

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE DAIRY DIVISION, UNITED STATES DEPARTMENT OF AGRICULTURE.]

PHTHALATE BUFFERS—SOME INCOMPATIBILITIES.

By HARPER F. ZOLLER.

Received January 15, 1921.

Certain phenomena associated with the conduct of buffer mixtures containing phthalic acid have impressed the writer with their importance, inasmuch as these buffer mixtures are rapidly gaining popularity in their application to biological problems.

When a solution of pure crystal violet (hexamethyl-tri-amino-tri-phenylmethane chloride) in 0.1% concentration was added to a set of Clark and Lubs buffer mixtures throughout the range of hydrogen-ion concentration from 1.0 to 10.0¹ the following changes were noted:

p_H .	Effect.	Color of solution.	Constitution.
1.0	Yellow-green		HCl-KCl
1.4	Light blue		HCl-KCl
1.8	Darker blue		HCl-KCl
2.0	Deeper blue		HCl-KCl
2.2	Dark blue		HCl-KCl
2.2	Greenish fluorescent with violet flocks		HCl-KH phthalate
2.4	Greenish fluorescent with violet flocks		HCl-KH phthalate
2.8	Greenish fluorescent with larger flocks		HCl-KH phthalate
3.0	Greenish fluorescent with larger flocks		HCl-KH phthalate
3.4	Clear liquid, heavy precipitate		HCl-KH phthalate
3.8	Clear liquid, heavy precipitate		HCl-KH phthalate
4.2	Violet solution less precipitate of pink flocks		NaOH-KH phthalate
4.4	Still less flocculation		NaOH-KH phthalate
4.8	Violet		NaOH-KH phthalate
5.0	Violet		NaOH-KH phthalate
5.4	Violet		NaOH-KH phthalate
6.0	Violet		NaOH-KH phthalate
6.4	Violet		NaOH-KH ₂ PO ₄
7.0	Violet		NaOH-KH ₂ PO ₄
8.0	Violet		NaOH-KH ₂ PO ₄
8.6	Violet		NaOH-HBO ₃
9.0	Violet		NaOH-HBO ₃
9.6	Color fading		NaOH-HBO ₃
10.0	Color fading		NaOH-HBO ₃

There is nothing remarkable in the simple color transformations noted in the scheme; these are well recognized changes. But attention is called to the precipitate which occurs in the zone described by p_H 2.2 to 4.4. The amount settling out seemed to bear a direct relation to the amount of dissociated free phthalic acid present.

At first it was believed to be a hydrogen-ion effect, but when comparable buffer solutions having a similar range of hydrogen-ion con-

¹ Five drops of the crystal violet solution was added to 10 cc. of the buffer mixtures which were themselves about 0.1 *N* concentration.

centration were prepared from acetic acid, citric acid, tartaric acid, or propionic acid, no such precipitation of dye took place, and the color transformations were normal. Buffer solutions prepared from salicylic acid showed a similar fluorescence and precipitation in the same range of hydrogen-ion concentration. The dye mixtures labeled gentian violet and methyl violet behave materially as crystal violet.

Evidence has been procured which shows that this reaction between crystal violet and the phthalate buffer solution yields an addition compound of phthalic acid with the triphenylmethane dye. The nature of this addition compound will not be dealt with in this paper. Certain other familiar tri-amino-triphenylmethane derivatives do not act in this manner with phthalic acid, *e. g.*, methyl green, dahlia, malachite green.

Albumin-Phthalic Acid.

Another reaction of similar character, but of far greater importance to biologists who are likely to use phthalate buffers in their studies, is that existing between albumin and phthalates. Impure lactalbumin in dilute concentration (0.01–0.001%) does not coagulate or flocculate at 60° in either citrate or acetate buffers, but when phthalate is substituted for these it flocculates readily at this temperature (also at lower temperatures) between p_H 2.4 and 5.0. Naturally as the temperature is raised we get a true coagulation (irreversible) around 80°, but then we find coagulation also at much higher hydrogen-ion concentration (5.0 and 6.5); with acetate buffers there is no coagulation at p_H 4.0 under these conditions.

Potassium phthalate also noticeably lowers the coagulation point (thermal) of egg albumin.

This finding will no doubt prove valuable in stimulating further attempts to separate and purify albumins. It was possible to precipitate some crude albumin with phthalate at p_H 3.0, filter off the precipitate and wash with the phthalate solution, and then suspend the precipitate in some distilled water in a collodion membrane, and dialyze against distilled water until free from salt. The albumin-phthalic acid dissolves readily in pure water. This suggests that the phenomenon may be classed by the rather general term, a "salt effect."

The iso-electric points of most of the body proteins and amino acids exist in the range of hydrogen-ion concentration covered by the phthalate series, and occasion is here taken to refer to the phenomena set forth in the above paragraphs and their significance in the operation of roughly locating the iso-electric point with these buffers. They can be employed to this end only with very judicious care, and even then may lead to error. Just what bearing these reactions may have on the use of phtha-

lates in bacteriological culture media can only be hypothesized at present, but they certainly must be taken into consideration.

DETROIT, MICHIGAN.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF JOHNS HOPKINS UNIVERSITY.]

TRIMETHYL PHOSPHINE AND ITS SELENIDE.¹

BY R. R. RENSHAW AND F. K. BELL.²

Received January 21, 1921.

In this paper the preparation of trimethyl phosphine and trimethyl phosphine selenide are described.

Trimethyl phosphine was first prepared by Cahours and Hofmann³ by the action of phosphorus trichloride on zinc methyl. They described it as a mobile, highly refractive liquid with an indescribable odor boiling between 40° and 42° and uniting with oxygen so readily that the substance fumes in the air and explodes easily. Later Hofmann⁴ prepared the substance by heating phosphonium iodide with various quantities of methyl alcohol in sealed tubes for 6 to 8 hours at 160–180°. He stated that this method is preferable to the zinc alkyl method in the ethyl series but not in the methyl because in the latter case the main product of the action is frequently the quaternary compound. Fireman⁵ claims that by substituting methyl ether for methyl alcohol in this reaction as complete a reaction can be brought about in less time and at lower temperatures, namely one to two hours at 120° to 140°.

Auger⁶ obtained a mixture of primary, secondary and tertiary phosphines when methyl iodide was treated with a solution of phosphorus in alcoholic sodium hydroxide containing water and when methyl iodide was heated at higher temperatures with phosphorus di-iodide, tribromide or trichloride.⁶ Apparently he did not obtain any better results for the preparation of trimethyl phosphine by the application of the Grignard reagent.⁷

Hibbert,⁸ however, using an excess of Grignard reagent and low temperatures has succeeded in isolating a very satisfactory yield (70%) of *tri-ethyl* phosphine as the carbon disulfide addition product.⁹

¹ Published by permission of Maj. Gen. W. L. Sibert, Director, C. W. S., U. S. Army.

² The authors wish to express their appreciation for the generosity shown by Dr. E. Emmet Reid, in offering the facilities of his laboratory for this work.

³ Cahours and Hofmann, *Ann.*, **104**, 29 (1857).

⁴ Hofmann, *Ber.*, **4**, 209, 373 (1871).

⁵ Fireman, *ibid.*, **30**, 1089 (1897).

⁶ Auger, *Compt. rend.*, **139**, 639 (1904).

⁷ Auger and Billy, *loc. cit.*, p. 597.

⁸ Hibbert, Dissertation "Ueber Additions produkte von Trialkyl-phosphinen-Arsinen und-Steibinen." See also *Ber.*, **39**, 160 (1906).

⁹ Application of this and of other methods of preparing the trialkyl derivatives of certain elements of Group V will be presented in a later paper.