Organic Syntheses

A Publication
of Reliable Methods
for the Preparation
of Organic Compounds

Working with Hazardous Chemicals

The procedures in *Organic Syntheses* are intended for use only by persons with proper training in experimental organic chemistry. All hazardous materials should be handled using the standard procedures for work with chemicals described in references such as "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at http://www.nap.edu/catalog.php?record_id=12654). All chemical waste should be disposed of in accordance with local regulations. For general guidelines for the management of chemical waste, see Chapter 8 of Prudent Practices.

In some articles in *Organic Syntheses*, chemical-specific hazards are highlighted in red "Caution Notes" within a procedure. It is important to recognize that the absence of a caution note does not imply that no significant hazards are associated with the chemicals involved in that procedure. Prior to performing a reaction, a thorough risk assessment should be carried out that includes a review of the potential hazards associated with each chemical and experimental operation on the scale that is planned for the procedure. Guidelines for carrying out a risk assessment and for analyzing the hazards associated with chemicals can be found in Chapter 4 of Prudent Practices.

The procedures described in *Organic Syntheses* are provided as published and are conducted at one's own risk. *Organic Syntheses, Inc.*, its Editors, and its Board of Directors do not warrant or guarantee the safety of individuals using these procedures and hereby disclaim any liability for any injuries or damages claimed to have resulted from or related in any way to the procedures herein.

These paragraphs were added in September 2014. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

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CASEIN

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1. Procedure

To 1 l. of milk, from which the cream has been largely separated (Note 1), 0.05 *M* hydrochloric acid is slowly added, with stirring, through a capillary tube extending to the bottom of the beaker. The addition is continued until the solution attains a *p*H of 4.6 (Note 2). The end point is determined by withdrawing 5-cc. samples, diluting to 50 cc., adding methyl red, and matching against a buffered series (Note 3). Approximately 1 l. of acid is required; the separation of the casein is practically complete at this point. Three liters of water is then added, stirring is discontinued, and the flocculent precipitate of casein is allowed to settle in the refrigerator for twelve to twenty-four hours. The clear supernatant liquid which contains soluble proteins and salts is removed as completely as possible by siphoning; the precipitate is collected on a suction funnel and washed with cold distilled water until the washings are free of calcium (give no precipitate with ammonium oxalate).

The casein, which is contaminated with calcium phosphate and fats, is filtered to as small a volume as possible (about 500 cc.) and transferred to a 2-l. beaker. It is then treated with 0.1 *M* sodium hydroxide, the alkali being added slowly and with stirring through a capillary extending to the bottom of the beaker (Note 4). The addition of alkali is continued until the *p*H of the mixture reaches 6.3 (Note 5); 100–150 cc. of the alkali is required. The end point is determined by matching against a buffered series (Note 6), employing dibromo-*o*-cresolsulfonphthalein ("bromocresol purple"). At this *p*H the casein is completely in solution in the form of its sodium salt; fats, calcium phosphate, and any calcium caseinate remain undissolved. Care must be taken not to add more alkali than is necessary to bring the *p*H to the above point (Note 4). The milky solution is filtered through a thick layer (10–15 mm.) of filter paper pulp tightly packed upon a suction funnel. The filtrate may be slightly opalescent; if it is less clear it is again filtered through a fresh layer of pulp.

The filtrate is brought to a *p*H of 4.6 with 0.05 *M* hydrochloric acid just as in the original precipitation, the necessary amount of acid being determined by titration of an aliquot portion, diluted fivefold, with 0.01 *M* hydrochloric acid; 220–250 cc. of 0.05 *M* acid is required. As the reprecipitation progresses, the rate at which the acid is added is decreased in order to prevent precipitation at the tip of the capillary tube; vigorous mechanical stirring is, of course, essential. When the acidification is complete, 5 l. of cold distilled water is added and the flocculent precipitate allowed to settle in the refrigerator. After siphoning off the clear supernatant liquid, the casein is collected on a suction funnel, using hardened paper, washed with cold distilled water until free of chloride, sucked as dry as possible, and dried over calcium chloride in a vacuum desiccator. The yield is 23–29 g. of a colorless coherent product which may readily be pulverized in a mortar.

2. Notes

1. The cream is satisfactorily removed by allowing the milk to stand in a refrigerator overnight and siphoning off the lower layer.
2. Casein exists in milk in the form of a calcium derivative; *p*H 4.6 is the isoelectric point of free casein, which is soluble to the extent of only 0.11 g. per liter.[1]
3. Buffers for this range may be made up as follows:

0.1 *M* Acetic Acid	0.1 *M* Sodium Acetate	*p*H
7.35 cc.	2.65 cc.	4.2
6.3	3.7	4.4
5.1	4.9	4.6
4.0	6.0	4.8

2.95	7.05	5.0

4. It is important to avoid a local excess of alkali, which would tend to denature the casein.[2]
5. At this *p*H sodium caseinate is largely dissolved, whereas calcium caseinate is largely undissolved.[3]
6. The buffer series may conveniently be prepared as follows:

Disodium Phosphate (*M*/15)	Monopotassium Phosphate (*M*/15)	*p*H
0.78 cc.	9.22 cc.	5.8
1.2	8.8	6.0
1.85	8.15	6.2
2.65	7.35	6.4
3.75	6.25	6.6
5.0	5.0	6.8

3. Discussion

The precipitation of casein in its uncombined form by the addition to milk of one or another acid forms the basis of all methods of preparation. These differ widely, however, in the subsequent purification. In the method of Hammarsten,[4] just enough alkali is added to dissolve this casein completely. The alkalinity reached in this process somewhat modifies its physical properties but probably not its composition. In the method of Van Slyke and Bosworth[5] the last trace of calcium is removed by adding oxalate to an ammoniacal solution of the casein, but this procedure was shown to be unnecessary by Van Slyke and Baker.[6]

The present process is based in large part upon that of Van Slyke and Baker, the modifications depending upon the observation that casein forms far more soluble compounds with univalent than with bivalent bases at neutral reactions.

References and Notes

1. Cohn, J. Gen. Physiol. **4**, 697 (1922).
2. Cohn and Hendry, ibid. **5**, 521 (1923).
3. Loeb, ibid. **3**, 547 (1920–21).
4. Hammarsten, "Textbook of Physiological Chemistry," translation of 7th Ed., p. 619, John Wiley & Sons, 1911.
5. Van Slyke and Bosworth, J. Biol. Chem. **14**, 211 (1913).
6. Van Slyke and Baker, ibid. **35**, 127 (1918).

Appendix
Chemical Abstracts Nomenclature (Collective Index Number); (Registry Number)

Methyl Red

casein

calcium caseinate

sodium caseinate

calcium chloride (10043-52-4)

hydrochloric acid (7647-01-0)

sodium acetate (127-09-3)

sodium hydroxide (1310-73-2)

calcium (7440-70-2)

calcium phosphate

oxalate

ammonium oxalate (1113-38-8)

chloride

bromocresol

Disodium Phosphate (7558-79-4)

Monopotassium Phosphate (7778-77-0)

dibromo-o-cresolsulfonphthalein