

An air-dried sample thus prepared contained 0.015% ash, and its rotation, after being completely dehydrated, was

$$[\alpha]_D^{20} = 123.23 \text{ (10.0136 g. per 100 cc.)}$$

$$[\alpha]_{\lambda 5461}^{20} \text{ A. u.} = 144.95 \text{ (10.0136 g. per 100 cc.)}$$

### Summary.

An improved method for preparing raffinose from cotton-seed meal has been developed.

Cotton-seed meal is extracted by percolation. The extract is purified with basic lead acetate and the excess of lead removed with oxalic acid. The sugar is then removed from the solution as the insoluble calcium raffinosate. To regenerate the raffinose this compound is decomposed with carbon dioxide. The resulting solution is evaporated under diminished pressure to 70–75% total solids and crystallized by the addition of alcohol.

A device is described for the convenient and rapid carbonation of the raffinosate; and other uses for this apparatus are suggested.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH.]

## THE SIGNIFICANCE OF THE ISO-ELECTRIC POINT FOR THE PREPARATION OF ASH-FREE GELATIN.

By JACQUES LOEB.

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Three years ago the writer published a paper on "the significance of the iso-electric point for the purification of amphoteric colloids"<sup>1</sup> in which it was shown that amphoteric colloids could be rendered ash-free in a simple and quick way by bringing the powdered ampholyte, *e. g.*, gelatin, to the iso-electric point and then washing a sufficient number of times with cold water. This method was based on the writer's observation that at the iso-electric point an amphoteric electrolyte like gelatin cannot combine with either anion or cation, and hence if iso-electric gelatin in powdered form be washed sufficiently all the ash formerly in combination with the gelatin can be removed. The usual procedure for preparing practically ash-free gelatin used in the writer's laboratory is as follows.

Fifty g. of commercial powdered Cooper's gelatin, which happened to possess a Sørensen value ( $P_H$ ) 6.0 to 7.0, is put into 3000 cc. of 0.0078 *M* acetic acid in a jar at 10°, and stirred frequently. After 30 minutes the supernatant liquid is decanted and fresh 0.0078 *M* acetic acid at 10° added to equal the original volume. The mass is frequently stirred and after 30 minutes the acid is again decanted and replaced by an equal volume of distilled water at 5° and a hydrogen-ion concentration a little above  $P_H$  5.0. The gelatin is well stirred and then filtered by suction through towel cloth in a Büchner funnel. It is then washed in the funnel 5 times each with 1000 cc. of water at 5°. After all the water is drained off the gelatin is transferred from the

<sup>1</sup> Loeb, *J. Gen. Physiol.*, **1**, 237 (1918–19).

Büchner funnel into a large beaker which is then heated in a water-bath to about 50° till the gelatin is melted.

The concentration of the gelatin is determined by evaporating to dryness 10 cc. of the melted gelatin in an electric oven at 90° to 100° for 24 hours.

A sample of such gelatin was taken at random and the ash determined by Dr. Hitchcock of this laboratory. The stock solution of gelatin contained 12.69% of gelatin. The results of the analysis are as follows.

	Sample 1	Sample 2
Volume of solution, cc. ....	20	10
Weight of dry gelatin, g. ....	2.535	1.269
Weight of ash, g. ....	0.0024	0.0012

Obtained qualitative tests for  $\text{Fe}^{+++}$ ,  $\text{Ca}^{++}$ , and  $\text{PO}_4^{\equiv}$ ; negative tests for  $\text{Cl}^-$  and  $\text{SO}_4^{\equiv}$ .

In the writer's experiments on swelling, osmotic pressure, and viscosity, 1 g. of originally iso-electric gelatin was as a rule contained in 100 cc. of solution or liquid. The ash content in this solution might have been about 1 mg. It was shown in the writer's experiments that that amount of ash (which equals roughly a 0.000033 *M* solution of tricalcium orthophosphate) has no influence on the physical properties of the proteins, such as osmotic pressure, swelling, viscosity, or potential difference.<sup>2</sup>

Miss Field<sup>3</sup> has shown that by carrying the washing process a step further, the last traces of ash can be removed from the powdered gelatin.

I had used the term "free from ionogenic impurities" instead of "ash-free" to signify the theoretical justification for the method. That my method was intended to yield ash-free gelatin was correctly understood by J. H. Northrop who used it for this purpose in his work on the digestion of gelatin<sup>4</sup> as well as by Miss Field who had seen me before the publication of her paper.

In a paper recently published in THIS JOURNAL, C. R. Smith<sup>5</sup> states that my results on the osmotic pressure and swelling of gelatin were vitiated by the fact that my gelatin solutions were not ash-free. This is contrary to fact as far as my papers published during the last three years are concerned. Smith refers to older papers published by me before September 1918 in the *Journal of Biological Chemistry*, and adds in the reference "also recent articles in *Am. J. Expt. Physiol.*" This is an error, since my papers on proteins during the last three years were published in the *Journal of General Physiology*, *Science*, and *Journal de chimie physique*.<sup>2</sup>

The values for the osmotic pressure of solutions containing 1 g. of originally iso-electric gelatin in 100 cc. are given in a paper published in the

<sup>2</sup> Loeb, *J. Gen. Physiol.*, 1, 39, 237, 363, 483, 559 (1918-19); 2, 87 (1919-20); 3, 85, 247, 391, 547, 557, 667, 691, 827 (1920-21); 4, 73, 97 (1921-22). *Science*, 42, 494 (1920). *J. chim. phys.*, 18, 283 (1920).

<sup>3</sup> Field, THIS JOURNAL, 43, 667 (1921).

<sup>4</sup> *J. Gen. Physiol.*, 3, 715 (1920-21).

<sup>5</sup> Smith, THIS JOURNAL, 43, 1350 (1921).

May number of the *Journal of General Physiology*. Former values were lower since the solutions contained less than 1 g. in 100 cc., usually 0.8 g., as was pointed out in a paper published in January, 1921, in the same *Journal*.

I have recently shown that the osmotic pressure of a gelatin solution of a given hydrogen-ion concentration is not a constant, since it diminishes on standing on account of the formation of aggregates.

NEW YORK CITY.

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### NOTES.

**Synthesis of Indigo from Fumaric Acid and Aniline.**—In the "Text-Book of Dye Chemistry" by G. von Georgevics and Grandmougin<sup>1</sup> there is the statement that indigo has never been obtained by fusion with potash followed by air oxidation from dianilido-succinic acid  $C_6H_5.NH.CH(COOH)CH(COOH).NH.C_6H_5$ . In the course of some work on the compounds derived from maleic acid the following experiments were made which show that appreciable yields of indigo may be obtained from this compound.

**Preparation of Dibrom-succinic Acid.**—The method given by Michael<sup>2</sup> was used. Thirty g. of fumaric acid, 41.4 g. of bromine, and 26 cc. of glacial acetic acid were heated in a sealed tube at 100° for 7 hours. The contents of the tube were washed into an evaporating dish with a little acetic acid and evaporated to dryness. The average yield on three runs was 96.5%.

For the preparation of dianilido-succinic acid the method given by Reissert<sup>3</sup> was followed. The yields were 37%, 38.7% and 41.5% of the theoretical.

**Fusion of Dianilido-succinic Acid to Indigo.**—Vorländer<sup>4</sup> recorded that he secured indigo in 3 out of 40 or 50 trials by fusing the above acid with potassium hydroxide. No yields were given. The fusion of the free dianilido-succinic acid by us with a molecular mixture of potassium and sodium hydroxide (9.6 parts to 1 part of acid) in a small crucible at 450° for 1½ hours followed by air blowing failed to give a trace of indigo. Similar negative results were obtained in a closed agitated pot with the mixed caustic. However, indigo was obtained by the following procedure. A molecular mixture (300 g.) of potassium and sodium hydroxides was dehydrated at 450° for 2½ hours in a closed agitated iron pot. Thirty g. of sodamide was then added and a stream of dry ammonia passed through

<sup>1</sup> Scott, Greenwood and Co., London, 1920. Trans. from 4th German ed. by F. A. Mason.

<sup>2</sup> Michael, *J. prakt. Chim.*, [2] 52, 295 (1851).

<sup>3</sup> Reissert, *Ber.*, 26, 1763 (1893).

<sup>4</sup> Vorländer, *Ber.*, 27, 1604 (1894).