

butylene, etc., will react with benzene under the same general conditions and it is intended to take up the study of these in this Laboratory.

### Summary.

1. By the aid of intensive stirring ethylene can be made to react with benzene in the presence of aluminum chloride so rapidly and completely that this becomes a practical method for the ethylation of benzene.

2. Naphthalene can be readily ethylated by heating it with poly-ethylbenzenes and aluminum chloride under intensive agitation.

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[CONTRIBUTION FROM THE POLARIMETRY SECTION, BUREAU OF STANDARDS, U. S. DEPARTMENT OF COMMERCE.]

## AN IMPROVED METHOD FOR PREPARING RAFFINOSE.<sup>1</sup>

BY E. P. CLARK.

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Several methods have been proposed for the preparation of raffinose.<sup>2</sup> All these procedures, however, with the exception of the method of Hudson and Harding, as these authors have well pointed out, have many serious disadvantages. The latter method, while it is a decided improvement over all others, and gives fairly good results on very small lots, nevertheless has objectionable features when applied to the preparation of larger quantities of the sugar. Chief among these may be mentioned the tedious procedure in extracting the meal, the necessity of evaporating large quantities of water, due to the excessive dilution of the extract, and the quantity and cost of the reagents required. The following method overcomes these disadvantages and enables one to prepare relatively large quantities of this sugar by a convenient and economical process.

Five kg. of coarsely ground cotton-seed meal is thoroughly moistened with 2 liters of water and allowed to stand overnight. It is then loosely packed in a cylindrical percolator, and sufficient water added to saturate the meal and leave a stratum above it. When the liquid begins to run from the percolator, more menstruum is added, from time to time, until a sample of the percolate, after defecation with dry basic lead acetate, has an optical rotation of not more than 1 circular degree in a 2dcm. tube.<sup>3</sup>

<sup>1</sup> Published by the permission of the Director, U. S. Bureau of Standards.

<sup>2</sup> von Lippmann, "Die Chemie der Zuckerarten," Friedrich Viewig und Sohn, Braunschweig, 1904, 3rd ed., pp. 1627-1630. Zitkowsky, *Am. Sugar Ind.*, 12, p. 324 (1910). Hudson and Harding, *THIS JOURNAL*, 36, 2110 (1914).

<sup>3</sup> As there are substances in the meal which are extracted much more slowly than the sugar, and which cause difficulty in the subsequent steps, as well as give an inferior product, it is essential to obtain a quite rapid percolation, consuming not more than 30 to 35 minutes; and it is not expedient to carry the extraction beyond where the optical rotation of the liquid is less than 1° in a 2dcm. tube.

The process is then stopped, and the percolate is treated with a solution of basic lead acetate until no more precipitate is formed. The yellow precipitate is filtered upon large folded filters and finally washed on the filter with a little water. The filtrate and washings, which should have a volume of 12 to 13 liters, are freed from the excess of lead with oxalic acid. The lead oxalate is removed and washed on the filter with a little water. The filtrate and washings are thoroughly mixed, measured, and the optical activity of the liquid determined. It is then made distinctly alkaline to litmus with sodium hydroxide. The precipitate thus formed flocks out and settles to the bottom of the vessel in a few minutes. The supernatant liquid is next filtered through a Büchner funnel provided with a thin layer of decolorizing carbon on filter paper. The filtration is rapid under these conditions, and when all has passed through, the precipitate is placed on the filter and drained.

The raffinose is next removed from the solution by forming the insoluble calcium raffinosate. To conveniently accomplish this, the liquor is cooled to  $10^{\circ}$ , or lower, placed in a jar or other suitable container, and rapidly stirred with a mechanical stirring device. As it is being agitated, a quantity of powdered active lime, preferably 200 mesh, but not coarser than 100 mesh, sufficient to precipitate all the sugar, is slowly sifted in. After all has been added, the stirring is continued for about 5 minutes. If the lime is active, and the optical activity of the solution is calculated to raffinose hydrate with a specific rotation of about  $105^{\circ}$ , 1 g. of lime to 1 g. of raffinose is sufficient for complete precipitation. However, unless the activity of the lime is known, it is advisable to test the liquid to see whether all the sugar has been removed; if not, more lime should be added. The calcium raffinosate is filtered off, washed by grinding up to a smooth paste with 2.5 liters of cold lime water, and again filtered.

It is next carbonated to neutrality. Emphasis is to be laid upon accomplishing this easily and quickly. The device illustrated in Fig. 1 was used for this purpose.<sup>4</sup> The cake of raffinosate is placed in a deep,

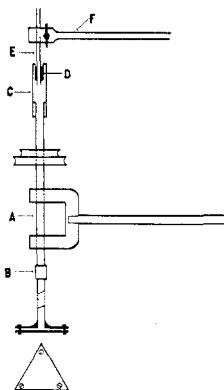


Fig. 1.

<sup>4</sup> The design is essentially that developed by Mr. C. W. Doherty of the Great Western Sugar Company for carbonating beet juices. It consists of an ordinary stirring apparatus that may be clamped to any rigid laboratory support stand. The stirring shaft, A, is made of steel tubing 8 mm. inside diameter. Just below the shaft support is a union, B, to which is joined another section of shafting of the desired length. For most laboratory work, a length of 45 cm. is sufficient. Attached to the lower end are 2 triangular plates. These plates, which are 2 mm. thick and whose sides are 75 mm. long, are held 2 mm. apart by 3 rivets near the apex of the angles. The plate attached

narrow can of about 8 liters' capacity and filled  $3/4$  full of water at  $50^{\circ}$ , and a lively stream of carbon dioxide is passed through the apparatus while it is being turned about 1500 r. p. m. In this way the gas is centrifugally distributed and neutralizes the lime in 4 or 5 minutes without any loss of carbon dioxide. The solution is filtered while hot and the precipitate is washed by grinding up with 2 liters of water and again filtering. The combined liquors are evaporated under diminished pressure to 70–75% total solids and warmed to about  $60^{\circ}$ . To this syrup 95% ethyl alcohol is added just to saturation. The alcoholic solution is then warmed on the water-bath to about  $60^{\circ}$  and filtered through a small Büchner funnel in which has been placed a mat of washed asbestos. The filtrate, which is brilliantly clear, is seeded and placed in the ice box to crystallize. Two days are generally sufficient for complete crystallization.

The raffinose is filtered from the mother liquor, washed, first with 80%, then 95% alcohol, and dried. The yield varies considerably, according to the amount of sugar in the meal; but from a number of different experiments in which different meals were used, yields from 2.3% to 4% were obtained. The crude sugar thus prepared is quite pure, containing only from 0.06 to 0.08% ash.

To purify crude raffinose, a 40% solution (anhydrous sugar) is made by dissolving it in distilled water at  $70^{\circ}$ . The warm solution is filtered through a mat of asbestos and placed in the ice-box to crystallize. The crystals are freed from mother liquor, washed with 80%, then 95% alcohol, and dried. A sample of air-dried material thus purified contained 0.005% ash.

An alternative method of recrystallization is to concentrate the above 40% solution under reduced pressure to 70% total solids, warm to  $70^{\circ}$ , and add two volumes of 95% alcohol with constant stirring. Crystallization begins almost at once and is complete in a few hours. The crystals are filtered off, washed with 95% alcohol, and dried.

to the shafting has an opening connecting with the stirring rod. The bottom plate has no opening. C is a stuffing box made by simply attaching to the stirring shaft a short piece of good rubber tubing in which is placed a well oiled, closely fitting cork cylinder, D, through which a piece of glass tubing, E, is inserted. The glass tube is held rigid by means of a clamp, F. The cork thus arranged may be made gas-tight, and still turn freely, by tightening the rubber tubing about it with a wire.

Incidentally, this apparatus may be used to advantage for a number of purposes. In the work described in this paper, when any stirring was done, or when precipitates were ground up with water in order to wash them, the stuffing box attachment was removed and the upper orifice of the turning shaft corked. The precipitate was placed in a suitable container with the liquid used to wash it and the apparatus turned for 1 or 2 minutes. At the end of this time, the mixture was a perfectly smooth paste, all lumps being completely broken up. It has also been used very effectively in decomposing lead precipitates with hydrogen sulfide. Quantities of lead precipitates, which would normally require from 3 to 4 hours for complete decomposition, have been decomposed completely in 15 to 20 minutes.

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.

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[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).