A STUDY OF THE PHARMACOPŒIAL METHOD FOR THE PREPARATION OF SOLUTION OF CHLOROXYLENOL

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Solution of chloroxylenol first became official in the Sixth Addendum to the British Pharmacopœia of 1932. It survived unchanged in the 1948 Pharmacopœia, but in 1953 an alteration was made in the method of preparing the soap. Previously this had been made by the neutralisation of ricinoleic acid with potassium hydroxide, but the present monograph specifies castor oil as the starting material.

In our hands, the old method produced a satisfactory product provided that the acid complied with the official standards, particularly with regard to acid value. Unfortunately, material of this quality was not always readily obtainable, and furthermore, samples satisfactory when taken into store, sometimes gave unsatisfactory results because of a tendency to separate into several layers on standing. If such samples were well mixed prior to use, no difficulties were encountered, but considerable practical problems arose when large bulk containers were involved. It is presumably for this reason that castor oil has now replaced ricinoleic acid as the starting material.

In the present B.P. monograph, a concentrated aqueous solution of potassium hydroxide is added to a mixture of castor oil and alcohol (95 per cent.). The mixture is set aside for 1 hour, or until a small portion diluted with 19 times its volume of water, gives a clear solution. Oleic acid is then added until a few drops of the soap solution give a bluish-green colour with solution of bromothymol blue. This soap solution is then added to a mixture of the remaining ingredients, and made up to volume with water.

In preparing routine batches by the official method, we have been surprised by the wide variation in the volume of oleic acid necessary to obtain the bluish-green colour with bromothymol blue, even when starting materials from the same batches have been employed. Some variation is to be expected, due to the unavoidable error in weighing exact amounts of potassium hydroxide, but it was felt that this could not entirely account for the addition of volumes of oleic acid up to 10 times that suggested in the official formula.

Experimental

In all our experimental work, we have used the amounts of materials specified in the official formula for 1000ml. of final solution. Castor oil is officially required to have a saponification value of 177 to 187, and therefore 63 g. should require between 11·15 g. and 11·78 g. of potassium hydroxide, KOH, for complete saponification. Potassium hydroxide B.P. is required to contain not less than 85 per cent. of total alkali calculated as

hydroxide, of which not more than 4 per cent. may be carbonate. Assuming that the latter enters into the reaction, 13·6 g. of potassium hydroxide B.P. may provide from 11·56 g. to 13·6 g. of potassium hydroxide. The maximum possible excess of potassium hydroxide is therefore 2·45 g., representing approximately 14 ml. of oleic acid. B.P. This maximum excess can only occur when 13·6 g. of real potassium hydroxide is used, and in practice this is unlikely unless an assayed solution is employed.

Saponification of Castor Oil. It was considered possible that the disparity might well be due to incomplete saponification of the castor oil. 13·6 g. of potassium hydroxide was dissolved in 15 ml. of water and the solution added whilst hot to 63 g. of castor oil mixed with 63 ml. of alcohol (95 per cent.) and the mixture stirred for 30 seconds. After an interval of 2 minutes, 0·5 ml. of the mixture was added to 9·5 ml. of water in a test tube, and the appearance noted. This test was repeated at 2 minute intervals.

A similar experiment was carried out in which the potassium hydroxide solution was allowed to cool to room temperature before adding to the castor oil/alcohol mixture. The results are shown in Table I.

TABLE I

CASTOR OIL—SAPONIFICATION TIME AS DETERMINED BY THE OFFICIAL DILUTION TEST

	Saponification with hot potassium hydroxide solution		Saponification with cold potassium hydroxide solution	
Time, minutes	Appearance on dilution	Appearance after 10 minutes	Appearance on dilution	Appearance after 10 minutes
2	Very turbid	Very turbid	Very turbid	Very turbid
4	Turbid	Turbid	,, ,,	,, ,,
6	Clear	Opalescent	Turbid	,, ,,
8	,,	Clear	,,	Turbid
10	,,	,,	,,	,,
12	,,	,,	Opalescent	,,
14	,,	,,	Clear	Opalescent
16	,,	,,	,,	Clear
18	,,	,,	,,	,,
20	,,	,,	,,	,,

As saponification approached completion, it was observed that turbidity did not appear immediately, but developed gradually over a period of 10 minutes. Dilutions which were clear after 10 minutes, remained clear for 24 hours. We have therefore concluded that under normal conditions, saponification by the pharmacopœial method proceeds rapidly and to completion, and that the cause of the variation in the volume of oleic acid used for subsequent neutralisation must be sought elsewhere.

The bromothymol blue end-point. Castor oil soap, as prepared by the official method, has a pronounced yellow colour which is bound to affect observations using bromothymol blue as an indicator, especially in its blue range. The Phamacopœia gives no specific directions for carrying out the test, and the colour observed may be expected to vary with the proportion of indicator to soap solution used. 5 samples of soap solution were prepared and to each sample a known volume of oleic acid was added. Each sample was used to prepare a series of tubes containing varying

proportions of indicator to soap solution, and the colours noted. The results are shown in Table II.

It will be noted that in each series at least one tube showed a bluish-green colour, including the sample to which no oleic acid had been added. From these results we have concluded that bromothymol blue is an unsatisfactory indicator for this purpose, unless strict conditions for performing

TABLE II

COLOURS OBSERVED USING VARYING PROPORTIONS OF BROMOTHYMOL BLUE TO SOAP SOLUTION

Sample	Added volume of oleic acid, ml.	Volume of bromothymol blue solution / Volume of soap solution					
		1/2	1/5	1/10	1/15	1/25	1/50
A	Nil	Blue	Blue	Blue	Greenish blue	Bluish green	Green
B	1·0	,,	,,	,,	,,	,,	,,
C	2·0	,,	,,	,,	,,	,,	,,
D	2·5	,,	,,	,,	,,	,,	,,
E	2·75	Bluish green	Bluish green	Bluish green	Bluish green	,,	,,

the test are laid down. We have found a "spotting out" technique using equal volumes of soap solution and indicator to be satisfactory in the hands of experienced operators, but it was nevertheless thought desirable to investigate the use of an alternative indicator which might prove more reliable for routine use.

Phenolphthalein. The colour change of phenolphthalein is not appreciably masked in yellow solutions. It is used in the official determinations of

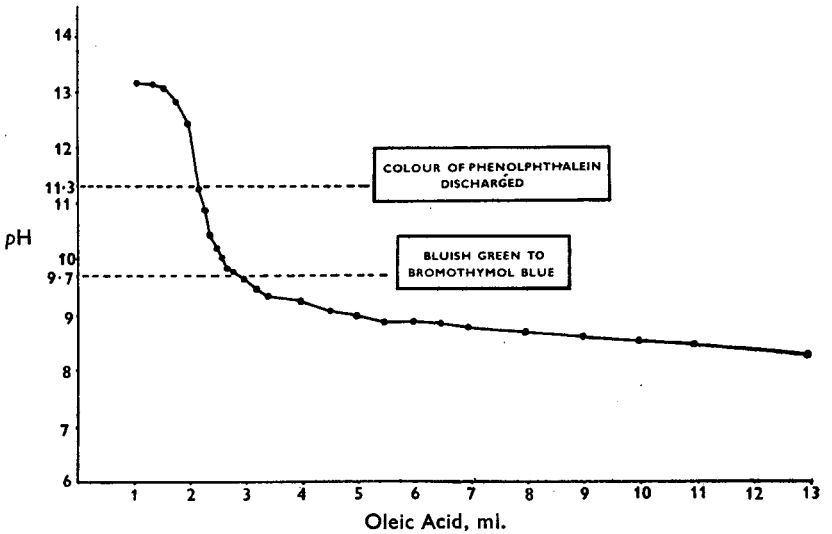

FIG. 1. Graph illustrating relationship between the *p*H value (glass electrode) of the soap solution, and the volume of oleic acid added.

saponification and acid values, and was used as the indicator in the former official method for preparing the soap for solution of chloroxylenol. The pink colour of phenolphthalein in alkaline solution is discharged at a *p*H value in the region of 8·2, which is somewhat higher than that of 7·6 corresponding to the bluish-green tint of bromothymol blue. In preparing the castor oil soap, the initial volume of oleic acid required to discharge the colour of phenolphthalein, will depend upon the excess of alkali present. After this point it should be possible to determine from a curve obtained by plotting the *p*H value of the soap solution against the volume of oleic acid added, what further volume is required to bring the *p*H value down to 7·6. This volume should be constant within the limits of experimental error. A sample of soap solution was prepared by the official method and oleic acid added from a burette, the *p*H value as determined by the glass electrode, being noted after each addition. A typical curve for the neutralisation of a strong base by a weak acid was obtained (Fig. 1).

A similar sample of soap solution was prepared, and oleic acid added until the pink colour of phenolphthalein was just discharged using the "spotting out" technique. At this point a glass electrode reading of 11·3 was recorded. The addition of oleic acid was continued until the bluish-green colour of bromothymol blue was obtained, using the spotting out technique previously described. The glass electrode reading at this point was found to be 9·7. Both these points have been marked on Figure 1. It will be noted that the phenolphthalein colour change occurs near to the point of complete neutralisation, and the bluish-green tint of bromothymol blue at the point on the curve where the gradient begins to diminish rapidly, the difference representing a volume of 0·6 ml. of oleic acid. An important point brought to light by this experiment is the discrepancy between the *p*H values, as determined by indicators and the glass electrode.

Discrepancy between indicator and glass electrode readings. We have not been able to trace any reference suggesting that this discrepancy has been observed by other workers. To ascertain whether the cause was the presence of a high proportion of ethanol in the mixture, a sample of soap solution was prepared and oleic acid added until it gave the bluish-green colour with bromothymol blue. At this point the glass electrode reading was 9·7. The sample was then heated on a water bath to remove the ethanol, and the loss in weight made up with water. On cooling, the product still gave a bluish-green colour with bromothymol blue, and a glass electrode reading of 9·6, suggesting that the presence of ethanol does not significantly account for the discrepancy. Palmer[1] describes experiments to demonstrate that the colours shown by indicators in certain heterogeneous systems, are affected by adsorption of the indicator ions onto the interfaces, and it is reasonable to surmise that a concentrated soap solution, because of its micellular structure, may exert a similar effect. If this is in fact the case, it is conceivable that the colour shown by an indicator when added to a soap solution may not be indicative of the *p*H value normally attributed to that colour in a homogeneous system. Furthermore, indicators may not agree amongst themselves, due to variations in the adsorption of their coloured ions.

9 samples of soap solution designated A to I were prepared, and adjusted by the addition of oleic acid, to the point at which the pink colour of phenolphthalein was just discharged. To sample A, the addition of acid was continued until the bluish-green colour with bromothymol blue was obtained by the "spotting out" technique. To each of the remaining samples, a known additional volume of oleic acid was added. The *p*H value of each sample was determined using the glass electrode. Because of the deep yellow colour of the soap solutions, small colour changes of indicators are difficult to observe, and it was therefore decided to use each sample for the preparation of 1000 ml. of solution of chloroxylenol, and to conduct the tests with these completed solutions where the colour does not interfere appreciably. The *p*H value of each solution was recorded using (*a*) the glass electrode, (*b*) bromothymol blue, and (*c*) cresol red. The colours given by the indicators, were compared with those obtained using buffer solutions of known *p*H value. The results are shown in Table III.

TABLE III

EFFECT OF ADDITIONAL OLEIC ACID ON *p*H VALUE. COMPARISON OF GLASS ELECTRODE AND INDICATOR READINGS

Sample	Additional volume of oleic acid added, ml.	*p*H value of soap solution (glass electrode)	*p*H value of completed solutions		
			Glass electrode	Bromothymol blue	Cresol red
A	0·60	9·7	9·3	7·4	8·6
B	1·0	9·5	9·0	7·2	8·6
C	2·5	9·0	8·6	6·8	8·6
D	5·0	8·8	8·4	6·6	8·3
E	7·5	8·5	8·2	6·6	8·0
F	10·0	8·35	8·1	6·4	7·8
G	12·5	8·3	8·0	6·3	7·6
H	15·0	8·2	7·9	6·2	7·6
I	17·5	8·1	7·8	6·1	7·5

It will be seen that bromothymol blue and cresol red give values which cannot be reconciled with each other, or with those given by the glass electrode. The further investigation of this discrepancy is beyond the scope of this paper, but it can be said that the generally accepted view, as put forward by Firth[2], that solution of chloroxylenol has a *p*H value in the region of 7 to 8, is at least open to some doubt. We ourselves feel that the weight of evidence is on the side of the glass electrode, and in consequence it was decided to investigate some of the properties of the 9 samples, A to I, with a view to the formulation of a less alkaline preparation likely to be less irritant when used in the undiluted form.

Bactericidal Activity. The following test was made to compare the bactericidal activity of the samples, and not to evaluate them as bactericides. Serial dilutions were made in a nutrient broth containing 2 per cent. of glucose and Andrade indicator, and tested against a number of common pathogens by adding to each tube a standard drop of a broth culture, and incubating for 48 hours. The production of colour was taken to be indicative of a heavy growth of the organism, but owing to the cloudiness of the dilutions, this was always confirmed by subculturing the 3 preceding tubes. The results are shown in Table IV.

Stability. The colour of each sample was noted (i) immediately after preparation, (ii) after storing at room temperature for 7 days, and (iii) after heating in sealed containers at 50° C. for 24 hours. It was observed that when freshly prepared, samples A, B, C, and D, were considerably darker in colour than E, F, G, H, and I, these being almost colourless. After storing for 7 days at room temperature, all samples showed some darkening in colour, with the division between the 2 groups still being very clearly marked. The heated samples showed still further darkening in the cases

TABLE IV

COMPARISON OF BACTERICIDAL ACTIVITY

Sample	Inhibitory concentration after 48 hours at 37° C.					
	Staphylococcus pyogenes	*Streptococcus hæmolyticus*	*Streptococcus fæcalis*	*Coliforms*	*Proteus*	*Pseudomonas pyocyanea*
A	1/800	1/1600	1/800	1/400	1/400	>1/50
B	1/800	1/1600	1/800	1/400	1/400	>1/50
C	1/800	1/800	1/800	1/400	1/400	>1/50
D	1/800	1/1600	1/800	1/400	1/400	>1/50
E	1/800	1/1600	1/800	1/400	1/400	>1/50
F	1/800	1/800	1/800	1/400	1/400	>1/50
G	1/800	1/1600	1/800	1/400	1/400	>1/50
H	1/800	1/1600	1/800	1/800	1/400	>1/50
I	1/800	1/1600	1/800	1/800	1/400	>1/50

of A, B, C, and D. Samples E, F, G, H, and I, showed slight darkening, but remained lighter in colour than samples A, B, C, and D, stored at room temperature. None of the samples stored at room temperature or at 50° C. showed evidence of separation into layers, and all remained clear. No deposition of soap or separation into layers was observed in samples stored at 2° to 4° C. for 48 hours.

Dilution Test. All the samples complied with the official dilution test. These results show that the addition of oleic acid to produce a final solution of *p*H 8·2 or below (glass electrode), not only reduces the alkalinity, but results in a more stable product. At the same time, the bactericidal activity remains unchanged.

CONCLUSIONS

We have concluded that the variation in the amount of oleic acid necessary to obtain the end-point with bromothymol blue, is not due to incomplete saponification, but to the difficulty in observing the colour change of the indicator in a yellow solution. The disappearance of the pink colour of phenolphthalein has been found to be more readily observed, although the resulting soap is more alkaline. After this point, an additional volume of 0·6 ml. of oleic acid per litre of final solution, results in a product equivalent to one prepared by the official method. By increasing this additional volume to 10 ml., a product having a *p*H value of 8·1 may be prepared. To obtain still lower values, comparatively large volumes of oleic acid are required.

The following formula and method of preparation gives a reproducible product having a *p*H value of 8·1 (glass electrode), which is more stable than the official preparation. The bactericidal activity is unaffected.

SOLUTION OF CHLOROXYLENOL

Chloroxylenol	50 g.
Terpineol	100 ml.
Alcohol (95 per cent.)	200 ml.
Castor Oil..	63 g.
Potassium Hydroxide ..	13·6 g.
Oleic Acid	a sufficient quantity.
Distilled Water, sufficient to produce	1000 ml.

Dissolve the potassium hydroxide in 15 ml. of distilled water, and add whilst hot to a solution of the castor oil in 63 ml. of the ethanol (95 per cent.). Mix, and set aside for 10 minutes or until a small portion remains clear for 10 minutes when diluted with 19 times its volume of water. Add, with stirring, sufficient oleic acid until a small volume of the soap solution just ceases to give a pink colur when mixed with an equal volume of solution of phenolphthalein. Add 10 ml. of oleic acid. Dissolve the chloroxylenol in the remainder of the ethanol (95 per cent.), mix with the terpineol and add to the soap solution; finally add sufficient distilled water to produce 1000 ml.

Summary

1. In preparing routine batches of solution of chloroxylenol, a wide variation from batch to batch has been observed in the volume of oleic acid required to adjust the soap solution to the official end-point.

2. Investigation has shown this to be due to the masking of the colour change of bromothymol blue in yellow solutions. The disappearance of the pink colour of phenolphthalein is more readily observed, and from a curve showing the relationship between the *p*H value and the volume of oleic acid added, it is possible to calculate what further volume is required to reach the official end-point, or for that matter any other desired point. During the course of the work, experimental evidence has been obtained to show that indicators do not provide a reliable guide to the *p*H value of soap solutions, and that solution of chloroxylenol may be much more alkaline than would be expected from the colour which it gives with bromothymol blue.

3. A formula and method of preparation is given for a product of constant and reproducible composition, which is less alkaline than the official preparation, paler in colour, and less likely to darken on storage. Its bactericidal activity is not affected by the change.

We are indebted to Mr. J. E. Southall of the Department of Clinical Pathology, Manchester Royal Infirmary, for the bacteriological investigations, and to Dr. D. C. Henry of the Department of Colloid Chemistry, University of Manchester, for discussing with us the physico-chemical aspects of some of the problems encountered. Our thanks are also due to Professor H. Berry for suggesting possible lines of investigation, and to Professor H. Brindle for his help and interest throughout the course of our work.

REFERENCES

1. Palmer, *Experimental Physical Chemistry*, Cambridge University Press, London, 1952, 281.
2. Firth, *Pharm. J.*, 1945, **100**, 318.

DISCUSSION

The paper was presented by MR. J. B. LLOYD.

MR. R. LEVIN (Liverpool) said that he had prepared samples of roxenol to the B.P. 1948 and 1953 requirements and by the authors' method. For the 1948 B.P. sample he used ricinoleic acid which was not of B.P. standard and obtained an inferior roxenol. It separated on standing, and had a *p*H of 9·85, which might have been accounted for by the fact that the acid was not B.P. The roxenol B.P. 1953, using castor oil soap, had a *p*H of 9·1; roxenol prepared by the authors' method had a *p*H of 8·05; and a proprietary preparation which he examined had a *p*H of 9·4. The *p*H of 5 per cent. dilutions were 9·73, 9·4, 9·18 and 9·1 respectively. The wide range in the *p*H values of the roxenols was thus considerably narrowed on dilution. This raised two questions: what was the optimum *p*H of a dilution of roxenol, in regard to skin sensitivity, and for maximum antibacterial activity?

DR. W. MITCHELL (London), said that in his experience the quantity of oleic acid used was remarkably constant. While he agreed that indicators might give unreliable *p*H results, did the glass electrode give an accurate value in soap solution? He had tested many batches which were *p*H 8 to bromothymol blue but which, on the glass electrode, were all 9 to 9·1. On dilution with London tap water, 1 to 19, these *p*H values were virtually unaltered. A proprietary preparation behaved in the same way. Using a batch made according to the author's formula, the glass electrode showed a *p*H of 8·1, but when diluted with London tap water, 1 to 19, the *p*H was 9·2. The unanswered question was, what was the optimum *p*H for maximum antiseptic activity?

PROFESSOR H. BRINDLE (Manchester) stated that 20 students had each prepared small batches by the process described in the B.P., and the amounts of oleic acid which individuals considered it necessary to add had varied even more widely than those described by the authors. It was clear that the B.P. process was not satisfactory, and the authors' suggestion was a great improvement.

MR. J. H. OAKLEY (London) recommended neutralisation to phenolphthalein first, followed by bromothymol blue. He was not happy that the dilution test was indicative of completion of saponification. He was not sure that satisfactory *p*H results were obtained by the glass electrode, but he agreed that indicators, too, were likely to give unsatisfactory results. He confirmed the authors' observations about darkening on storage and agreed that the more alkaline the preparation the greater the darkening. Had the authors experienced any layering with their preparations?

MR. G. SYKES (Nottingham) pointed out that it was not correct to deduce bactericidal activity from bacteriostatic tests. Further bacteriological work was needed.

DR. R. E. STUCKEY (London) said that although there was a drift with many *p*H meters and many types of electrode, the *p*H meter should, nevertheless, be used for determinations in solutions of this type. It was wrong to use an indicator in a system which would suppress the ionisation of the indicator.

MR. G. RIGBY (Manchester) asked if the authors could confirm his belief that a serum-containing medium was used for *Staphylococcus pyogenes* in Table IV. If so, the results were not strictly comparable.

MR. LLOYD, in reply, said that dilution seemed to bring all solutions, no matter how much oleic acid had been added, to about the same *p*H value. In some hospitals roxenol was used undiluted, and it was felt, therefore, that it should be as nearly neutral as possible. Furthermore, as some speakers had confirmed, they had been able to show that the solution was clearer, colourless and, generally, a more stable product. Whether all the oleic acid recommended should, in fact, be added, depended on the *p*H required for the undiluted product. He agreed that the dilution test did not measure complete saponification, but the test was used because it was given in the B.P. They had prepared hundreds of samples of the solution, and in no case had they found layering, although they had looked for it. Neither the heated nor the cooled samples layered. Very little drift on the final solution had been found on the glass electrode. There was a definite drift on dilution. however, and the further one diluted the greater the drift—until at 1 to 100 dilution it was impossible to measure the *p*H of the solution by this method. He did not know whether serum had been added to the medium in the case mentioned, and he agreed that better bacteriological investigations could have been conducted before the statement was made which Mr. Sykes had criticised.