COUNTERCURRENT ELECTROPHORESIS ON PAPER

VIII. THE NATURE OF THE SO-CALLED "DECREASE IN RELATIVE THERMODYNAMIC ACTIVITY" IN PAPER ELECTROPHORESIS*

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INTRODUCTION

In addition to the concept whereby the influence of the paper on the electrophoretic mobilities of substances is characterised by a tortuosity factor and a separation function[1-5], there is also a concept of "barrier effect" introduced by McDONALD[6]. According to this theory, cellulose fibers act as a barrier to migrating particles. The slowing down of the particles is proportional to the number of barriers, characterised by the value "concentration of paper in the solution", P. This term is determined by the ratio of the weight of paper to the weight of solution. For quantitative expression of this idea, a quantity "relative thermodynamic activity" a_{ct} was introduced, and has unit value for solutions containing no paper ($P = 0$). The presence of paper in the solution ($P > 0$) produces a decrease in the quantity a_{ct}. The values of the quantity a_{ct} of the ions are given by the ratio of the conductivity K of the system with paper ($P > 0$) to the conductivity K_0 of the same system without paper ($P = 0$)[6]. In addition according to ref. 6,

$$U_0 = U_{cor}/^a_{ct} \tag{1}$$

where U_0 is the electrophoretic mobility in the free solution and U_{cor} the macroscopic electrophoretic mobility on paper corrected for electro-osmosis.

MARBACH[6] estimated the "relative thermodynamic activity" from measurements of conductivity. He measured the conductivity of the solution in a vessel which was gradually filled with chromatographic paper. The measured conductivity values were multiplied by the ratio ϑ of the volume of the vessel to the volume of solution actually present in the vessel. From this were obtained results corresponding to the same amount of solution. He calculated further the quantity a_{ct} and plotted the values obtained against the ratio P. A falling straight line resulted. Such results were obtained for papers free of conducting impurities and with solutions of specific conductivities $\varkappa > 4.10^{-4}\Omega^{-1}\text{cm}^{-1}$. When $\varkappa < 4.10^{-4}\Omega^{-1}\text{cm}^{-1}$ (*e.g.* for 0.001 N KCl) MARBACH found even for washed papers (ref. 6, *e.g.* Fig. 24) an increase of specific conductivity with the ratio P. In these cases he did not plot the dependence of a_{ct}

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* For Part VII, see ref. 5.

on P. The decrease of "relative thermodynamic activity" obviously did not occur in this case. This purely experimental correlation of U_0 and U without physical analysis was criticized by WALDMANN-MEYER AND SCHILLING[7].

In order to determine the physical meaning of the quantity a_{ct}, the conductivity in MARBACH's experimental arrangement was measured. The cross section of the vessel was denoted by A, the cross section and the weight of liquid in the paper pores by a_1 and g_1 respectively and the cross section and the weight of the liquid in the vessel apart from the paper by a_2 and g_2. For the conductivity of a pure liquid, K_0, and for the conductivity when paper is present, K, the following equations may be written:

$$K_0 = \varkappa_0 A/L; \qquad K = \varkappa_0[a_2 + (a_1/f)]/L \tag{2}$$

where L is the length of the vessel, f the tortuosity factor and $\varkappa_0$ the specific conductivity of the given liquid. If it is assumed that the conductivity $\varkappa_0$ is not influenced by the presence of the paper, it follows that:

$$a_{ct} = K\vartheta/K_0 = 1 - [a_1\vartheta(1 - 1/f)/A] \tag{3}$$

because $\vartheta = A/(a_1 - a_2)$. In addition, where H represents the weight of the water already filling all the pores of 1 g of the paper calculated on the dry paper and neglecting the swelling water, and since $P = g_p/(g_1 + g_2)$ (g_p is the weight of paper in the vessel) it follows that $a_1\vartheta/A = g_1/(g_1 + g_2) = g_1P/g_p = HP$. For the quantity a_{ct}, therefore,:

$$a_{ct} = 1 - HP + (HP/f) \tag{4a}$$

If the specific conductivity of the solution changes from the value $\varkappa_0$ to the value $\varkappa_b$ when paper is present, then:

$$a_{ct} = \varkappa_b[1 - HP + (HP/f)]/\varkappa_0 \tag{4b}$$

The specific conductivity of the solution changes from $\varkappa_0$ to $\varkappa_b$ for different reasons. When this change is due to the washing out of soluble conducting impurities from the paper the total bulk-conductivity $\varkappa_b$ is given by the sum of the original value, $\varkappa_0$, and of the conductivity of the impurities, $\varkappa_i$ ($\varkappa_b = \varkappa_0 + \varkappa_i$). The interaction of a dissolved substance with the paper (e.g. the adsorption of a part of the dissolved conducting substance by the paper or an exchange adsorption of ions of different conductivity on the paper) can also change the original value of the conductivity. Furthermore if, in dilute solutions, the surface-conductivity $\varkappa_s$ in the paper pores is taken into consideration, as well as the bulk-conductivity $\varkappa_b$ (ref. 8) then:

$$K = [(\varkappa_b + \varkappa_s)a_1 + \varkappa_b a_2 f]/fL$$

and:

$$a_{ct} = \varkappa_b[1 - HP + (HP/f)]/\varkappa_0 - \varkappa_s HP/\varkappa_0 f \tag{4c}$$

Equations (4a, 4b, 4c) are valid for $HP \geqslant 1$, i.e. the given experimental conditions. Under the normal conditions of electrophoretic experiments HP is nearly equal to unity. Then:

$$a_{ct} = (\varkappa_b + \varkappa_s)/\varkappa_0 f \tag{5}$$

If the concentration of the solution is large ($\varkappa_t, \varkappa_s \ll \varkappa_0$) and if no interaction takes place between the dissolved substance and the paper ($\varkappa_b = \varkappa_0$), the term a_{ct} becomes the reciprocal of the tortuosity factor and in this case $a_{ct} < 1$. If there is an interaction leading to decrease in specific conductivity and if the concentration of the solution is high, then $\varkappa_b < \varkappa_0$ and $a_{ct} < 1$ again. But when the concentration of the solution is small ($\varkappa_0$ does not differ much from $\varkappa_t$ and $\varkappa_s$) and the interaction with the paper leading to decrease in the specific conductivity is not too large ($\varkappa_b$ does not differ too much from $\varkappa_0$ and f varies from 1.1 to 1.4—see Table I) then $a_{ct} > 1$. When the interaction with the paper leads to the increase of specific conductivity ($\varkappa_b > \varkappa_0$) (exchange adsorption) then it is possible that $a_{ct} > 1$ for concentrated as well as dilute solutions.

The physical meaning of the quantity a_{ct} is consequently far from clear. It is furthermore evident that this quantity is not suitable for correlation of the values U_0 and U_{cor} by equation (1). According to this equation a_{ct} should always be smaller than unity ($a_{ct} < 1$), because in fact U_0 is always greater than U_{cor}. This, however, obviously contradicts the conclusions of eqn. (5).

It is, however, evident, that it is possible to use conductivity measurements of this kind for the estimation of the values of the quantities on the right hand side of eqn. (5). When the solution does not contain any substance which could interact with the paper (in the sense of chromatographic distribution), the difference between the values $\varkappa_b$ and $\varkappa_0$ may be ascribed to impurities in the paper and it is possible to calculate their conductivity $\varkappa_t$. (For $HP = 1$, $\varkappa_t$ becomes the specific conductivity of impurities from the given sample of paper dissolved in the amount of water which just fills all its pores.) On the other hand if the paper is free from impurities, but the solution contains substances which can interact with the paper, it is possible to find the corresponding distribution isotherm.

EXPERIMENTAL

Measurements were performed with Whatman No. 1, 2, 3, 4, 31, 52, Schleicher & Schüll, No. 604, 2043A, 2043B and Ederol No. 202 chromatographic papers. Methylene Blue $C_{16}H_{18}N_3Cl$, Neptun Blue $Ca(C_{37}H_{35}N_2O_2S_2)_2$ and freeze-dried albumin (Imuna, Michalany) were studied. All other substances were of analytical reagent purity. The conductivity measurements were performed in the experimental arrangement described by MARBACH[6]. A thermostated conductivity cell of dimensions $57 \times 14 \times 21$ mm was used and resistances were measured with a Tesla RLC bridge.

RESULTS

The dependence of resistance on the amount of paper added was measured. All measurements were carried out using papers with no previous treatment as well as with papers, which had been freed from soluble impurities. The impurities were removed by decanting for two hours and by washing the paper with a solution of a given concentration. The calculated values of the quantity a_{ct} were plotted against the ratio HP (values H for different kinds of chromatographic papers were taken from the previous paper[9]).

The reciprocal values of the quantity a_{ct} for $HP = 1$ from the measurement in

o.1 N KCl solution ($\varkappa_b = \varkappa_0$) corresponding to the tortuosity factor are shown for different chromatographic papers in Table I. The dependence of the quantity a_{ct} on the ratio HP in solutions of different concentrations of potassium chloride for Whatman No. 4 paper are shown in Fig. 1. Similar dependences were obtained also
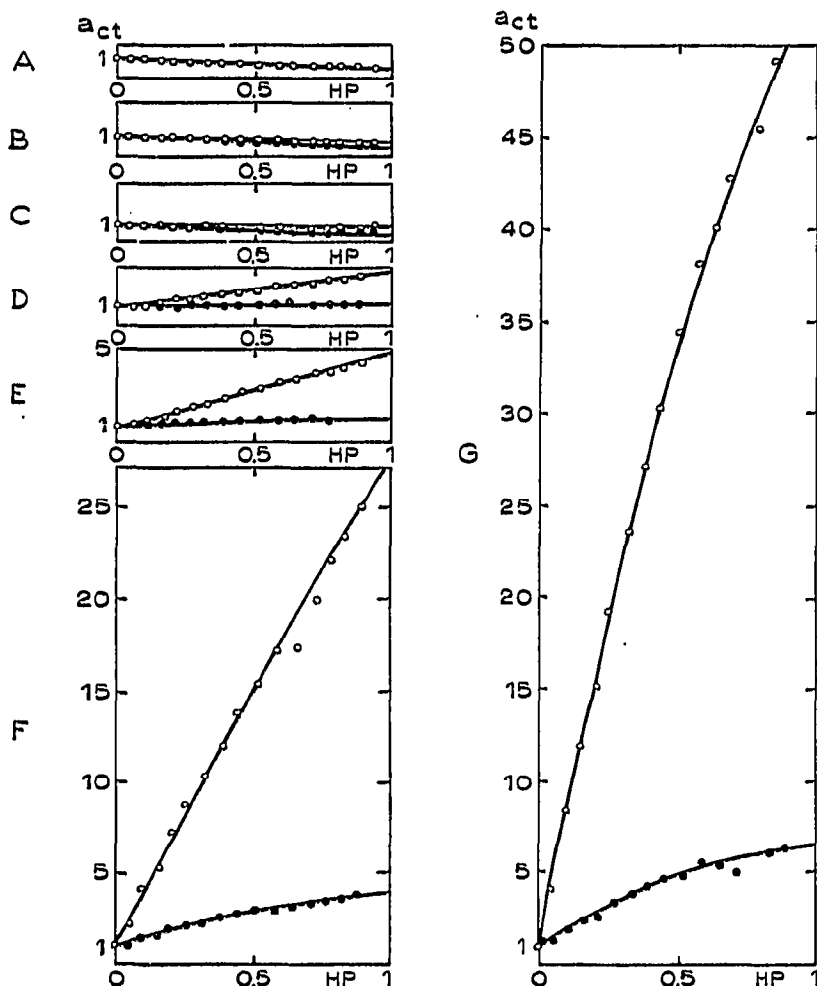

Fig. 1. The dependence of a_{ct} on the quantity HP for different concentrations of potassium chloride solution: A = o.1, B = o.o1, C = o.oo5, D = o.oo1, E = o.ooo5, F = o.ooo1, G = o.ooo05 M. ● washed paper, ○ unwashed paper.

for other kinds of chromatographic papers. The surface-conductivity $\varkappa_s$ was calculated from eqn. (5) using the values of a_{ct} which were obtained from the measurements of washed papers ($\varkappa_0 = \varkappa_s$). From the values of a_{ct} obtained from the measurements with papers without previous treatment ($\varkappa_b = \varkappa_i + \varkappa_0$) conductivities of impurities $\varkappa_i$ were calculated and these results are shown in Table I. Whatman No. 4 paper was also measured in different electrolytes; the results of these measurements are shown in Tables I and II.

In order to study the interaction of the substance with paper, solutions were prepared containing different amounts of the interacting substance in the same amount of electrolyte. Solutions obtained in this way had different specific conductivities. Another series of measurements was performed with solutions also containing differ-

TABLE I

TORTUOSITY FACTOR f, SURFACE CONDUCTIVITY $\varkappa_s$ AND CONDUCTIVITY OF IMPURITIES $\varkappa_i$ OF SOME CHROMATOGRAPHIC PAPERS

Paper		Whatman No.						Schleicher & Schüll No.			Ederol No. 202
		1	2	3	4	31	52	604	2043A	2043B	
f		1.28	1.31	1.31	1.28	1.22	1.25	1.25	1.25	—	1.39
$\varkappa_s \cdot 10^{-5} [\Omega^{-1} cm^{-1}]$		4.64	3.93	3.19	4.80	3.59	2.10	6.76	3.19	3.56	3.05
	$5.10^{-3} N$ KCl	2.37	1.37	—	2.56	0.85	—	3.52	2.88	1.95	—
$\varkappa_i \cdot 10^{-4}$	$5.10^{-4} N$ KCl	2.34	2.25	0.97	2.74	0.93	—	3.61	3.45	2.50	1.96
$[\Omega^{-1} cm^{-1}]$	$5.10^{-5} N$ KCl	2.17	2.41	1.48	2.90	1.24	3.09	3.81	3.09	2.03	2.65
	Average values[*]	2.29	2.01	1.22	2.80	1.01	3.09	3.65	3.14	2.16	2.30

[*] Average values are taken from all measurements performed, not only from those in Table I.
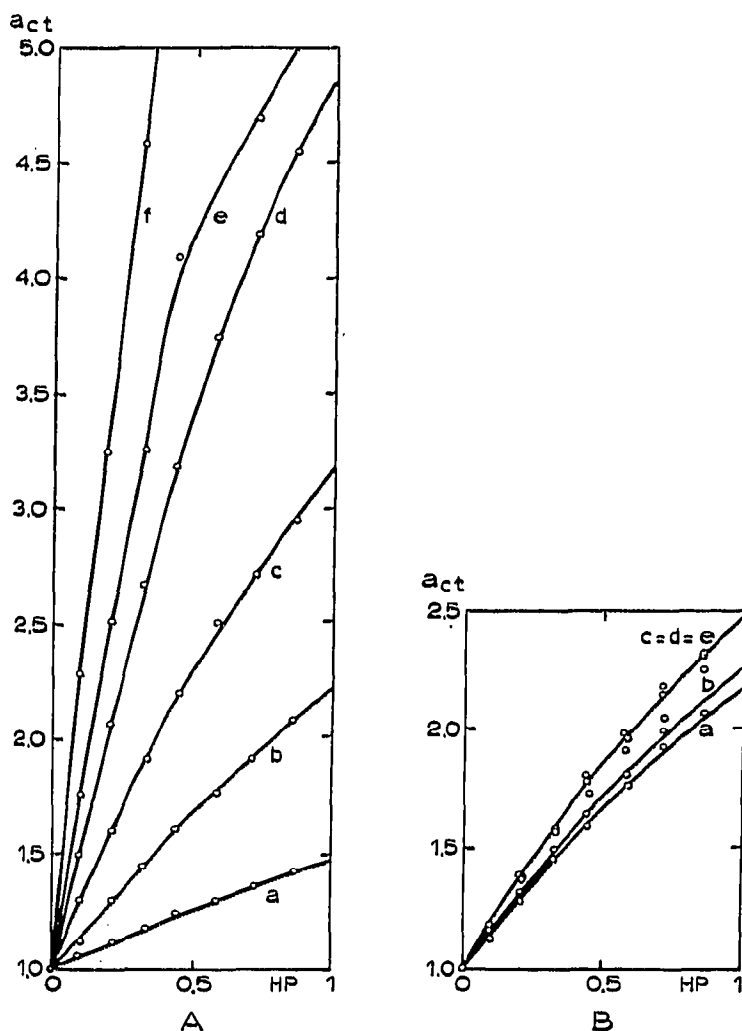

Fig. 2. The dependence of a_{ct} on the quantity HP. (A) Solutions of albumin in standard acetate buffer diluted with water (1:99). Concentration of albumin: a = 1, b = 0.5, c = 0.25, d = 0.1, e = 0.05%, f = buffer without albumin. (B) Analogous solutions with constant conductivity; curve f corresponds to the buffer solution used for dilution.

TABLE II

CONDUCTIVITY OF IMPURITIES $\varkappa_i \cdot 10^{-4}[\Omega^{-1}cm^{-1}]$ FOR WHATMAN NO. 4 PAPER IN DIFFERENT SOLUTIONS

Base solution	Dilution base solution: water				Average values
	1:1	1:9	1:99	1:999	
0.1 N Potassium chloride	—	—	2.84	2.97	2.80
0.1 N Acetic acid	2.04	2.95	3.04	2.76	2.70
Veronal buffer[a]	1.57	3.64	2.71	3.19	2.81
Phosphate–borate buffer[b]	—	3.14	2.83	3.59	3.08
Acetic acid–formic acid[c]	—	—	1.04	3.17	2.10

[a] 2.06% veronal-Na, 0.368% barbituric acid.
[b] 0.466% $NaH_2PO_4 \cdot H_2O$, 0.85% $Na_2B_4O_7 \cdot 10H_2O$.
[c] 17.7% acetic acid, 5.1% formic acid.

ent amounts of the interacting substance but adjusted (by changing the concentration of the electrolyte) to the same specific conductivity. The dependence of the quantity a_{ct} on the ratio HP obtained from these measurements is plotted in Figs. 2–4.

DISCUSSION

The advantage of the method described for measuring the tortuosity factor is that it is not obtained from a single measurement, but from a concentration dependence, where random experimental errors can be eliminated graphically. A further advantage is that it is possible also to determine the correcting factor for moistures of paper larger than H.
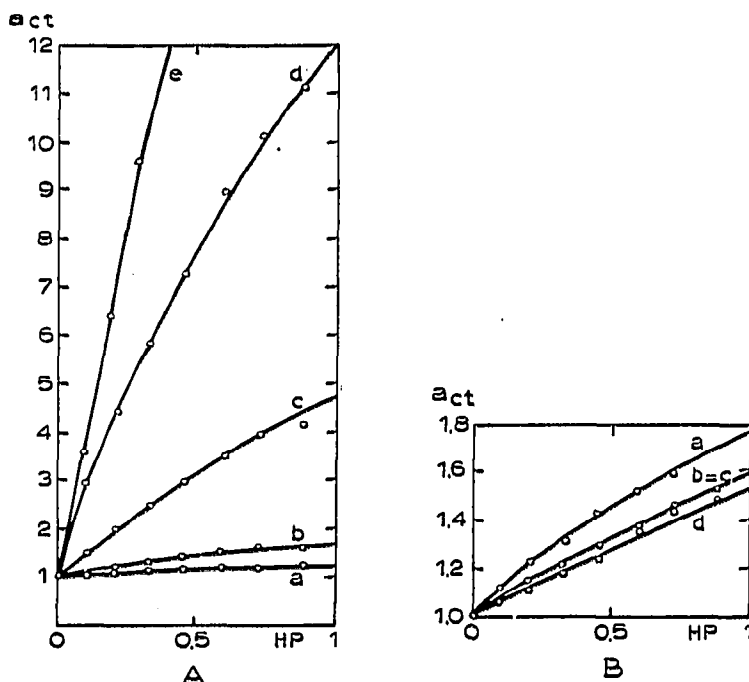

Fig. 3. The dependence of a_{ct} on the quantity HP for a solution of Methylene Blue in 0.0001 N acetic acid. (A) Concentration of the dye: a = 0.1, b = 0.05, c = 0.01, d = 0.005%, e = pure acetic acid. (B) Concentration of the dye: a = 0.05, b = 0.025, c = 0.01%, d = pure 0.03 N acetic acid. Constant specific conductivity.

It was found that papers contain conducting impurities in such amounts that $\varkappa_l$ for all of them is in the order of $10^{-4}\Omega^{-1}$ cm^{-1}.

According to the literature[8], it was found that surface-conductivity in the pores of the paper is practically independent of the concentration of the electrolyte. Moreover, the surface conductivity of all the papers used in all the electrolytes tested was found to be practically the same, *viz.* of the order $10^{-5}\Omega^{-1}$ cm^{-1}.

The increase in the quantity a_{ct} due to the conductivity of impurities and surface-conductivity is seen in Fig..1. The influence of impurities and of surface-conductivity is negligible in concentrated potassium chloride, and increases with dilution of the solution. The effect of interaction between the substance and the paper on the value of a_{ct} is clearly seen only under experimental conditions in which the concentration of interacting substance varies, while the specific conductivity of the solution is kept constant (Figs. 2B, 3B, 4B). It is otherwise impossible to determine to what extent the change of the quantity a_{ct} is due to the interaction and to what extent to the increase in bulk-conductivity of the solution. It is, however, shown that a change (increase or decrease) of the quantity a_{ct} due to the interaction is always much smaller than the decrease of a_{ct} exhibited by the increase in the specific conductivity of the solution (Figs. 2A, 3A, 4A).
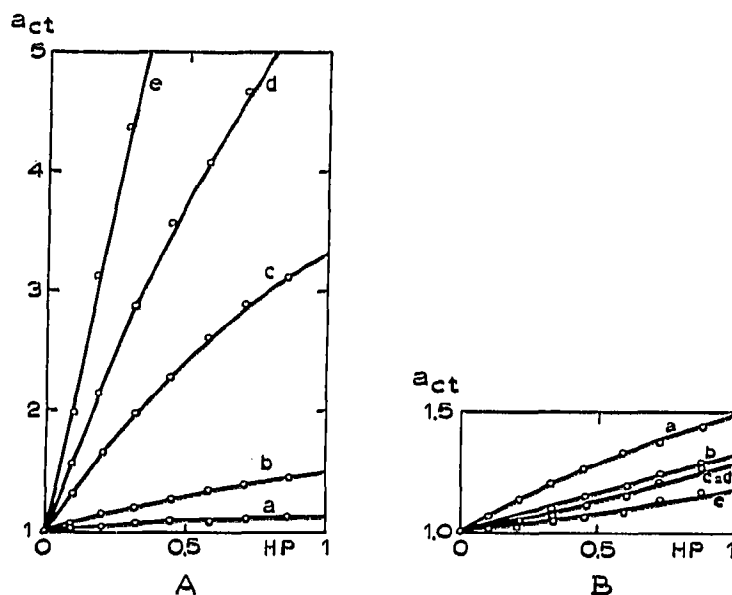
Fig. 4. The dependence of a_{ct} on the quantity HP for a solution of Neptun Blue in 0.0001 N acetic acid. (A) Concentration of the dye: a = 0.1, b = 0.05, c = 0.01, d = 0.005 %, e = pure acetic acid. (B) Concentration of the dye: a = 0.05, b = 0.025, c = 0.01, d = 0.005 %, e = pure 0.06N acetic acid. Constant specific conductivity.

It is evident that the interaction of the substance with paper can lead both to a decrease in conductivity of the solution (albumin), and also to its increase (Methylene Blue and Neptun Blue). The main role is played by the substance, which has a direct share in the interaction. In exchange adsorption on paper, where an electric double-layer structure is involved, a cation exchange can be assumed. Methylene Blue therefore exhibits an increase of specific conductivity due to the exchange of the coloured organic cation for the more mobile ion from the outer part of the electric

double-layer. This is also in agreement with the relatively smaller R_F value of Methylene Blue in the given solution (R_F = 0.03–0.3. The R_F values were obtained by frontal chromatography). With Neptun Blue the coloured part is an organic anion which is not adsorbed on the negatively charged cellulose (R_F approx. = 1). In this solution calcium cations take part in the adsorption leading to an increase in $\varkappa_b$. It is seen also that in systems in which the influence of surface conductivity of impurities is eliminated the interaction indicated by conductivity measurements of a given substance need not be the same as that indicated by chromatographic distribution.

SUMMARY

It has been shown that, under special conditions, the so-called "relative thermodynamic activity" can be expressed as the reciprocal value of the tortuosity factor. The value of this "activity" can not only decrease with the "paper concentration", but can also increase to values above unity. It is therefore not possible to consider the term "relative thermodynamic acitivity" as a general characteristic of the system solution–paper. On the other hand changes in electrical conductivity can indicate the interaction of the dissolved substance with paper and/or the presence of impurities. Measurements of this kind lead also to an evaluation of surface conductivity.

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