TABLE 1

COLOR AND LOCATION OF SPOTS IN THE PAPER CHROMATOGRAM OF ESTROGENS

Compounds	Color of spots	*Rf* values in New solvent	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.

(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.

(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]

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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

(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).