A NEW NON-AQUEOUS METHOD OF ASSAY FOR THE BARBITURIC ACIDS AND SOME COMMERCIAL PRODUCTS

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Non-aqueous titrimetry has been applied to the assay of barbiturates by several groups of workers. Autian and Allen[1] developed a potentiometric method using a solvent system of chloroform and polyethylene glycol 400. Their studies were modified and extended by Swartz and Foss[2], but these workers also failed to find a suitable indicator for this solvent system. They expressed a desire to employ chloroform alone, which is an excellent solvent for most barbituric acids, but found that such a titration was not suitable as precipitation of the barbiturate salts obscured the indicator end-point. Because of its low dielectric constant, chloroform was also a poor solvent for potentiometric titrations. They circumvented this difficulty by adding 10 per cent. polyethylene glycol 400, but were then forced to use a potentiometric titration method. Roland[3] used 75 per cent. ethanol in water and titrated the salts potentiometrically with 0·1N hydrochloric acid. He deplored the use of indicators and stated that in his opinion they were not accurate. This, of course, is understandable for such weak acids in a system containing 25 per cent. water.

Vespe and Fritz[4] titrated barbituric acids in dimethylformamide as solvent and their investigation was confirmed and expanded by Ryan, Yanowski and Pifer[5]. It was noted in this laboratory, however, that the end-point in such a system faded rapidly even when the titration flask was protected carefully from the atmosphere. It was noted, furthermore, that when using this solvent, there was a distinct tendency to overestimate the barbiturate content of certain coloured tablet preparations. In addition, dimethylformamide is unpleasant to work with and constitutes an actual danger to the respiratory system.

The main objection to the methods employed by other workers[1,2,3] was that the titration could be carried out only potentiometrically. It was decided, therefore, to seek a solvent system which did not possess the disadvantages that are inherent in dimethylformamide, but which yet permitted a visual titration of the barbiturates.

For the titration of weak acids in nonaqueous solvents, most workers have employed a methoxide titrant which is prepared with either metallic sodium, potassium or lithium. A recent publication by Crisafio and Chatten[6] has shown that a 0·1N solution of potassium hydroxide in anhydrous methanol was equally satisfactory for the titration of the bile acids and was much simpler to prepare than the conventional methoxides. Caldin and Long[7] proved that, in reality, a solution of sodium hydroxide in methanol did not differ in methoxide content from one prepared by using metallic sodium. It would appear reasonable to extend their findings on sodium hydroxide to include potassium hydroxide.

Experimental

Apparatus. 5 or 10 ml. burette, graduated in 0·02 ml., electromagnetic stirrer, 125 ml. suction flask and a small Büchner funnel.

Reagents. (1) chloroform, A.C.S. grade, (2) anhydrous methanol, A.C.S. grade, (3) benzoic acid, A.C.S. grade, (4) potassium hydroxide, A.C.S. grade, (5) dimethylformamide, Eastman white label, (6) potassium hydroxide 0·1N in anhydrous methanol, and (7) thymol blue indicator in 0·5 per cent. in anhydrous methanol.

Standardisation of titrate. Accurately weigh approximately 200 mg. of benzoic acid and dissolve in 50 ml. of chloroform, and 1 ml. of methanol and 4 drops of thymol blue indicator and titrate to a violet colour. A blank with the solvent system used here was approximately 0·10 ml. of titrant.

The contents of the beaker or flask can be conveniently protected from the atmosphere by using a piece of rubber dental dam or cardboard with a hole sufficiently large to permit the burette tip to pass through.

Procedures. (*a*) To assay bulk barbiturates, accurately weigh a sample of 40 to 50 mg. into a 150 ml. beaker, dissolve in 50 ml. of chloroform by stirring electromagnetically, add 1 ml. of anhydrous methanol, 4 drops of thymol blue indicator and cover the beaker. Titrate to a violet end-point with 0·1N potassium hydroxide in methanol.

(*b*) For commercial barbiturate samples, weigh and powder 20 tablets. Place an accurately weighed sample equivalent to 40 or 50 mg. of the drug in a 150 ml. beaker. Add 40 ml. of chloroform and stir electromagnetically for 10 minutes. Filter under suction into a 125 ml. flask using a Büchner funnel with Whatman No. 1 filter paper. Wash the beaker and funnel with 10 ml. of chloroform, add to the filtrate 1 ml. of anhydrous methanol and 4 drops of thymol blue solution. Cover the flask and titrate to a violet end-point with 0·1N potassium hydroxide solution in methanol.

Experimental Results and Discussion

The precision of the method for barbituric acids was tested by titrating 5 consecutive samples of U.S.P. grade phenobarbitone and calculating the standard deviation. These results are recorded in Table I. The study was extended to a number of other pure barbituric acids and triplicate assays for each are shown in Table II. The criterion of purity for these samples is shown by their melting points as well as by comparative duplicate assays utilising the dimethylformamide procedure[4]. Potentiometric titrations were performed on a Fisher titrator which was equipped with the conventional glass-calomel electrode system and a suitable arrangement to preclude the atmosphere. Figure 1 illustrates the excellent agreement that was obtained between the potentiometric and visual end-points for phenobarbitone in the chloroform-methanol system.

TABLE I

Titration of U.S.P. grade phenobarbitone using a chloroform-methanol solvent system

Mg. taken	Mg. recovered	Per cent. recovery
45·3	45·3	100·0
44·1	43·9	99·5
46·2	46·3	100·2
53·3	53·2	99·8
47·6	47·7	100·2

Mean—99·9±0·3

TABLE II

COMPARATIVE RESULTS OF THE TITRATION OF PURE BARBITURIC ACIDS IN CHLOROFORM-METHANOL AND IN DIMETHYLFORMAMIDE

Barbituric acid	M.pt.° C.	In chloroform-methanol		Dimethylforformamide	
		mg. taken	mg. recovered	per cent.	per cent.
Amylobarbitone	156·3–156·9	48·0	47·9	99·8	100·4
		41·8	41·7	99·8	100·0
		44·7	44·8	100·2	
Barbitone	189·2–189·8	43·3	43·0	99·4	100·2
		41·1	40·8	99·3	99·2
		56·4	55·8	98·9	
Cyclobarbitone	171·3–172·8	41·7	41·4	99·3	99·2
		49·8	49·6	99·6	99·4
		46·0	45·8	99·6	
5-Allyl-5-phenylbarbituric acid	156–156·7	44·0	44·2	100·4	100·4
		45·8	45·9	100·2	100·2
		42·3	42·4	100·2	
Aprobarbital	140–142	46·7	46·7	100·0	100·0
		46·0	45·8	99·6	100·4
		42·7	42·4	99·3	
Propallylonal	180–181·2	51·7	51·0	98·6	99·8
		46·6	46·4	99·6	100·4
		47·7	47·5	99·6	
Hexethal	124·8–126·5	52·9	52·1	98·5	99·6
		49·0	48·6	99·2	99·3
		52·4	51·7	98·8	
Thiamylal	133·7–134·5	51·9	52·5	101·1	99·1
		45·8	46·3	101·1	99·4
		48·7	49·4	101·4	
5 - Methyl - 5 - phenylbarbituric acid	225·5–226·2	45·3	45·2	99·8	100·0
		49·4	49·4	100·0	100·0
		44·1	44·2	100·2	
5 - Ethyl - 5 - (1-*cyclo*hepten - 1 - yl) barbituric acid	170–172·2	43·0	42·7	99·3	100·4
		43·3	43·2	99·8	100·8
		44·1	44·0	99·8	
Pentobarbitone	128·2–129·4	50·6	49·9	98·6	98·8
		54·2	53·9	99·4	98·4
		49·9	49·4	99·0	

The precipitation of the barbiturate salts, when the acids were titrated in chloroform alone, as reported by Swartz and Foss[2] was confirmed in this laboratory. It was noted, however, that if 1 ml. of anhydrous methanol was added to the chloroform before commencing the titration precipitation did not occur. Since the titrant employed in this investigation was potassium hydroxide in anhydrous methanol, the addition of a further 2·5 ml., the amount required for most titrations, provided sufficient methanol to keep the barbiturate salts in solution. It was further noted that the precipitate which formed, when 1 ml. of methanol was not added, frequently redissolved before the titration was completed. This did not occur in every instance, however, and consequently it was deemed advisable to add the 1 ml. of methanol as a step in the standard procedure. It is the author's opinion that this resulted in a sharper visual end-point than that obtained in chloroform alone. It was also observed that the end-point was more permanent in either the chloroform or the chloroform-methanol system than in dimethylformamide.

TABLE III

STANDARDISATION OF POTASSIUM HYDROXIDE IN METHANOL

Mg. benzoic acid	Ml. titrant	Normality
202·2	21·74	0·0762
182·3	19·66	0·0760
180·3	19·37	0·0762
180·7	19·42	0·0762
193·6	20·80	0·0762

As outlined in the procedure, the titrant was standardised by dissolving approximately 200 mg. of benzoic acid, accurately weighed, in 50 ml. of chloroform, adding 1 ml. of anhydrous methanol and 4 drops of thymol blue solution. The 1 ml. of methanol, in this instance, serves no purpose except to keep the solvent system constant. The results of 5 consecutive titrations are given in Table III, which shows the reproducibility of the method.

It was reported earlier that the use of dimethylformamide as solvent resulted, in almost every instance, in the overestimation of the barbiturate content of coloured tablet preparations. As a result of this finding, a comparison was conducted between the two solvent systems. The results appear in Table IV. It is noted that the recoveries with dimethylformamide are not only higher than expected but are erratic. This may be accounted for by the fact that the extracts with this solvent were intensely coloured by the tablet dye, whereas the chloroform extracts were only faintly coloured or colourless. It had been hoped to perform comparative assays on these products by some other standard method, but owing to the other ingredients in the tablet, none were found to be suitable. In order to justify the results obtained with the two solvent systems, therefore, the tablet residues from the chloroform extracts were saved and dried. Known weights of phenobarbitone of U.S.P. grade were added to the residues and the phenobarbitone determined by the chloroform-methanol procedure. The recoveries were quantitative. The residues were again dried, further known weights of the phenobarbitone added and the assays carried out using dimethylformamide. In every instance, overestimation occurred with this latter solvent which was about the same as that found when the unknown tablets were assayed.

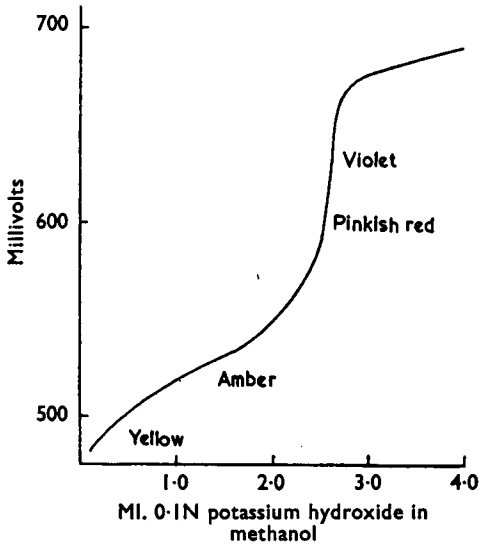

FIG. 1. Titration of phenobarbitone in chloroform-methanol system.

Vespe and Fritz[4] noted that when phenobarbitone was combined with aminophylline, the baribituric acid content could not be determined by extraction with dimethylformamide, due to interference by the theophylline. Although the same drug also interferes with direct chloroform extraction, the use of this latter solvent enables the analyst to apply a modification of David's[8] method to such mixtures. By treating the preparation with ammonia and silver nitrate as instructed by David, the procedure need be

carried only to the stage where the barbituric acid is extracted by shaking with 25 ml. portions of chloroform. When this has been accomplished, the drug can be determined by the direct titration previously outlined. It is necessary to use 1 ml. of methanol for each 50 ml.of chloroform extract. The results of such a modification, when applied to commercial products, are given in Table V along with the analyses of certain other phenobarbitone (uncoloured) preparations.

Comparative assays were performed, where possible, by the B.P. 1953 method for the phenobarbitone tablets. Agreement between the non-aqueous and the official procedure appears to be quite satisfactory in all but three instances. The preparations in question, which are a half grain tablet, a one grain and a grain and a half, are those of one manufacturer. For each the recovery by the official procedure was considerably higher than that which was obtained when using the non-aqueous technique. Titration of the phenobarbitone residues revealed that some additional substance was being weighed as phenobarbitone and that the actual amount of drug present in the residue agreed very closely with the non-aqueous values. The phenobarbitone residues of all other

TABLE IV

COMPARISON BETWEEN PER CENT. RECOVERIES IN THE CHLOROFORM-METHANOL SOLVENT SYSTEM AND DIMETHYLFORMAMIDE FOR COLOURED PRODUCTS

Product	Chloroform-methanol	Dimethylformamide
I	98·0	109·5
	98·2	102·5
II	100·0	107·8
	100·8	102·2
III	100·2	105·7
	100·0	105·8
IV	96·8	122·0
	95·6	113·4

TABLE V

RECOVERY OF PHENOBARBITONE FROM COMMERCIAL TABLETS

Product	Per cent recovery	
	Non-aqueous	B.P. 1953
Aminophylline and phenobarbitone ..	98·6	•
	96·8	
Aminophylline and phenobarbitone ..	92·1	•
	91·8	
Aminophylline and phenobarbitone ..	90·4	•
	90·5	
Aminophylline and Phenobarbitone ..	101·9	•
	101·4	
Phenobarbitone gr 1½	98·1	98·5
	98·3	
Phenobarbitone gr. 1½	98·0	98·3
	98·2	
Phenobarbitone gr. 1¼	89·0	97·9†
	89·1	
Phenobarbitone gr. 1	91·3	90·5
	90·9	
Phenobarbitone gr. 1	94·5	97·8†
	94·4	
Phenobarbitone gr. ½	97·5	97·3
	97·7	
Phenobarbitone gr. ½	92·9	99·1†
	92·6	
Phenobarbitone gr. ½	94·6	95·0
	94·4	
Phenobarbitone gr. ¼	91·1	91·4
	91·5	
Phenobarbitone gr. ¼	96·1	97·6
	96·6	
Phenobarbitone with sodium pentobarbitone	87·2	•
	87·7	

• Not official in B.P. † Overestimated by B.P. procedure.

tablets, when also determined volumetrically, were found to be reasonably pure drug. These results indicate that the official procedure, which is much slower than the non-aqueous method, may lead to overestimation with certain products.

Sodium salts of barbituric acids, as bulk or in tablets or capsules, were extracted as the free acid by Swartz and Foss[2], who used the "Schmall apparatus". These workers acidified an aqueous solution of the salt and extracted the drug with chloroform. Evaporation of the solvent to 50 ml. was followed by the addition of 10 per cent., polyethlene glycol 400. The titration was performed potentiometrically. To use the procedure described in this report, it is necessary to add 1 ml. of anhydrous methanol after the chloroform extract has been evaporated to 50 ml. A visual titration can then be performed as previously described.

Summary

1. A new non-aqueous technique has been devised for the barbituric acids which is rapid, accurate and can be performed visually.

2. The procedure has been successfully applied to commercial samples of phenobarbitone tablets as well as those of phenobarbitone and aminophylline. The advantages over existing methods have been discussed.

3. The use of potassium hydroxide in methanol as a titrant in non-aqueous titrimetry has been extended to the barbiturates.

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