

Sample II

A 0.1% solution of the L isomer which had stood 8 months.
 $[\alpha]_D +360^\circ$
 Cell constant, 0.3920
 Measured resistance, 1050 ohms
 Specific conductance, 3.74×10^{-4}
 Molar conductance, mhos/cm. 126.5

a Freas conductivity cell were used. The determinations were run at 18° .

TABLE I

These data are shown graphically in Fig. 1.

Wave length, Å.	Specific rotation [Co _D -pn ₂ CO ₂]Cl·2H ₂ O	Specific rotation [Co _L -pn ₂ CO ₂]Cl·H ₂ O
6360	+109°	-91°
6180	+75	-215
6000	+71	-273
5840	+62	-504
5680	+90	-594
5540	+100	-566
5420	+111	-536
5320	+127	-433
5230	+143	-380
5140	+160	-245
5060	+294	+92
4980	+400	+294
4900	+600	+550
4740	+758
4600	+94	+690
4450	+536

Werner¹⁵ carried out conductivity determinations on a series of coordination compounds to find the molar conduc-

(15) A. Werner, "Neuere Anschauungen auf dem Gebiete der anorganischen Chemie," Vieweg, Braunschweig, ed. 4, 1920, page 46; D. A. MacInnes, "The Principles of Electrochemistry," Reinhold Publishing Corp., New York, N. Y., 1939, p. 381.

TABLE II

These data are shown graphically in Fig. 1

Wave length, Å.	Extinction coefficient [Co _D -pn ₂ CO ₂]Cl·2H ₂ O	Extinction coefficient [Co _L -pn ₂ CO ₂]Cl·H ₂ O
3500	3.28	3.88
3550	3.54	4.08
3600	3.62	4.10
3700	3.26	3.72
3800	2.62	2.96
3900	1.96	2.20
4000	1.30	1.60
4100	0.84	1.02
4200	0.54	0.72
4300	0.52	0.66
4400	0.64	0.82
4500	1.02	1.18
4600	1.48	1.72
4700	2.08	2.38
4800	2.72	3.02
4900	3.34	3.62
5000	3.74	4.08
5080	3.92	4.30
5100	3.96	4.30
5160	3.88	4.22
5200	3.78	4.04
5300	3.38	3.78

tance values. He showed the values for solutions of 2 ion complexes to be in the neighborhood of 105. If, on standing in solution, the L isomer was aquated, rather than converted to the D isomer as the authors propose, the molar conductance would have been in the neighborhood of 450, the approximate value for a 4 ion complex. Even after standing 8 months the molar conductance values of the complex indicate only slight aquation.

URBANA, ILLINOIS

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Diffusion-Convection. A New Method for the Fractionation of Macromolecules

BY JOHN G. KIRKWOOD^{1a} AND RAYMOND A. BROWN^{1b}

A new method of fractionation of macromolecules in solution based upon the principles of the Clusius column is proposed. Horizontal transport of the macromolecular components in a vertical convection channel is produced by thermodynamic interaction with a diffusing low molecular weight substance to which the channel walls are permeable. Vertical convective transport under gravity is produced by the horizontal density gradient of the solution arising from the concentration gradient of the diffusible components. Preliminary experiments on the transport of serum albumin, which demonstrate the practical possibilities of the method, are described.

We wish to describe a new method of fractionation of macromolecules in solution, which we shall call diffusion-convection. Like the method of electrophoresis convection,² the new method is based upon the principles of the Clusius column. A solution of the macromolecular components is contained in two reservoirs connected by a vertical convection channel, the walls of which are impermeable to the passage of the macromolecules but permeable to a diffusible component of low molecular weight, the concentration of which is maintained at a level $C_1^{(1)}$ in an exterior reservoir on one side of the convection channel and at a concentra-

tion $C_1^{(2)}$ in a similar reservoir on the other side. The low molecular weight component, diffusing horizontally across the channel under its concentration gradient, produces by virtue of its effect on the density of the solution, a horizontal density gradient leading under the action of gravity to counter-current convective circulation in the channel between the top and bottom reservoirs. If the diffusible component interacts thermodynamically with a macromolecular component, the concentration gradient of the former will produce a horizontal chemical potential gradient of the latter. As a consequence, the macromolecular component will diffuse horizontally toward the channel wall at which its chemical potential is the lowest. Such transport may also be produced in part by co-diffusion arising directly from the chemical po-

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(2) J. R. Cann, J. G. Kirkwood, R. A. Brown and O. Plescia, THIS JOURNAL, 71, 1630 (1949).

tential gradient of the diffusible component. Superposition of horizontal diffusive transport on the vertical countercurrent convective circulation of the solution in the channel leads to differential transfer of the macromolecular components from the top to bottom reservoir at rates depending on their thermodynamic interaction with the diffusible component. As a result the top reservoir is enriched in the weakly interacting components and the bottom reservoir in the strongly interacting components.

Although general thermodynamic interaction suffices to produce the operation of the column, the process is perhaps more easily envisaged, if we suppose that one of the macromolecular components complexes strongly with the diffusible component. The macromolecular component in question then diffuses to the side of the channel at which the concentration of the diffusible component is high and descends or ascends in the channel in the form of the complex, depending upon the effect of the diffusible component on the density of the solution. In order to prevent blocking of the circulation between the channel and the bottom reservoir arising from the establishment of unfavorable vertical density gradients, it is important to provide a sink of diffusible component in the bottom reservoir. This may be accomplished by a connection through a semi-permeable membrane between the bottom reservoir and the exterior reservoir containing the diffusible component at low concentration.

A quantitative description of the operation of the diffusion-convection column becomes formally identical with that of the operation of the thermal diffusion column,³ under the condition the horizontal density gradient in the channel is controlled by the diffusible component alone and that this component diffuses as an ideal solute. This condition implies that the solution is dilute with respect to both the diffusible component and the macromolecular components and that the molar concentration of the latter is small relative to that of the diffusible component. A macromolecular component initially present at concentration C_0 is then transported according to the following relation expressing its concentrations C_T in the top reservoir and C_B in the bottom reservoir as functions of the time of transport, t

$$\frac{C_T}{C_0} = 1 - (1 - e^{-t/\theta}) \tanh \sigma$$

$$\frac{C_B}{C_0} = 1 + (1 - e^{-t/\theta}) \tanh \sigma \quad (1)$$

The characteristic time θ and the transport parameter σ are related to the volume V of each reservoir, the channel wall separation a , the channel length h , the channel width b , and the properties of the components of solution in the following manner

$$\sigma = \frac{252\eta_0 D h \beta_{21}}{\alpha_1 \rho_0 g a^4}$$

$$\theta = \frac{720\eta_0 V}{\alpha_1 \rho_0 g a^3 b \beta_{21}} \frac{\tanh \sigma}{(\partial C_1 / \partial x)^2} \quad (2)$$

(3) See L'Effet Soret, "Diffusion Thermique dans les Phases Condensées" by S. R. De Groot N. V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam, 1945.

where η_0 is the viscosity coefficient of the solvent, ρ_0 the solvent density, g the acceleration of gravity, D the diffusion coefficient of the macromolecular component, and $\partial C_1 / \partial x$ is the horizontal concentration gradient of the diffusible component. The coefficients α_1 and β_{21} are

$$\beta_{21} = - \left(\overline{\frac{\partial \log \gamma_2}{\partial C_1}} \right)_{T,p,C_1} \quad (3)$$

$$\alpha_1 = \left(\frac{\partial \rho}{\partial C_1} \right)_{T,p}$$

where γ_2 is the activity coefficient of the macromolecular component, the density of the solution and the bar denotes the horizontal average value of the derivative of the logarithm of the activity coefficient of the macromolecular component, at constant temperature, pressure, and concentrations of the macromolecular components.

With increasing time a stationary state is approached with a ratio of concentrations given by

$$C_B / C_T = (1 + \tan \sigma) / (1 - \tanh \sigma) \quad (4)$$

Although practical operating conditions would in general lie outside the range of applicability of equations (1), (2), (3) and (4), calculations based on them demonstrate that significant transport of a macromolecular component, which interacts strongly with the diffusible component should be realized under attainable conditions.

Experimental

Apparatus.—A modified electrophoresis-convection apparatus² has been used for a preliminary study of transport of a single component by diffusion-convection. The small feet at the bottom of the cell were removed and the cell inserted into a grooved box so constructed that the box was separated into two compartments by the cell. Separate circulation systems were used for each side of the box with buffer being introduced through tubes at the bottom of the box and overflowing through tubes at the top. The buffer in both reservoirs was changed every 24 hours. Better support of the membranes was provided by replacing the face plate struts with a honeycomb grid of one-half inch holes drilled in lucite. A sink for the diffusible component was established in the bottom reservoir by removing the reservoir wall opposite the tap and replacing it by a membrane gasketed in place by a lucite frame.

Materials.—In these experiments Armour crystalline bovine plasma albumin served as the macromolecular component and sodium chloride as the diffusible component. One circulation system contained phosphate buffer, ionic strength 0.01 and pH 7.6, to which had been added 0.15 mole of sodium chloride per liter and the other phosphate buffer without the sodium chloride. A buffer was thought necessary since the interaction of the components is strongly dependent upon the charge of the protein. A 1.2% solution of the protein was dialyzed overnight against the phosphate-chloride buffer before being placed in the apparatus. Transport of the protein was followed by removing small samples from the top reservoir for analysis.

Theory predicts θ 's of the order of 3 to 24 hours and presumably the apparatus should come to a steady state in several days. The fractionations were stopped after 9 days on the assumption that a steady state had been reached. The contents were removed from the top reservoir and the contents of the channel were combined with those of the bottom reservoir.

Ten per cent. of the protein was transported out of the top reservoir in an experiment with $a = 0.09$ cm. and 15% in an experiment with $a = 0.07$ cm. If one calculates an effective channel width, a^* , from the theory for these experiments using Scatchard's data⁴ for

$$\left(\frac{\partial \log \gamma_2}{\partial C_1} \right)_{T,p,C_1}$$

(4) G. Scatchard, A. C. Bacheider and A. Brown, *THIS JOURNAL*, **68**, 2320 (1946).

and appropriate values for the other parameters, he finds σ^* to be 0.18 cm. in the first experiment and 0.16 cm. in the second. Since there are a number of disturbing factors which will cause departure from the ideal theory the agreement with experiment must be considered good.

The amount of transport achieved in the experiments is small but significant. An apparatus with a narrower and longer channel should be capable of making practical separations.

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Conjugative Effects of Methylsulfonyl and Methylthio Groupings¹

BY F. G. BORDWELL AND GLENN D. COOPER

The effect of *m*- and *p*-CH₃SO₂ and CH₃S groups on the acid dissociation constants of benzoic acid, phenol and the anilinium ion have been determined. The necessity for using a larger Hammett sigma constant to express the effect of the *p*-CH₃SO₂ group on the acidity of phenol and the anilinium ion than for its effect on the acidity of benzoic acid is interpreted as evidence for an appreciable resonance effect (involving ten-electron structures). The CH₃S group resembles CH₃O in increasing the acidity of benzoic acid when substituted in the meta position and decreasing the acidity when in the para position, which points to a resonance effect for CH₃S similar to that for CH₃O but smaller in magnitude.

Chemical evidence, such as the ability of the CH₃SO₂ group to increase the acidity of α -hydrogens, its power in promoting addition of basic reagents to an α,β -carbon-carbon double bond, its strong accelerating effect on the loss of carbon dioxide from a β -carboxyl group, and its role as a meta director in aromatic substitution, suggests a close similarity in electronic effects between the CH₃SO₂ group and CH₃CO, CN, NO₂, etc., groups. Until recently it has been felt, however, that the CH₃SO₂ group differed in that it must exert its influence primarily through induction rather than by resonance. The recent physical evidence which may be interpreted as indicating considerable double bond character for the sulfur-oxygen bond in CH₃SO₂, etc., groups² has led to a reconsideration and renewed interest in the ability of the sulfur in such groups to expand its valence shell and to conjugate with other unsaturated groupings.

The present investigation of the acid dissociation constants of *m*- and *p*-CH₃SO₂ and CH₃S benzoic acids, phenols and anilinium ions was undertaken with two objectives in mind. First, the determinations would furnish quantitative data as to the electronic effects of CH₃SO₂ and CH₃S relative to that of other groups. Second, it should provide additional evidence as to the conjugative effect of CH₃SO₂, since it is known that para groups which can enter into strong resonance interaction with the -O⁻ and -NH₂ groupings, such as NO₂, increase the acidity of phenol and [the anilinium ion much more than would be expected from their acidifying effect on benzoic acid (where resonance is a minor factor).³ As a result of this resonance effect, two Hammett sigma constants are needed to describe the electronic effect of the *p*-NO₂ group (and similar groups) one for reactions of benzoic acids and other benzene derivatives, and one for reactions of phenols and anilines ($\sigma =$

0.778 and 1.27, respectively).⁴⁻⁶ The difference in sigma constants for the *p*-CH₃SO₂ group determined for benzoic acids vs. phenols and anilines should then be indicative of the ability of this group to conjugate with -O⁻ and NH₂. Determinations were also made for the CH₃CO group to serve as a comparison.

Experimental

Preparation of Materials.—The compounds used were, for the most part, either Eastman Kodak Co. White Label products, further purified by recrystallization or rectification, or compounds obtained by procedures given in the literature. In a few instances new synthetic routes were used, and these are given below.

***p*-Methylsulfonylbenzoic Acid.**—Twenty grams (0.145 mole) of methyl *p*-tolyl sulfide and 100 g. of potassium permanganate were refluxed in 800 ml. of water to which 10 ml. of 10% sodium hydroxide was added. After two hours the mixture was cooled, acidified with 96% sulfuric acid, and decolorized with sodium bisulfite. The precipitate was collected and dissolved in 200 ml. of 2 *N* sodium hydroxide solution; this solution was treated with charcoal and filtered. The filtrate was acidified and the colorless needles of *p*-methylsulfonylbenzoic acid were collected on a filter and recrystallized from glacial acetic acid; yield 18 g. (62%), m.p. (uncor.) 264–265°.

***m*-Methylsulfonylbenzoic Acid.**—*m*-Methylsulfonylbenzoic acid, m.p. 234–235° (uncor.), was obtained in 72% yield by oxidation of methyl *m*-tolyl sulfide by the procedure described above.

***p*-Methylsulfonylphenol.**—Eight and eight-tenths grams (0.05 mole) of *p*-methylsulfonylanisole was refluxed for two hours with 50 ml. of 48% hydrobromic acid. The solution was cooled and extracted with a total of 250 ml. of chloroform in 50-ml. portions. Evaporation of the chloroform left a white solid which on crystallization from benzene yielded 6.5 g. (40%) of colorless needles of *p*-methylsulfonylphenol, m.p. 95.5–96.5°.

***m*-Methylsulfonylphenol.**—One and seven-tenths grams (0.01 mole) of *m*-methylsulfonylaniline was diazotized and the diazonium salt decomposed in aqueous sulfuric acid according to the directions given for the preparation of *m*-nitrophenol.⁸ The reaction mixture was extracted with chloroform and the chloroform extracted with 5% sodium hydroxide solution. The sodium hydroxide solution was acidified and extracted with chloroform. Evaporation of the chloroform and crystallization of the residue from ben-

(1) This investigation was supported by the Office of Naval Research under Contract No. N7onr-45007. An account of this work was presented at the Conference on Organic Sulfur Compounds held at Indiana University, Aug. 29–Sept. 1, 1951.

(2) G. M. Phillips, J. S. Hunter and L. E. Sutton, *J. Chem. Soc.*, 146 (1945); D. Barnard, J. M. Fahian and H. P. Koch, *ibid.*, 2442 (1949).

(3) G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1941, pp. 257, 417.

(4) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chapter VII.

(5) Using $\sigma = 0.778$ and a rho value for phenols determined for groups having small resonance effects ($\rho = 2.0$), the calculated K_A for *p*-NO₂C₆H₄OH is less than one-tenth the observed value.

(6) H. Gilman, "Organic Syntheses," Coll. Vol. I. John Wiley and Sons, Inc., New York, N. Y., 1941, p. 404.