diameter of droplet	Time to move 200μ	Diameter of droplet	Time to move 200μ
μ	*sec.*	μ	*sec.*
30	15.2	0.5	14.5
1	15.3	13	14.5
5	15.2	13	14.6
3	14.6	5	14.4
13	14.7	1	14.4

TABLE II.

Benzyl Alcohol + Small Amount of Sugar + Few Drops pH 7.4 Buffer.

Diameter of droplet	Time to move 300μ	Diameter of droplet	Time to move 300μ
μ	*sec.*	μ	*sec.*
45	13.5	20	14.0
5	14.2	20	13.8
2	14.2	2	14.4
25	14.2	15	14.0
2	14.2	3	13.8
1	14.0	1	13.6

TABLE III.

Paraffin Oil in Ethyl Alcohol-Water Mixture + Few Drops pH 7.4 Phosphate Buffer.

Diameter of droplet	Time to move 100μ	Diameter of droplet	Time to move 100μ
μ	*sec.*	μ	*sec.*
25	9.8	20	8.4
2	9.0	2	8.2
5	8.8		
20	9.4	2	6.0
		15	6.2
15	12.0		
3	12.2		

The brackets are groups from different levels in the cataphoresis cell.

TABLE IV.

Coca Butter.

Experiment (*a*). In distilled water without protein film			*Experiment* (*b*). The suspension was here in 0.05 per cent gelatin + dilute HCl (about N/1000)		
Diameter of droplet	Time to move same distance	Remarks	Diameter of droplet	Time to move same distance	Remarks
μ	*sec.*		μ	*sec.*	
8	5.2	Note a greater variation than in part (*b*)	8	4.6	The speed of the droplets is more uniform and independent of the size with the protein film
5	5.4		1	4.2	
3	6.2		4	4.4	
2	6.0		4	4.6	
2	6.0		2	4.6	
4	5.2		7	4.4	
7	5.4		5	4.6	
2	5.6		1	4.4	
			5	4.4	
			1	4.4	

TABLE V.

Castor Oil in Distilled Water + Few Drops of pH 7.4 Phosphate Buffer.

Diameter of droplet	Time to move 200μ	Diameter of droplet	Time to move 200μ
μ	*sec.*	μ	*sec.*
2	5.0	1	5.4
3	5.2	7	5.2
2	5.0	1	5.4
1	5.2	10	5.5
1	5.4	5	5.5

therefore used to study needles and droplets simultaneously. Asbestos needles of various lengths remained fairly well suspended in watery and dilute protein solutions, as did recrystallized *m*-aminobenzoic acid crystalline needles. In all experiments the following precautions were observed:

1. The vertical movements of the particles due to gravity were eliminated as far as possible, by having the density of the medium and of the particle similar.

2. Measurements were always made at the stationary water layer in the cell to make sure that the mobility observed in the mid-regions

(where most observations reported have been made) was not simply due to electroendosmotic streaming of the water within the cell.

EXPERIMENTAL.

1. The Effect of Particle Diameter on Cataphoretic Mobility.—Droplets of the following substances were studied: The details are in the tables where typical protocols are presented.

Nujol 0.5 μ to 35 μ Table I.
Benzyl alcohol 0.5μ to 45μ Table II.
Paraffin oil 2μ to 25μ Table III.
Coca butter 1μ to 8μ Table IV.
Castor oil 1μ to 10μ Table V.

All our experiments have been performed in the presence of sufficient electrolyte or protein to avoid the complications found in Mooney's experiments. As mentioned previously, this author noted that in electrolyte-poor emulsions, droplets having larger diameters move more rapidly than smaller droplets. Mooney also noted that this difference in velocity was less in the presence of electrolytes. The observations of Mooney have been confirmed by the authors in very electrolyte-poor emulsions containing no proteins on a few occasions.

An analysis of the five tables just mentioned discloses that under the stated conditions *the cataphoretic velocity of oil droplets is independent of their diameter.* The larger drops were easily studied and as has been described under methods, media of almost identical specific gravity with that of the droplets were used. Very rarely (less than about $\frac{1}{5}$ of 1 per cent of all observations) anomalous behavior of droplets was observed. A droplet would migrate very quickly or very slowly. The relatively large changes produced by small amounts of impurities in the system is sufficient to account for these variations which are in no way related to their size or shape.

2. The Effect of Particle Length on Cataphoretic Velocity.—Asbestos pulp in dilute suspensions gives needles of easily studied lengths from 3μ to 200μ. The diameter of the needles was from about 1μ to 3μ. Long cylinders of very small radius were, therefore, available for study. These needles were studied suspended in distilled water and in dilute

protein solutions. Fig. 1 gives the data for these two types of media. In the figure the orientation of the particle is given by its position, the length of the particle by the scale and the time in seconds taken for the particles to migrate a given distance, the same for all particles, by

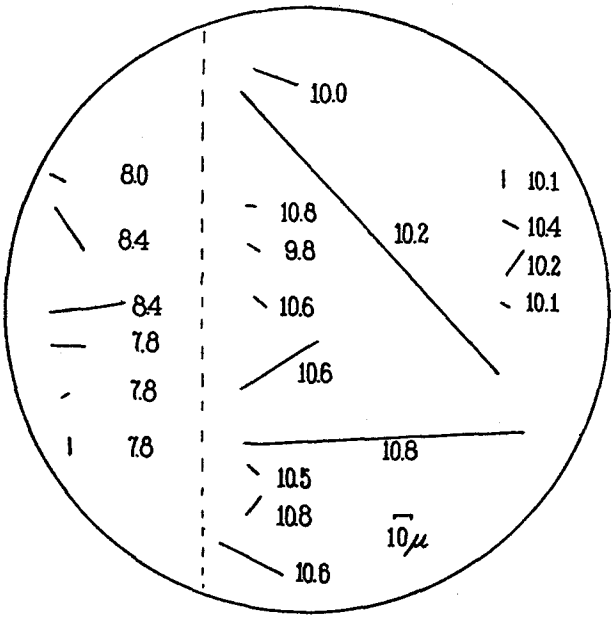

FIG. 1. The field is divided into two parts. In right lower hand corner is the scale to which the asbestos needles have been drawn. The slight differences in radius were neglected. The group of needles to the left of the dotted line were studied in distilled water with the orientation as when observed; the number to the right of each needle is the time in seconds required to migrate a given distance which was the same for the whole group. The needles to the right of the dotted line were studied in N/1000 HCl containing 0.05 per cent gelatin which coats the needles with a protein film. The numbers have the previous significance. This figure demonstrates that neither size nor orientation of particle changes the cataphoretic velocity.

the number next to the particle. It is evident that for such particles *the cataphoretic velocity is independent of particle size,* thus confirming with particles of entirely different shape the results obtained with oil droplets.

Orientation of Needle-Shaped Particles during Migration.

Most of the needle-shaped particles long enough to have no brownian movement are found in horizontal planes, but oriented at random within these planes. During the migration this random orientation is not disturbed over distance of 500μ or so.[1] These observations demonstrate that the streaming of fluid within the cell is practically laminar, making possible the application of von Smoluchowski's formula, indicating the relationship of the velocity of a particle to its depth within the cell.

The Effect of the Shape of the Particle on Cataphoretic Velocity.

The following needle-shaped particles were investigated:

(*a*) Asbestos needles from just visible lengths up to 70μ long.

(*b*) *m*-Aminobenzoic acid crystals (needles up to 100μ long). These were compared in the same medium (protein solutions) with small paraffin oil or mastix globules 1μ to 5μ in diameter. When such substances are suspended in protein-free media they have different cataphoretic velocities because of their chemically different surfaces. When suspended in dilute protein (*e.g.* 0.1 per cent gelatin) solutions they adsorb a protein film and in this way, the needles and oil globules are given chemically similar surfaces. That the protein film in such instances may give values of cataphoretic velocity remarkably close to the native protein has been shown by Abramson (9) and by Freundlich and Abramson (8). These experiments have been confirmed by Briggs (13) using an entirely different method.

Fig. 2 shows groups of particles studied at the same level. The particles are drawn to the scale indicated or are of the size noted. An oil droplet and a needle were always studied at the same level at the same time. The time of migration over a given distance is given in seconds next to the particle. These experiments demonstrate that *for very extreme instances of particle size and shape the velocity of cataphoretic migration is here determined solely by the surface characteristics of the particle and is independent of both size and shape within the limits studied.*

[1] Red cell rouleaux have also been observed to exhibit the same phenomenon in serum and other liquids. The same was true for gelatin gels (8, 9). See also Abramson, H. A., *Proc. Soc. Exp. Biol. and Med.*, 1928, xxvi, 147.

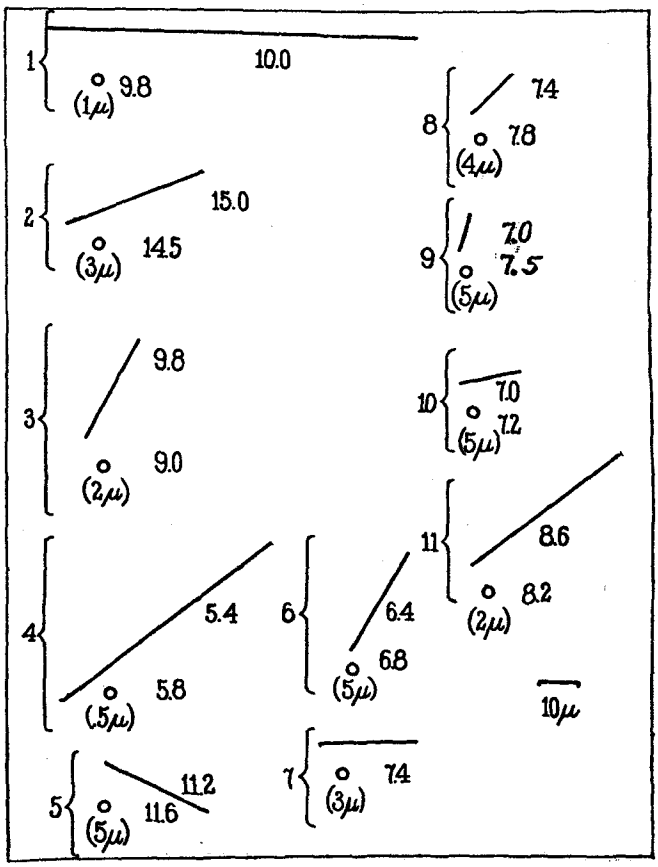

FIG. 2. The brackets indicate needles and globules studied in pairs. Typical experiments, Nos. 1–3, are *m*-aminobenzoic acid crystals and mastic globules covered with gelatin. Nos. 4–10 are asbestos needles and paraffin oil globules covered with albumin. No. 11 is a *m*-aminobenzoic acid crystal and a paraffin oil globule covered with gelatin. The oil droplets and mastic particles are all drawn the same size but their diameter is given in parentheses near the particle. The needles are drawn to the scale in the lower right hand corner.

Each pair was studied at the same level in the cell. The numbers following each particle are the relative speeds. It is evident that there is no change in cataphoretic velocity even for these limiting cases.

TABLE VI.

The Migration Velocity of Quartz and Benzyl Alcohol Droplets in Approximately N/100 *and* N/1000 *HCl When Both Quartz and Benzyl Alcohol Have Similar Adsorbed Gelatin Films (Initial Concentration Gelatin 0.2 Per Cent).*

N/100 HCl		N/1000 HCl		N/100 HCl		N/1000 HCl	
Time to migrate given distance		Time to migrate given distance		Time to migrate given distance		Time to migrate given distance	
	sec.		*sec.*		*sec.*		*sec.*
B	9.4	Q	5.8	B	9.5	Q	6.2
Q	9.0	Q	6.0	Q	10.2	B	6.0
Q	9.4	B	5.8	Q	9.2	B	5.8
B	9.0	Q	6.2	Q	9.4	Q	6.2
Q	8.8	Q	5.8	Q	9.4	Q	5.8
Q	9.6	B	6.0	B	10.0	Q	5.4
B	9.2	Q	5.6	Q	9.8	Q	5.8
Q	9.4	Q	6.0	Q	9.6	Q	5.5
B	9.1	B	5.8	Q	9.5		

B = benzyl alcohol droplet. Q = quartz particle.

TABLE VII.

Agar Particles in Equilibrium with N/50 *Acetate Buffer, pH = 3.6, in ½ Per Cent Gelatin Solution Migrate with the Same Speed as Paraffin Oil Droplets.*

Time to migrate given distance		Time to migrate given distance	
	sec.		*sec.*
P	6.4	A	6.4
A	6.4	A	6.2
A	6.4	A	6.0
A	6.6	P	6.2
P	6.7	P	6.0
A	7.0	P	6.0
A	6.8	A	6.8
		A	6.0

The Same Experiment with Hemocyanine, pH = 4.0

	sec.		*sec.*
P	7.0	P	7.8
A	7.4	A	7.0
A	6.5	A	7.0
A	6.4		
A	6.8	P	9.0
P	7.2	A	8.4
A	7.0		
A	7.0		

Brackets refer to droplets studied at particular levels in the cataphoresis cell. P = oil. A = agar.

The Influence of Particle Conductivity.

The following kinds of particles, all covered with protein films, were studied simultaneously in dilute electrolyte solutions: paraffin droplets, quartz particles, droplets of benzyl alcohol, carbon particles, particles of agar. The conductivity of the material of these particles varies from virtually zero in the case of quartz, up to a conductivity practically equal to that of the surrounding medium in the case of agar. The protein film was obtained either with gelatin or with

TABLE VIII.

Carbon Particles Suspended in N/100 *HCl* + ⅛ *Per Cent Gelatin Move with the Same Speed As Paraffin Oil Droplets.*

Time to migrate given distance		Time to migrate given distance		Remarks
	sec.		*sec.*	
P	6.0	P	5.8	Brackets refer to particles studied at different levels in cataphoresis cell
C	6.0	C	6.0	
P	5.8	P	9.0	
C	5.8	C	9.4	
P	4.5	P	8.8	
C	4.8	C	9.4	
P	6.6	P	5.6	
C	7.0	C	5.4	

P = oil. C = Carbon.

hemocyanine (*Limulus*). Tables VI, VII and VIII show that *the velocity of cataphoresis does not depend to any measurable extent on the bulk conductivity of the particle.*

CONCLUSION.

The electrophoretic mobility of microscopically visible particles is independent of size, shape and conductivity of the particle within the limits of the experimental error. This is valid for extreme variations in size, shape and conductivity.

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