

This method has been successfully used on the urine by Henderson¹⁴ and by Walpole.¹⁵ As proteins interfere with the colors of many indicators, and as both blood and serum possess color, it has been impossible to apply the method directly to the blood.¹⁶

It seemed probable that the indicator method might be utilized for blood, provided coloring matters and proteins could be excluded by means of dialysis.¹⁷ If blood is dropped into collodion sacs and dialyzed for five minutes, the dialysate is free from protein and coloring matter, but contains salts, and is well adapted to the use of indicators.

Since phenolsulphonephthalein exhibits definite variations in quality of color, with very minute differences in hydrogen-ion concentration between pH 6.4 and 8.4, it was adopted as the indicator in this method.

PREPARATION OF STANDARD COLORS

Standard phosphate mixtures are prepared according to Sorensen's¹⁸ directions as follows:

1. 15 mol. acid or primary potassium phosphate. 9.078 grams of the pure recrystallized salt (KH_2PO_4) is dissolved in freshly distilled water and made up to 1 liter.

1. 15 mol. alkaline or secondary sodium phosphate. The pure recrystallized salt ($NaH_2PO_4 \cdot 12H_2O$) is exposed to the air for from ten days to two weeks, protected from dust. Ten molecules of water of crystallization are given off and a salt of the formula $NaHPO_4 \cdot 2H_2O$ is obtained; 11.876 grams of this is dissolved in freshly distilled water and made up to 1 liter. The solution should give a deep rose red color with phenolsulphonephthalein. If only a faint pink color is obtained, the salt is not sufficiently pure.

The solutions are mixed in the proportions indicated below to obtain the desired pH.

pH	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4
Primary Potas. Phos., c.c.	73	63	51	37	22	9	1	0	0	0	0
Secondary Sodium Phos., c.c.	0	0	0	0	0	0	0	0	0	0	0

Three c.c. of each of the solutions are placed in suitable small test tubes (100 — 10 mm., inside measurement). Five drops of an aqueous

¹⁴ Henderson: *Biochem. Ztschr.*, 1910, xxiv, 40.

¹⁵ Walpole: *Biochem. Jour.*, 1910, v, 207.

¹⁶ Sorensen (*Biochem. Ztschr.*, 1909, xxii, 238) has applied the indicator method to the filtrate obtained after precipitating blood or serum by boiling with three volumes of dilute hydrochloric acid. Adler (*Am. Jour. Physiol.*, 1907, xix, 1) has tested the reaction of the serum with paper dyed pink with rosolic acid. The objections to these methods are obvious.

¹⁷ Preliminary experiments showed that it was impossible, by the indicator method, to obtain concordant results on dilutions of serum.

¹⁸ Sorensen: *Biochem. Ztschr.*, 1909, xxii, 352.