

well as in view of our more complete spectra of both acids.

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Summary

1. A new line 480 cm.^{-1} was found in the Raman spectrum of anhydrous nitric acid and interpreted as the frequency of the restricted internal rotation of the hydrogen.

2. Anhydrous deuteronic acid, free of lower oxides, has been prepared, and its Raman spectrum was determined.

3. All nine fundamentals were found in the spectra of nitric acid and deuteronic acid. A complete interpretation is presented which, except for minor details, is believed to be a necessary consequence of the experimental results.

4. The results are in accord with the product formula applied to symmetry C_s (plane configuration).

5. The density of deuteronic acid at 25.00° is 1.5228 .

6. The refractivities of nitric and deuteronic acids at 25.0° are $n_D = 1.3920$ and $n_D = 1.3909$, respectively.

PULLMAN, WASHINGTON

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OHIO STATE UNIVERSITY]

Deviations between Observed and Calculated Polarographic Diffusion Currents¹

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This paper presents evidence to show that the deviations of observed from calculated polarographic diffusion currents have not been satisfactorily explained up to the present time. Further evidence is presented to show that the deviations are chiefly the result of a combination of the properties of ions and of stirring effects produced by the dropping electrode. The assumption of perfect drops at the electrode has been experimentally checked and validity of the Ilkovic equation has been reaffirmed.

Polarographic diffusion currents can be calculated from the Ilkovic equation,⁴ $i_d = 605nD^{1/2}Cm^{2/3}t^{1/6}$ in which n is the number of faradays of electricity involved per mole of reactant, D is the diffusion coefficient of the reacting ion or molecule, C is the concentration in millimoles per liter of the reacting substance, m is the weight of mercury in milligrams flowing from the capillary per second, and t is the drop life in seconds; i_d is the average diffusion current in microamperes. Lingane and Kolthoff⁵ have examined the Ilkovic equation at length and have concluded that it is essentially correct. Deviations, however, of the

diffusion current from the theoretical have been observed by Ilkovic⁴ and by Kolthoff and Lingane.⁶ The latter authors report deviations as high as 8% between calculated and experimental diffusion currents. These authors have attributed the deviations to uncertainties in the values of the diffusion coefficients used in calculating the currents by the Ilkovic equation. An earlier suggestion was advanced by Lingane and Kolthoff⁵ that the formation of the complex ions, CdCl^+ and ZnCl^+ may be responsible for the deviations found for these two metals.

In order to determine whether the deviations noted by Kolthoff and Lingane are peculiar to chloride solutions, we have determined the deviations using the nitrates in 0.1 molar potassium nitrate solution. It has been shown by Harned and Fitzgerald,⁷ Bates,⁸ and Robinson⁹ that the activity coefficient for cadmium nitrate is quite different from that for cadmium chloride. Robinson, Wilson and Ayling¹⁰ recently concluded from their values of the activity coefficient of cadmium nitrate that a complex nitrate ion does not exist.

Our data are shown in Table I together with

(1) From a thesis submitted by E. W. Balis in partial fulfillment of the requirements for the Ph.D. Original manuscript received August 4, 1941.

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(4) D. Ilkovic, *Coll. Czech. Chem. Comm.*, **6**, 498 (1934).

(5) J. J. Lingane and I. M. Kolthoff, *THIS JOURNAL*, **61**, 825 (1939).

(6) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941, p. 79.

(7) H. S. Harned and M. E. Fitzgerald, *THIS JOURNAL*, **58**, 2624 (1936).

(8) R. G. Bates, *ibid.*, **61**, 308 (1939); **63**, 399 (1941).

(9) R. A. Robinson, *Trans. Faraday Soc.*, **36**, 738, 1135 (1940).

(10) R. A. Robinson, J. N. Wilson and H. S. Ayling, *THIS JOURNAL*, **64**, 1469 (1942).

TABLE I
COMPARISON OF OBSERVED AND CALCULATED DIFFUSION CURRENTS

Ion	D° , cm. ² sec. ⁻¹ $\times 10^6$	$m^{3/2}/t^{1/2}$ (mg. ^{3/2} /s. sec. ^{-1/2})	i_d , microamperes		% Deviation	
			Observed	Calculated	K. & L. (0.1 M chloride)	(Observed in 0.1 M nitrate)
Ni ⁺⁺	0.69				6.3	
Cd ⁺⁺	.72	1.56	5.33	5.08	8.1	4.9
Zn ⁺⁺	.73 ^a	1.54	5.16 ^a	5.04 ^a	5.2	2.4
Cu ⁺⁺	.73 ^a	1.57	5.34 ^a	5.13 ^a	4.4 ^b	4.1
Pb ⁺⁺	.98	2.03	7.72	7.68	1.3	0.7
					-0.5	
Tl ⁺	2.00	2.02	5.46	5.46	-0.7	0
					-1.6	
Fe(CN) ₆ ⁼	0.90 ^a	2.01	3.43 ^{a,c}	3.67 ^a	-8.3	-6.5 ^c
CrO ₄ ⁼ ($n = 3$)	1.07				-0.4	
($n = 6$)	1.07				1.0	
IO ₃ ⁻	1.09				0.4	

^a At 26°. ^b Measured in 0.1 M KNO₃. ^c Measured in 0.1 M KCl.

the observations of Kolthoff and Lingane.⁶ The deviations recorded are somewhat smaller than those observed by Kolthoff and Lingane but it should be noted here that many of the data of Kolthoff and Lingane were obtained with a three-second capillary while we used one with a drop life greater than four seconds. Thus it appears that the deviations found by us in nitrate solutions closely parallel those observed by Kolthoff and Lingane in chloride solutions and that the deviations cannot be explained as being due to a peculiarity of chloride solutions or to complex formation.

Lingane and Kolthoff⁶ have also suggested that the negative error for the Fe(CN)₆⁼ ion may be due to the reaction of this ion with mercury. Since concentration enters in the Ilkovic equation as the first power, an error of 8% (6.5% in our data) in the diffusion current requires that 8% of the Fe(CN)₆⁼ originally present must react with the mercury before the voltage is reached at which the diffusion current is measured. The current-voltage curves following the initial rise to the diffusion current would then slope distinctly downward. No indication of such a downward slope was found and therefore the reaction of Fe(CN)₆⁼ with mercury in the cell cannot be rapid enough to account for the large negative deviation found.

A study of polarographic phenomena and of the available literature led to the consideration of two factors other than uncertainties in the calculation of diffusion coefficients which appeared likely to have some effect on the magnitude of the deviations: (1) possible irregularities in the formation of the drop and (2) stirring effects produced in the solution by the growing and

falling drop. A study of drop formation is also important as an experimental check on the assumption of perfect drops in the derivation of the Ilkovic equation.

Irregularities in Drop Formation.—The derivation of the Ilkovic equation assumes a spherical drop which grows in mass at a uniform rate and breaks away cleanly at the tip of the capillary. In several cases we had observed that the drop was non-spherical, that it grew at an irregular rate and that it often hung from the end of a thread of mercury which formed the beginning of the next drop. Figure 1 shows an extreme case of this phenomenon observed in 0.1 molar chloride solution with the drop acting as anode. The drops fell from the end of the connecting thread and this thread then formed the beginning of the next drop.

The dropping electrode was therefore examined for irregularities during the passage of the diffusion current for those ions whose deviations have been noted. As a preliminary step an equation¹¹ was derived for the diffusion current which accounted for a residual drop left on the capillary tip each time the main drop fell away. Calculations show that the area of a residual mercury surface must be greater than that of a sphere one-tenth the diameter of the maximum drop in order to increase the average current one per cent. Although the amount of mercury in a residual drop of this size is quite easy to see through a low power microscope, the motion and growth of the drop are so rapid that an estimate of its initial size cannot be reached by visual observation. Hence motion pictures were taken of the drops of mercury with a high-speed motion-

(11) E. W. Ballis, Ph.D. Thesis, Ohio State University, 1941.

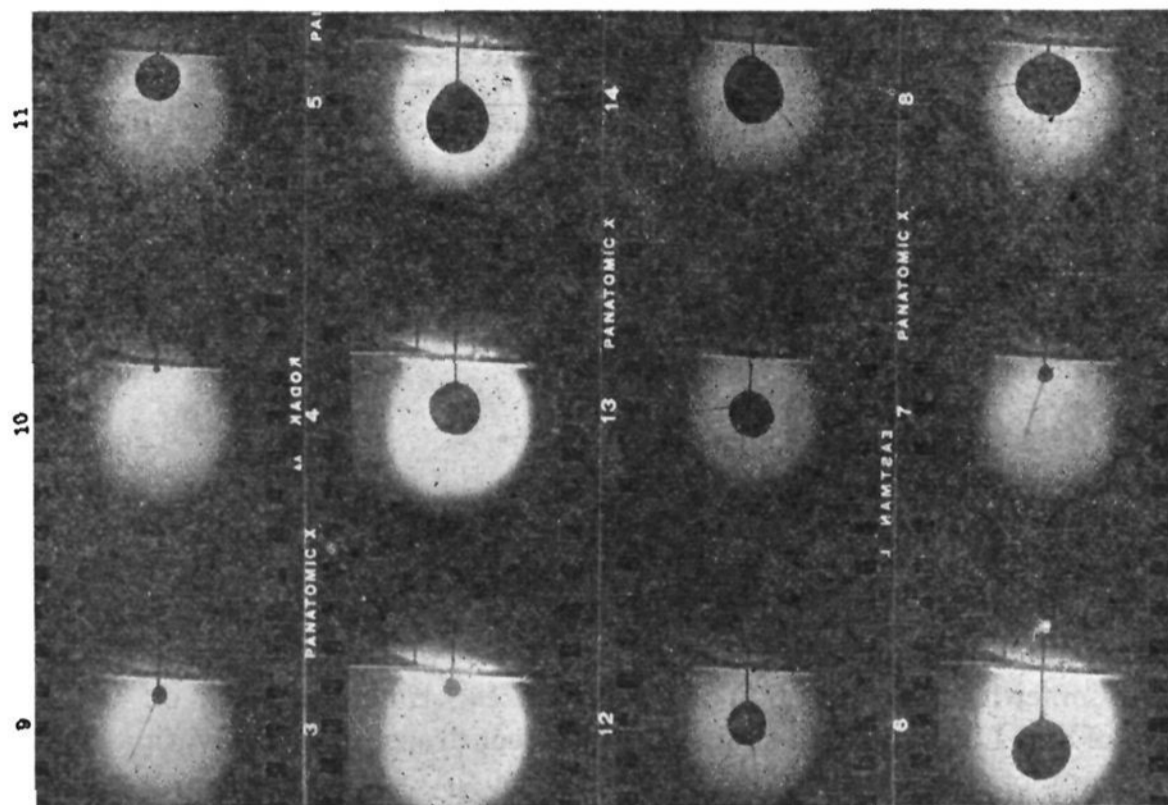


Fig. 1.—Photographs of mercury drop as anode in 0.1 *N* potassium chloride. Exposures 10 and 11 represent the drop without applied potential. In all other cases a potential of 1.5 to 3.0 volts (with reference to the normal calomel electrode) was applied.

picture camera (125 frames per second). This has been done for those ions noted in Table I (except ferricyanide) where large deviations occurred and where it seemed possible that noticeable irregularities might exist. In each case the measurement of i_d , m , and v and the photographs were taken simultaneously so that it is known that the deviations occurred under the drop conditions shown by the photographs.

In none of these cases was any appreciable irregularity in the shape of the drop found such as was observed in Fig. 1. The drop mass increased at a uniform rate, the drops were spherical and broke away cleanly at the capillary. Figure 2 shows one series of successive photographs of the drop in a solution of 0.001 molar cadmium nitrate in 0.1 molar potassium nitrate. This is typical of all the photographs of all the ions studied, namely, Cd^{++} , Zn^{++} , Cu^{++} , Pb^{++} and Tl^+ . For this group of ions, these experiments justify the assumption of perfect drops made by Ilkovic⁴ and by MacGillavry and Rideal¹² in their respective derivations of the Ilkovic equation. It must therefore be concluded that the irregularities in drop formation shown in Fig. 1 represent a very extreme case and are probably due to the effects of depositing solid mercurous

(12) D. MacGillavry and E. K. Rideal, *Rec. trav. chim.*, **56**, 1013 (1937).

chloride on the surface of the drop, as has been observed by Majer.¹³

Evidence of Deviations Due to Stirring.—

The second possible source of deviations investigated was the effect produced on the current by stirring. Stirring effects have been observed by Frumkin and Bruns,¹⁴ Antweiler,¹⁵ Jofa and Frumkin,¹⁶ and Krinkova and Kabanov,¹⁷ in their studies of maxima and Antweiler has photographically recorded streaming in the solution.

It is obvious that a certain amount of motion of the solution is inherent in the operation of the dropping mercury electrode. If the solution is agitated sufficiently to disturb the diffusion layer resulting from concentration polarization, then the discharging ions can reach the electrode not only by diffusion but also by electrical migration and by mechanical transportation.

(13) V. Majer, *Coll. Czech. Chem. Comm.*, **7**, 215 (1935).

(14) A. Frumkin and B. Bruns, *Acta Physicochem. U. S. S. R.*, **1**, 232 (1934).

(15) H. J. Antweiler, *et al.*, *Z. Elektrochem.*, **43**, 596 (1937); **44**, 663, 719, 888 (1938).

(16) S. Jofa and A. Frumkin, *Compt. rend. acad. sciences U. S. S. R.*, **20**, 293 (1938).

(17) T. A. Krinkova and B. N. Kabanov, *J. Phys. Chem. U. S. S. R.*, **13**, 455 (1939).

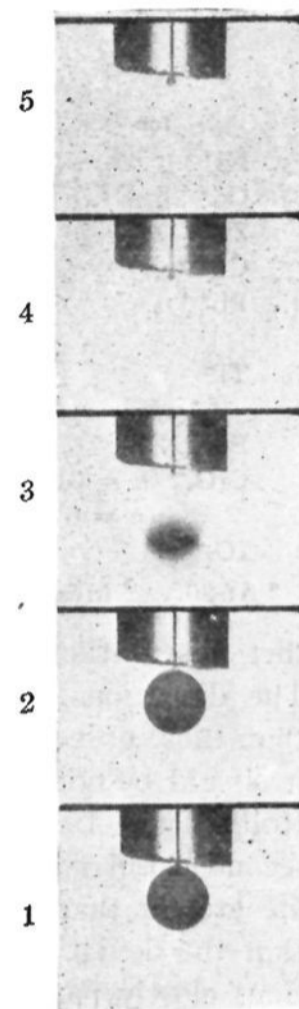


Fig. 2.—Photographs of mercury drop in 0.001 *M* cadmium nitrate in 0.1 *M* potassium nitrate. The numbers indicate order of sequence.

Listed below are several direct pieces of evidence that this motion does result in a disturbance of the diffusion layer and is responsible for the observed deviations of the diffusion current.

(1) Within the experimental error, positive ions discharged at the cathode give positive deviations and negative ions, negative deviations (see Table I).

(2) The data of Maas¹⁸ are used to show that the deviations for a single ion increase with increasing drop rate. Table II is calculated from Kolthoff and Lingane's summary¹⁹ of Maas' data.

TABLE II
COMPARISON OF DEVIATIONS AND DROP TIME
(Calculated from data of Maas^{18,19})

Capillary	<i>t</i> , seconds	<i>i</i> _d observed	Calculated from Maas' data	% Deviation calculated from Maas' data
1	6.43	6.92	6.36	8.8
2	6.28	7.26	6.61	9.8
3	5.90	7.88	6.93	13.7
4	4.89	10.05	8.99	11.7
5	4.10	11.73	10.45	12.3
6	3.17	13.82	12.24	12.9

The value for capillary 3 in Table II is out of order. Recently, however, Mr. S. R. Steele of this Laboratory using a similar set of capillaries determined the deviations and does not find the irregularity noted in the calculations made from Maas' data.

Further, the deviations found by Kolthoff and Lingane (Table I) with a 3 second drop rate are in general greater than the deviations which we found at a 4 second rate.

(3) A study of Table I shows that the major deviations occur for those ions having a small diffusion rate (*i. e.*, coefficient).

(4) It has been shown by Heyrovsky²⁰ that where maxima occur, their magnitudes are greater at shorter drop times.

(5) Experiments with mechanical stirring in this laboratory show an increase in the current measured, as would be expected.

Efforts to apply, regulate, and evaluate the amount of stirring produced at the surface of the drop have not been successful. An effort to eliminate stirring by the use of a quiet, fixed drop has met with difficulties since, as has been pre-

viously observed with platinum microelectrodes, unavoidable building vibrations are enough to raise the current appreciably. For example, a current observed with a fixed hanging drop during the day would be very erratic and greater by as much as 30% than that observed when the building was relatively quiet. The alternative was a regulated amount of stirring which would be great enough to absorb all effects of building vibration.

The only method of mechanical stirring which was found to be practical was to bubble nitrogen at a fixed rate into the solution from a small orifice so arranged as to give a slow steady flow in the solution. This resulted in marked increases in the currents measured. The results of these experiments are shown in Table III. The effect of vibration from the thermostat stirrer motor is also shown in Table III.

TABLE III^a
EFFECT OF VIBRATION AND SLOW STIRRING ON DIFFUSION CURRENTS (*i*_d in microamperes)

Ion	No vibration	Motor vibration	% increase	Gas stirring	% increase
Cd ⁺⁺	6.71	6.90	3	14.1	110
Zn ⁺⁺	6.61	6.61	0	13.3	101
Cu ⁺⁺	7.09	7.28	3	15.0	112
Pb ⁺⁺	7.67	7.95	4	15.9	108
Tl ⁺	5.46	5.46	0	12.0	119
Fe(CN) ₆ ⁴⁻	3.43	3.43	0	5.8	71

^a Same capillary used for Pb⁺⁺, Tl⁺, and Fe(CN)₆⁴⁻ in Table I.

The same dropping electrode was used in all the stirring experiments, the same cell with a fixed gas orifice was used and nitrogen was bubbled through at the same rate. The increases noted with gas stirring are due to stirring effects and not to any increase in *m* and *t* values. The quantity *m* was found to be 1.973 mg./sec. during gas stirring as compared with 1.982 mg./sec. without stirring. The drop time measured at the same potential as the diffusion current changes in one case as an example from 4.4 seconds to 3.8 seconds during stirring but since *t* enters the Ilkovic equation as the one-sixth power, this difference is insignificant compared with the increases found.

As previously discussed, there are two effects involved in stirring the Fe(CN)₆⁴⁻ ion. A breakdown in concentration polarization tends to decrease the current as a result of repulsion of the negative ions by the cathode charge. Stirring also transports more ions mechanically to the electrode which tends to increase the current.

(18) J. Maas, "De Polarografische Methode met de druppelnde kwickelektrode ten dienste van het Pharmaceutisch Onderzoek," Dissertation, Amsterdam, 1937., *Coll. Czech. Chem. Comm.*, **10**, 42 (1938).

(19) I. M. Kolthoff and J. J. Lingane, *Chem. Rev.*, **24**, 23 (1938).

(20) J. Heyrovsky, "Actualités scientifiques et industrielles," No. 90, Paris, 1934.

With strong stirring the latter effect predominates although the increase is appreciably less than for the reducible cations.

The vibration due to the thermostat stirrer motor was great enough to cause an easily observable though slight motion of the drop but the polarization layer was not greatly disturbed as shown by the data of Table III. Knowledge of this fact excludes any possibility that building vibrations play a part in the deviations reported in Table I.

Experimental

A Heyrovsky-Nejedly Polarograph, Type X, more commonly known as the Heyrovsky Micro Polarograph, was used in this work. The characteristics of the galvanometer in this instrument were: Sensitivity = 6.38×10^{-9} ampere per millimeter on the photographic drum located at a distance of 27.5 centimeters; coil resistance = 32 ohms; damping resistance = 256 ohms; period = 9 seconds. The response of the galvanometer was controlled by means of a constant damping multi-ratio shunt. An auxiliary circuit was incorporated to facilitate the checking of potentials and the calibration of the polarograms. The same technique which has since been described by Kolthoff and Lingane⁶ was used for the latter operation.

Cross section of Optical Cell

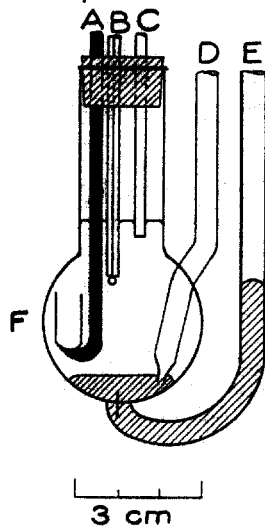


Fig. 3.

Both faces at F are made of plane pieces of Pyrex glass sealed in as integral parts of the cell. The optical cell was used for the stirring experiments. Due to the cylindrical

shape of this cell, a uniform circular flow of the solution was easily obtained.

The average swing of the galvanometer has been used in estimating the currents. Correction has been made for the residual current which has been determined in the same way and at the same potentials at which the diffusion current was observed. Maxima were suppressed with 0.1 ml. of a 0.1% solution of potassium methyl red added to 50 ml. of the test solution.

Since the thermostat could not be used when photographs were taken, the data in Table I for zinc, copper, and ferricyanide were obtained at a room temperature of 26°. The temperature did not change more than 0.2° during these experiments.

In the experiments with ferricyanide care was taken to minimize any reaction with the mercury. There was no contact between the solution and mercury until the curve was run; the point at which i_d was measured was reached in about two minutes after bringing the mercury and solution in contact.

Summary

1. Experimental deviations from the Ilkovic equation, previously reported by Kolthoff and Lingane in chloride solutions, have been checked in nitrate solutions. The postulation (a) of complex formation to explain the deviations for cadmium and for zinc ions and (b) of reaction with mercury to explain the deviation for ferricyanide ion has been shown to be unsatisfactory.

2. The possibility that irregular drop formation might account for the deviations from the Ilkovic equation was eliminated. In the specific cases observed, a spherical drop was formed which grew in mass at a uniform rate and broke away cleanly at the tip of the capillary, as the Ilkovic equation assumes.

3. Recalculation of the Ilkovic equation to account for a residual drop has shown that such a drop must be very large to affect appreciably the average current. This fact coupled with photographs of the drops establishes that the dropping electrode is mechanically satisfactory under all ordinary conditions.

4. The deviations from the Ilkovic equation have been shown to be to a large degree the result of the properties of the ions and of stirring effects produced by the dropping electrode.

5. The validity of the Ilkovic equation has been reaffirmed. In view of the effect of drop time on the deviations, capillaries with the slower drop rates should be employed whenever the data are to be used in the Ilkovic equation, as, for example, in the calculation of diffusion coefficients.

6. Although the calculated values of ionic diffusion coefficients are not exact, the uncertainties in these values are relatively small.

COLUMBUS, OHIO

RECEIVED NOVEMBER 20, 1942

[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

Chromatography as a Means of Separating Amino Acids¹BY JACQUES L. WACHTEL² AND HAROLD G. CASSIDY

The separation of several binary mixtures of amino acids was reported in a preliminary paper from this Laboratory.³ The method of separation used was that of Tswett-column adsorption analysis⁴ employing a special brand of commercial charcoal as the adsorbent. This method has now been extended to the separation of a quaternary mixture of amino acids, and this separation will be described, giving full details of the method. It is hoped to extend the method to the separation of more complex mixtures.

Other investigators have used adsorption analysis for the separation of amino acids. Whitehorn⁵ and Felix and Lang⁶ used permutit for the separation of the basic amino acids from the neutral and the acidic amino acids. Johnson⁷ separated glutamic acid from histidine on adsorption columns of titania gel. Tiselius,⁸ using a modified Tswett-column technique in combination with the Toepler schlieren method for following changes in refractive index, analyzed a mixture of alanine, valine and leucine. Martin and Synge⁹ described the separation of the acetyl derivatives of certain amino acids using a "chromatogram" employing two immiscible liquid phases one of which was supported on a solid phase.¹⁰

(1) Material for this paper was taken from the dissertation submitted by J. L. Wachtel to the faculty of the Graduate School, Yale University, 1942, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Present address: United States Department of Agriculture, Bureau of Agricultural Chemistry and Engineering, Northern Regional Research Laboratory, Peoria, Illinois.

(3) J. Wachtel and H. G. Cassidy, *Science*, **95**, 233 (1942).

(4) L. Zechmeister and L. Chohnoky, "Principles and Practice of Chromatography," John Wiley and Sons, Inc., New York, N. Y., 1941; H. H. Strain, "Chromatographic Adsorption Analysis," Interscience Publishers, Inc., New York, N. Y., 1942.

(5) J. C. Whitehorn, *J. Biol. Chem.*, **56**, 751 (1923).

(6) K. Felix and A. Lang, *Z. physiol. Chem.*, **182**, 125 (1929).

(7) Sr. M. B. Johnson, Dissertation, Catholic University of America, Washington, 1938.

(8) A. Tiselius, *Arkiv. Kemi, Mineral., Geol.*, **14B**, #22 (1940); **14B**, #32 (1941); **15B**, #6 (1941); *Science*, **94**, 145 (1941); "Advances in Colloid Science," ed. E. O. Kraemer, Interscience Publishers, Inc., New York, N. Y., 1942, pp. 81-97.

(9) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941).

(10) It is unfortunate that Martin and Synge⁹ have used the term "chromatography" to describe their new and ingenious method. The name chromatography has hitherto been used to describe processes

involving countercurrent adsorption of the Tswett-column type. In a "chromatogram" of the Martin and Synge type separations "depend upon differences in the partition between two liquid phases of the substances to be separated, and not, as in all previously described chromatograms, on differences in adsorption between liquid and solid phases." The method of Martin and Synge is analogous to chromatography in the sense that all separation processes which depend upon partitions are analogous; however, it appears more nearly to be a special kind of countercurrent liquid-liquid extraction process, in which one liquid phase is held substantially stationary. Indeed this is indicated by their "Theory of Chromatography." For the purposes of this theory their partition coefficient is assumed to be linear. It is well known that the "partition coefficients" of adsorption processes are not linear. It is true that their "theory of chromatography" could be made to describe an adsorption partition by suitable modification of the partition coefficient, but as mentioned above this is because of the general analogy between partition processes (see, for example, M. Randall and B. Longtin, *Ind. Eng. Chem.*, **30**, 1063 (1938)). It may be hoped that the term chromatography will not come into general use to describe the process of Martin and Synge, for this will lead to unnecessary confusion in terminology.

(11) See M. S. Dunn and A. Loshakoff, *J. Biol. Chem.*, **113**, 359 (1936).