THE COLORIMETRIC DETERMINATION OF PHENOLPHTHALEIN

BY J. ALLEN*, (MISS) B. GARTSIDE† AND C. A. JOHNSON‡

*From the *British Drug Houses Ltd., Graham Street, London, N.1, †The Pharmaceutical Society of Great Britain and ‡Boots Pure Drug Co. Ltd.*

Received May 23, 1962

The conditions governing the intensity and stability of the pink colour given by phenolphthalein in alkaline solution have been examined and a procedure for its colorimetric determination proposed. The results of collaborative trials of this method and of its application to certain pharmaceutical preparations are presented.

DURING revision of the B.P.C. 1954, difficulties with the gravimetric assay of phenolphthalein in Emulsion of Liquid Paraffin and Phenolphthalein made it necessary to investigate an alternative procedure, and a colorimetric method appeared to offer the most appropriate solution to the problem. In the present paper, conditions governing the development of the pink colour of phenolphthalein in alkaline solution have been investigated.

EXPERIMENTAL

Initially, the procedure of Frederick and Koff (1946) was examined, in which a solution of phenolphthalein in ethanol is treated with 0·5N ethanolic potassium hydroxide. Differences in the intensity of the colours produced by different batches of phenolphthalein were excessive however, and agreement between different laboratories with the same sample was poor. Typical figures are shown in Table I.

TABLE I

RESULTS FOR PHENOPHTHALEIN OBTAINED BY THE METHOD OF FREDERICK AND KOFF (1946)

	E (1 per cent, 1 cm.) at max. 560–564 mμ	
Sample	Laboratory A	Laboratory B
1	273·4 267·5	297·6 318·6
2	262·2	311·1
3	252·5 277·0	291·8 314·8

An attempt was then made to apply the method of the Bureau of Industrial Alcohol (1931) in which an aqueous solution of phenolphthalein containing 5 per cent ethanol is made alkaline with 10 per cent ammonia. This gave a considerably greater colour intensity than the method of Frederick and Koff. Values for *E* (1 per cent, 1 cm.) at the maximum at about 550 mμ ranged from 977 to 1002; the colour, however, was not sufficiently stable for routine use. Consideration of these findings, combined with the known instability of the colour given by aqueous sodium

hydroxide, prompted an examination of the effect of pH on the intensity and stability of the colour of alkaline phenolphthalein. During this it was found that even small amounts of ethanol in the final solution markedly reduced the colour intensity, and preliminary evaporation to dryness of all alcoholic solutions was carried out. If, however, this evaporation with ethanol is omitted, maximum colour development is not achieved (but see Discussion).

Nine samples of phenolphthalein from several sources were examined using glycine/sodium hydroxide buffer solutions in the pH range from 10·0 to 12·5; the extinctions obtained with a typical selection of these are given in Table II.

TABLE II

E (1 PER CENT, 1 CM.) OF PHENOLPHTHALEIN AT VARIOUS pH VALUES

	pH										
Sample	10·0	10·45	10·7	10·9	11·1	11·3	11·5	11·7	11·9	12·1	12·5
A	862	1012	1046	1052	1056	1056	1056	1052	1051	1036	964
B	858	1010	1048	1057	1061	1061	1061	1061	1057	1034	948
C	820	990	1053	1053	1055	1055	1055	1051	1047	1018	907

In the pH range where extinction values were greatest, the colour is stable for periods up to 15 min.; after 30 min., the intensity had fallen by about 3 per cent.

The recommended method of colour development is given below. Under these conditions, the curve relating intensity of colour produced to the weight of phenolphthalein up to 0·9 mg. is rectilinear.

Reagents

Buffer solution (pH 11·1). Mix a solution containing 7·5 g. of amino-acetic acid and 5·8 g. of sodium chloride per litre with an equal volume of 0·1N sodium hydroxide. The pH of this solution should be checked electrometrically, and, if necessary, adjusted to 11·1.

Ethanol. 95 per cent Industrial Methylated Spirit.

Procedure

Evaporate a volume of ethanol containing about 0·5 mg. of phenol-phthalein to dryness in a small beaker on a boiling water-bath. Dissolve the residue in buffer solution and transfer to a 100 ml. calibrated flask; wash the beaker with successive quantities of buffer solution, adding the washings to the calibrated flask until a volume of 100 ml. is obtained. Measure the extinction of a 1 cm. layer of this solution at the maximum at about 555 mμ. This measurement must be completed within 10 min. of the first addition of buffer solution to the residue of phenolphthalein. For the purposes of calculation, assume the *E* (1 per cent, 1 cm.) of phenol-phthalein under these conditions to be 1055.

This method has been applied to pharmaceutical formulations as follows:

Emulsion of Liquid Paraffin and Phenolphthalein B.P.C.

Transfer about 3 g., accurately weighed to a 100 ml. basin and mix to a stiff paste with about 1 g. of Filtercel.* Add ethanol in increments, continuing the stirring to maintain a uniform smooth paste, until the volume of the mixture is about 25 ml. Transfer with the aid of ethanol to a 40 ml. centrifuge tube and centrifuge at 2500 r.p.m. for 10 min. Decant the clear supernatant liquid into a 100 ml. calibrated flask and wash the basin, centrifuge tube and residue by repeating this procedure with 10 ml. portions of ethanol until the extraction is complete. Add the washings to the 100 ml. flask and dilute to volume with ethanol. Apply the general method for colour development to 5 ml. of this solution.

Compound Pills of Phenolphthalein B.P.C.; Compound Tablets of Phenolphthalein B.P.C.; Tablets of Phenolphthalein B.P.

Weigh and powder 20 pills or tablets. Dissolve as completely as possible in 100 ml. of ethanol, a quantity of the powder expected to contain about 10 mg. of phenolphthalein. Allow any insoluble matter to settle and apply the general method for colour development to 5 ml. of the clear supernatant liquid.

Chocolate Preparations

Reduce the sample to coarse granules and chill by immersing in a freezing mixture until brittle. Grind the sample to a fine powder with a chilled pestle and mortar, transfer an accurately weighed quantity of the powder, expected to contain about 20 mg. of phenolphthalein, to a prepared Gooch crucible and extract the fat with three portions, each of 5 ml., of carbon tetrachloride, using slight suction, if necessary, towards the end of the extraction. Extract phenolphthalein from the residue with about 100 ml. of hot ethanol, applied in successive portions, until extraction is complete; transfer the mixed extracts to a 200 ml. calibrated flask, cool and dilute to volume. Apply the general method for colour development to 5 ml. of this solution.

TABLE III

E (1 PER CENT, 1 CM.) VALUES OBTAINED BY THE RECOMMENDED METHOD

Sample	Laboratory		
	A	B	C
British A	1054	1056	1053
,, B	1056	1059	1055
,, C	1062	1060	1062
Italian	1055	1068	1057
German	1058	1063	1057
Origin unknown (at least 8 years old)	1055	1053	1055

RESULTS AND DISCUSSION

Samples of phenolphthalein from various sources have been examined in three laboratories by the recommended method. The results on six are given in Table III.

* Johns-Manville Co. Ltd.

From all the figures obtained in the three laboratories, comprising a total of 57 determinations on 39 different samples, the mean value for the E (1 per cent, 1 cm.) of phenolphthalein at the maximum at about 555 mμ is 1055·35 with a standard deviation of 3·84.

Similar extinction values to those quoted above can be obtained by using acetone in place of ethanol in the preliminary evaporation stage; indeed, the same effect can be achieved by heating untreated phenolphthalein in the buffer solution. Dissolving phenolphthalein in the buffer solution without heating, however, gives low and variable extinction values. Moreover, when a solution prepared by any of these methods has faded on standing, the full intensity can be restored by heating in a water bath for 5–10 min. In the recommended methods, ethanol is preferred to acetone because of its more selective solvent properties.

We consider it to be of sufficient importance to repeat our statement that the presence of even a few per cent of ethanol in the final solution is sufficient to reduce the intensity of the pink colour to a marked extent and it is therefore essential that all traces of the solvent be removed at the evaporation stage.

TABLE IV

APPLICATION TO PHARMACEUTICAL PREPARATIONS

	Laboratories			Nominal content
	A	B	C	
Emulsion of Liquid Paraffin and Phenolphthalein B.P.C.				0·36 per cent w/w
Manufacturer I	0·370	0·375	0·360	
II	0·342	0·340	0·341	
Compound Pills of Phenolphthalein B.P.C.				32·4 mg./pill
Manufacturer I	31·5	33·1	32·2	
II	30·6	31·3	31·1	
Compound Tablets of Phenolphthalein B.P.C.				32·5 mg./tablet
Manufacturer II	33·5	34·4	33·5	
II	31·4	31·8	31·0	
Tablets of Phenolphthalein B.P.				129·6 mg./tablet
Manufacturer I	129	130	129	
II	126	128	127	
Chocolate Laxative Type (a)	7·85	8·10	7·97	8·0 per cent
Type (b)	3·87	3·80	3·77	4·0 per cent

The effect of temperature on the colour intensity is negligible over the range of 8° to 28°.

No interference with the determination of phenolphthalein was experienced from other constituents present when the methods described above were applied to pharmaceutical preparations from each of two manufacturers. All were assayed in each laboratory and the results are given in Table IV.

Acknowledgements. We wish to express our thanks to Miss C. J. Lloyd and Miss M. Warrenne for considerable technical assistance thoughout.

REFERENCES

Frederick, M. and Koff, A. (1946). *J. Amer. pharm. Ass., Sci. Ed.*, **35**, 158–159.
The Bureau of Industrial Alcohol, Washington, D.C. (1931). *Amer. J. Pharm.*, **103**, 609–610.

The paper was presented by MR. ALLEN.