TABLE I

COLOR AND LOCATION OF SPOTS IN THE PAPER CHROMATOGRAM OF ESTROGENS

Compounds	Color of spots	*Rf* values in New solvent	*Rf* values in Old solvent[1]
Estradiol-17α[3]	Purple	0.32	0.96
Estradiol-17β	Purple	.09	.82
Estradiol-17α + estradiol-17β	Purple	.35 + 0.08	.96 + 0.83
Equilin	Purple	.49	.96
Equilenin[3]	Blue	.05	.80
Equilin + equilenin	Purple + blue	.45 + 0.05	.97 + 0.81
Estrone	Purple	.29	.96
Estriol	Purple	.00	.10

Discussion.—Paper partition chromatography is capable of resolving such closely related pairs as the epimeric estradiols and the equine estrogens equilin and equilenin, which differ only in the degree of saturation in ring B. The new solvent improves the separation of estradiol-17β from estrone and removes the latter farther from the yellow solvent front, but the estriol spot is not moved. This makes the identification of estriol difficult if other immobile pigments are present, as in urine. By using both the new and the old solvent mixtures in a two-dimensional chromatogram this difficulty can be avoided and the resolution further increased.

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(3) Provided by Ayerst, McKenna and Harrison, Ltd., through the courtesy of Dr. Gordon Grant.

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Specific Gravities of Ferrous Sulfate Solutions

BY HARRIS HOLTZMAN AND LEONARD MAY

In view of the variable degree of hydration of commercial grade ferrous sulfate heptahydrate crystals known as copperas, it is necessary that each individual lot be tested before use. The compositions of different lots of copperas may run as low as $FeSO_4 \cdot 3\text{-}4H_2O$ instead of $FeSO_4 \cdot 7H_2O$.

Routine evaluations of commercial salts such as copperas are most readily performed by use of specific gravity or Baumé tables for aqueous solutions. However, when the currently accepted tables for aqueous ferrous sulfate solutions are used,[1] we have found that the values for composition of the salt are approximately 9% in error over values as determined by oxidimetric titrations of the same solutions, lower values being obtained by the chemical analysis.

An investigation of the literature indicated that the values given in the "International Critical Tables"[1] were based almost entirely on work performed prior to 1900.[2,3,4] Except for some work at very low concentrations,[5,6] it would appear that chemical analytical procedures were not used. It is also possible that, in the very earliest work, the monohydrate ($FeSO_4 \cdot H_2O$), which breaks down only at high temperatures,[7] was mistaken for the anhydrous salt. Such a discrepancy would result in an error of 6.5% (based on $FeSO_4 \cdot 7H_2O$), or 11.8% (based on $FeSO_4$).

In view of the discrepancies encountered in our laboratory, we decided to set up a specific gravity *vs.* concentration curve based on new analytical data.

Experimental Work.—Baker C.P. ferrous sulfate heptahydrate was used for this work. Analyses were run, using 0.100 *N* $KMnO_4$ and $K_2Cr_2O_7$ solutions for oxidation of ferrous iron. Titanous chloride titrations indicated less than 0.01% ferric iron, so that direct titrations with $KMnO_4$ or $K_2Cr_2O_7$ gave the total amount of iron without significant error. The differences between permanganate and dichromate titrations were less than 1 part in 400. Specific gravity determinations using pycnometer and hydrometer were made on solutions of several concentrations, temperature being maintained at 18°.

Results.—Titrations indicated that the C.P. ferrous sulfate was 99.0% pure, as $FeSO_4 \cdot 7H_2O$.

Table I, column A gives the concentration of $FeSO_4 \cdot 7H_2O$, in g. per liter at 18°, by actual analysis. Column D gives the concentration of $FeSO_4 \cdot 7H_2O$, in g. per liter at 18°, on the basis of "I.C.T." values,[1] and column E indicates the percentage error in composition introduced by use of "I.C.T." or chemical handbook tables.

TABLE I

A $FeSO_4 \cdot 7H_2O$ g. per l. by analysis	B Specific gravity 18°/18°	C Baumé = 145 − 145/B	D $FeSO_4 \cdot 7H_2O$ g. per l. ("I.C.T." values)	E % error in "I.C.T." tables
180.5	1.099	13.0	198.0	+ 9.9
198.2	1.107	14.0	215.2	+ 8.6
215.4	1.115	15.0	232.6	+ 8.0
230.5	1.124	16.0	250.2	+ 7.8
244.2	1.133	17.0	268.9	+10.1
			Av. deviation	+ 8.9

From these results, it is evident that the values given in the literature for specific gravities of ferrous sulfate solutions are in error, particularly for ranges around 10 to 20% or higher.

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(1) "International Critical Tables," Vol. III, 1929. These values have been repeated and recalculated in terms of Baumé readings in several handbooks.

(2) Klein, *Ann. Physik*, **27**, 151 (1886).

(3) Gerlach, *Dinglers Polytechnisches Journal*, **181**, 129 (1866).

(4) Gerlach, *Z. Anal. Chem.*, **8**, 245 (1869).

(5) Trotsch, *Ann. Physik*, **41**, 259 (1890).

(6) Pasea, *Proc. Roy. Soc. Can.*, **6-III**, 27 (1900); MacGregor, *Chem. News* (Nov. 7, 1890).

(7) Mellor, "Treatise on Inorganic Chemistry," Vol. 14, p. 248.

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Paper Partition Chromatography of Simple Phenols[1]

BY RALPH L. HOSSFELD

The application of paper partition chromatography to the qualitative separation of phenolic compounds has been reported.[2–5] However, the results indicated that the method did not effectively separate mixtures of simple phenols of closely

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(1) University of Minnesota Agricultural Experiment Station Scientific Journal Series Paper No. 2584.

(2) R. A. Evans, W. H. Paar and W. C. Evans, *Nature*, **164**, No. 4172, 675 (1949).

(3) E. C. Bate-Smith and R. G. Weltall, *Biochim. Biophys. Acta*, **4**, 427 (1950).

(4) A. E. Bradfield and E. C. Bate-Smith, *ibid.*, **4**, 441 (1950).

(5) G. Lindstedt, *Acta Chem. Scand.*, **4**, 448 (1950).

related structure. The adsorption chromatography of the azo dyes derived by coupling simple phenols with diazotized *p*-nitroaniline was reported by Bielenberg[6] who also found that practical separations were limited to relatively few simple mixtures.

In the course of studying the phenolic degradation products of lignin[7] an urgent need was felt for a supplementary technique to fractional distillation which would increase the ease and thoroughness of identification of the constituents of the complex mixtures. It has been found that satisfactory qualitative separation of many simple phenols can be accomplished by the application of paper partition chromatography to a mixture of the sodium salts of phenyl azo dyes derived by coupling the phenols, pure or in mixture, with diazotized sulfanilic acid.

In preliminary experiments, dyes were prepared by coupling *o*-cresol with diazotized sulfanilic acid, anthranilic acid, *p*-nitroaniline and *β*-naphthylamine-6-sulfonic acid. The dyes were then chromatographed both as the free acids and as the sodium salts. In the first instance, untreated Whatman No. 1 filter paper was used, and in the second the paper was first sprayed to fiber saturation with 4% sodium carbonate and dried before spotting. *n*-Butanol–water, methyl ethyl ketone–water and *n*-butanol (5 vol.)–water (5 vol.)–ethanol (1.5 vol.) were studied as irrigating solvents.

Of these various combinations of experimental conditions the development of the sodium salt of *o*-cresyl-azo-benzenesulfonic acid on the carbonate treated paper using methyl ethyl ketone–water proved most effective. Spots developed with methyl ethyl ketone–water showed less tendency to trail than when developed with other solvents, and development of the dyes as the sodium salts both reduced the tendency to trail and resulted in a more favorable spread of R_f values.

The separation of dye mixtures was observed both by superimposed spotting of the individual dyes at a single location on the paper, and by spotting the mixed dye resulting from the coupling of a mixture of phenols with the diazotized sulfanilic acid. In the latter case, it was not always possible to isolate the mixed dyes from the aqueous reaction mixture. In these instances the reaction mixture, after acidification, was extracted with a suitable solvent such as benzene or chloroform to remove the unreacted phenol; then a small portion of the aqueous solution was diluted with alcohol and used in spotting the paper strip.

The results of this technique as it was applied to a mixture of phenol, guaiacol and *o*-cresol are illustrated in Fig. 1. Figure 2 illustrates the degree of differentiation which is possible with guaiacol, phenol, *o*-cresol, *p*-cresol and 2-hydroxy-1,4-dimethylbenzene. It is apparent, however, that mixtures of 4-hydroxy-1,2-dimethylbenzene with *p*-cresol,

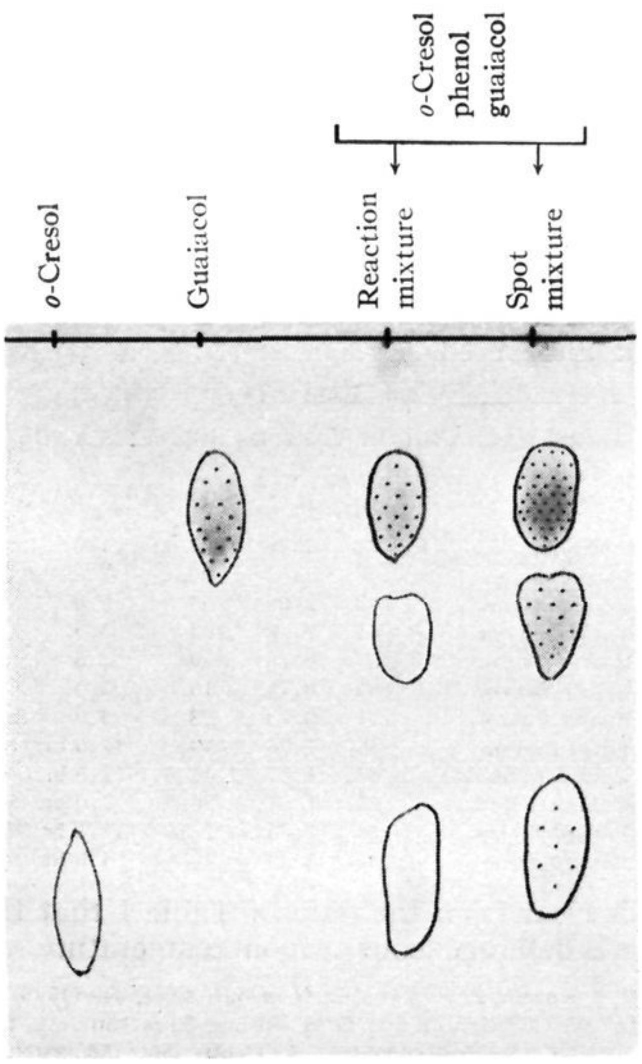

Fig. 1.—*n*-Butanol–H_2O.

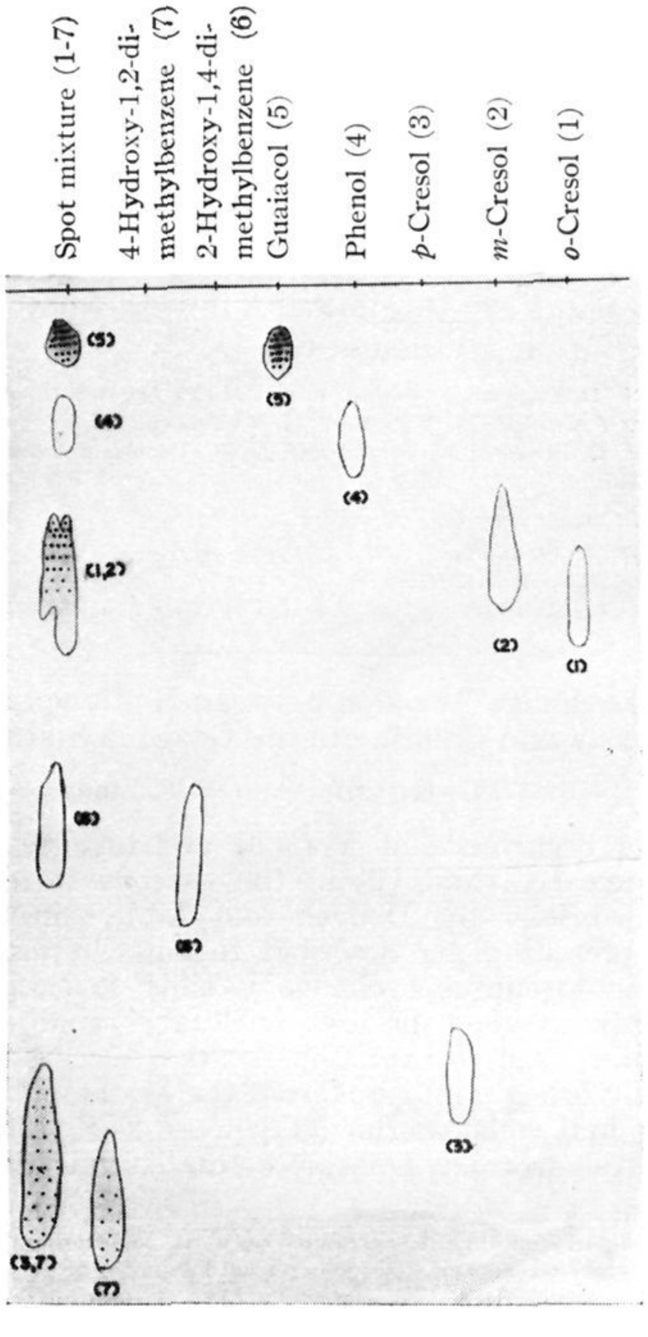

Fig. 2.—Methyl ethyl ketone–H_2O.

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[6] W. Bielenberg and L. Fischer, *Brennstoff-Chem.*, **23**, 283 (1942).

[7] D. L. Brink, R. L. Hossfeld and W. M. Sandstrom, THIS JOURNAL, **71**, 2275 (1949).

and of *o*-cresol with *m*-cresol were not separated under the conditions of these experiments.

Experimental

Preparation of Azo Dyes.—The method of Cheronis[8] was followed using 0.01 molar quantities of reactants. In many cases it was found that the dyes could be recovered in almost pure state by acidifying the final reaction mixture and filtering the product. The dyes so recovered were recrystallized from alcohol–benzene or alcohol alone as required. In other cases the dyes could not be isolated in crystalline form either as the free acid or by salting out of the sodium salts. The reaction mixtures containing these dyes were treated in the same manner as the mixtures described below.

The preparation of dyes from phenol mixtures was accomplished by the same method except that no attempt was made to isolate the crystalline products. The resulting reaction mixture was acidified with an excess of hydrochloric acid and extracted with benzene or chloroform to remove unreacted phenol. A small portion (1–3 drops) of the aqueous solution was then diluted with 2 ml. of ethanol and spotted, by means of a small platinum wire loop, at the appropriate position on the paper strip.

Development of the chromatograms was accomplished by the usual methods with an apparatus similar to that of Steward,[9] consisting of a stainless steel trough (2.25 × 1.5 × 6.5 in.) suspended by brackets from the lugs in the top of a conventional museum specimen jar (20 × 60 cm.). The irrigating solvents (*vidi supra*) were prepared by thoroughly shaking the mixture in a separatory funnel and allowing to stand until completely separated. The organic phase was used for irrigation. The paper strips (14 × 56 cm. Whatman No. 1 filter paper) were first sprayed to the point of fiber saturation with aqueous 4% sodium carbonate, dried, spotted with the dye and suspended from the trough in the jar. They were allowed to remain undisturbed for one hour before adding the irrigating solvent in order to allow them to attain equilibrium with the aqueous phase of the solvent mixture which had previously been placed in the bottom of the jar.

Acknowledgment.—The author wishes to express appreciation of a Grant-in-Aid of Research by the Graduate School of the University of Minnesota. Thanks are also due Mr. Wayne L. Meek for preparation of the dyes.

(8) N. D. Cheronis, "Semimicro and Macro Organic Chemistry," Thomas Y. Crowell Co., New York, N. Y., 1942, p. 285.

(9) F. C. Steward, W. Stepka and J. F. Thompson, *Science*, **107**, 451 (1948).

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The Exchange Reaction between Hydrogen Chloride and Chlorine in the Gaseous State

BY W. H. JOHNSTON[1] AND W. F. LIBBY

The occurrence of a rapid exchange reaction between HCl and Cl_2 in the gaseous state was noted briefly by Dodgen and Libby[2] in 1949. The present paper describes the discoveries that the homogeneous exchange is slow, is catalyzed by ordinary glass surfaces, and that a rapid photochemical exchange reaction occurs.

Anhydrous tank gases from the Matheson Company and radiochlorine-36 ($t_{1/2} = 2 \times 10^6$ yr.) from the Isotopes Division of the Atomic Energy Commission were used. The gases were measured with a Bourdon gage in a known volume of Pyrex vacuum line. The reactions were carried out generally in 500-cc. Pyrex flasks some of which were coated with a mixture of saturated long-chain fluorocarbons (du Pont perfluorolube oil, FCD 335). Following the reaction the mixed gases were expanded into a trap at liquid nitrogen temperature and separated by distillation under vacuum with a bath of ethanol and water at −130°. The HCl^{36} and $ClCl^{36}$ were counted in a cylindrical annular volume surrounding a Geiger counter. The specific activities of the initial gases were measured in units of counts per minute per millimole of "Cl." Pure chlorine free of HCl was obtained by the distillation following the reaction and its specific activity was measured in the same manner. The over-all pressure balance and activity balance were measured and were found to be between 90 and 100%. The extrapolation of the data to zero exchange at zero time was a further check on the separation.

In the photochemical studies nearly monochromatic light of 3650 Å. wave length was obtained with a medium pressure mercury arc (G. E. lamp, AH-4) and appropriate filters. Relative intensities were obtained with a Bowen type Photometer[3] and 931-A photomultiplier tube. Absolute intensities were measured with the uranyl oxalate actinometer[4] and, independently, with a thermopile and moving coil galvanometer.[5] All readings were referred to a reference light source which was known to be stable. All photochemical studies were done in a fluorocarbon coated vessel where the rate of the dark reaction was negligible by comparison.

Most of the experimental data on the dark exchange reaction are shown in Table I. The sparsely covered surface was a Pyrex glass bulb which was uniformly spotted with very small droplets of the fluorocarbon. The largely covered surface had a maximum of four per cent. of the Pyrex uncovered.

TABLE I

DARK EXCHANGE IN VARIOUS SURFACES AT 25°

Run	Surface	HCl m./l. × 10^3	Cl_2 m./l. × 10^3	Exchange, %	Time, min.	Half-time, min.
6	Pyrex	0.52	1.48	64	4.0	3
7	Pyrex	.46	1.54	90	7.5	3
8	Packed pyrex	.43	2.26	100	9.0	2
9	Packed pyrex	.45	2.29	103	5.0	2
10	Sparse fluoro.	.83	0.78	9	2.5	19
11	Sparse fluoro.	.85	0.74	16	5.0	20
12	Sparse fluoro.	.81	0.73	28	9.0	21
5	Large fluoro.	.88	1.66	49	11.0 hr.	11 hr.
1	Complete fluoro.	.85	1.52	42.5	12.5 hr.	16 hr.
2	Complete fluoro.	.87	1.64	26.5	7.0 hr.	16 hr.
3	Packed fluoro.	.88	1.35	78.5	11.1 hr.	5 hr.
4	Packed fluoro.	.87	1.36	43	4.0 hr.	5 hr.

It is clear from the data of Table I that the reaction is heterogeneous at room temperature.

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(1) Atomic Energy Commission Predoctoral Fellow. This paper is taken in part from the thesis presented by W. H. Johnston to the Faculty of the University of Chicago in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Present address: Department of Chemistry, Purdue University, Lafayette, Indiana.

(2) H. Dodgen and W. F. Libby, *J. Chem. Phys.*, **17**, 951 (1949).

(3) E. J. Bowen, *Proc. Roy. Soc.* (*London*), **A154**, 349 (1936).

(4) (a) W. G. Leighton and G. S. Forbes, THIS JOURNAL, **52**, 3139 (1930); (b) G. S. Forbes and L. J. Heidt, *ibid.*, **56**, 2363 (1934); (c) L. J. Heidt and F. Daniels, *ibid.*, **54**, 2384 (1932).

(5) (a) A. Farkas and H. W. Melville, "Experimental Methods in Gas Reactions," Macmillan, Ltd., London, 1939, pp. 250–254. (b) J. D. Strong, "Procedures in Experimental Physics," Prentice-Hall, Inc., New York, N. Y., 1938, pp. 305–341.