THE DETERMINATION OF PHENOLIC COMPOUNDS IN PHARMACEUTICAL PREPARATIONS USING 4-AMINOPHENAZONE

By C. A. Johnson and R. A. Savidge

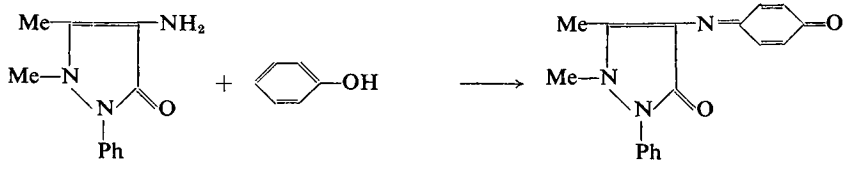
From the Analytical Development Group, Standards Department, Boots Pure Drug Co., Ltd.

Received May 30, 1958

The determination of phenols based upon coupling with 4-aminophenazone in the presence of an alkaline oxidising agent has been examined. The importance of pH has been studied and a suitable buffering agent has been recommended. The reactivity of many phenols of pharmaceutical interest has been investigated and the application of the method to a number of pharmaceutical preparations is described.

Until recently phenolic preservatives in injection solutions were determined exclusively by steam distillation of the phenolic material followed by bromination. An ultra-violet absorption method was frequently invalidated because the other ingredients of the injection solution themselves absorbed strongly at the critical wavelength, but a considerable advance was made in the application of this technique when the use of oxidised cellulose for the removal of certain interfering substances was introduced[1]. Many injection solutions still remain, however, to which this technique is inapplicable and a search was therefore made for a suitable method of determining phenolic substances which would be both accurate and rapid.

Such a method is based on the use of 4-aminophenazone[2]; the determination depends upon the production of a dye by the action of 4-aminophenazone on the phenol in the presence of an alkaline oxidising agent, usually potassium ferricyanide.



The method was first described by Emerson[2], discussed by Ettinger and others[3], and applied to the determination of phenols in waters from coke wastes by Shaw[4], and in industrial waste waters by Mohler and Jacobs[5]; much valuable information has been published by these workers. The present paper reviews the necessary conditions for quantitative reaction and in particular stresses the careful control of pH which is required; it also examines the applicability of the method to many phenols likely to be met in pharmaceutical practice and describes methods for the determination of many of these substances in typical pharmaceutical preparations.

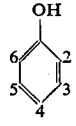
Scope and Applicability of the Method

In general substances having a free phenolic hydroxyl group and a free *para* position or a *para* position which is substituted by a halogen, hydroxyl or alkoxyl, sulphonic acid or carboxylic acid group give a positive reaction. Presumably these substituents are expelled in the reaction, for thymol and chlorothymol, which has the halogen in the *para* position to the hydroxyl, give coloured products which have identical absorption curves except that the molecular extinctions differ, consistent with the difference in molecular weights. A nitro or a carboxylic acid group in the *ortho* position relative to the free hydroxyl prevents the formation of colour and this is probably due to hydrogen bonding with consequent loss of phenolic character.

The reaction apparently occurs only at the *para* position, since there are several examples of substances having free *ortho* positions which give no colour, for example, methyl *p*-hydroxybenzoate, stilboestrol and *p*-cresol.

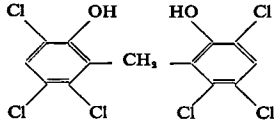
TABLE I

COLOURS DEVELOPED BY PHENOLIC COMPOUNDS OF PHARMACEUTICAL INTEREST



Substance	Substituents					Resulting colour in chloroform
	2	3	4	5	6	
Phenol						Strong orange colour
Chlorocresol		CH_3	Cl			Strong orange colour
o-Cresol	CH_3					Strong orange colour
m-Cresol		CH_3				Strong orange colour
p-Cresol			CH_3			No reaction
Chloroxylenol		CH_3	Cl	CH_3		Strong plum colour
Thymol	$CH(CH_3)_2$			CH_3		Moderately strong yellow colour
Chlorothymol	$CH(CH_3)_2$		Cl	CH_3		Moderately strong yellow colour
o-Amyl *m*-cresol	C_5H_{11}			CH_3		Strong orange colour
Salicylic acid	COOH					No reaction
Methyl salicylate	$COOCH_3$					Strong reddish-orange colour
Salol	$COOC_6H_5$					Strong reddish-orange colour
Salicylamide	$CONH_2$					Strong reddish-orange colour
p-Hydroxybenzoic acid			COOH			Strong orange colour
Methyl *p*-hydroxybenzoate			$COOCH_3$			No reaction
Propyl *p*-hydroxybenzoate			$COOC_3H_7$			No reaction
Resorcinol		OH				Brownish-orange colour
Hexylresorcinol	C_6H_{13}			OH		Brownish-orange colour
Guaiacol	OCH_3					Strong orange colour
Vanillin	OCH_3		CHO			No reaction
2:4:6-Trichlorophenol	Cl		Cl		Cl	Strong orange colour
Stilboestrol			X			No reaction
Hexoestrol			X			No reaction
Morphine			X	X	X	No reaction
Hexachlorophene*						Strong orange colours—
Dichlorophene†						insoluble in chloroform

* Hexachlorophene is



† Dichlorophene is

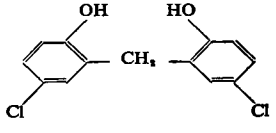


Table I shows the results obtained with a number of phenolic substances of pharmaceutical interest. In addition to the compounds mentioned, pyridoxine hydrochloride yields a strong plum colour which fades rapidly.

One or two of the substances listed call for special mention. As is to be expected, both *o* and *m*-cresol give strong colours while pure *p*-cresol does not react; this means that Cresol B.P., which is a mixture in which the *meta* isomer predominates, can only be determined in preparations with accuracy if a sample of the batch used in manufacture is available for use as a standard. The two dihydric phenols listed give rather unsatisfactory colours since the darkening characteristic of these substances in alkaline solution considerably modifies the orange colour. In general the colours produced are unstable in aqueous solution, some loss in intensity being observable after 15 minutes, but most of those deriving from pharmaceutical phenols may be readily extracted into chloroform, in which solvent they form stable solutions.

General Method

For Phenols giving Aminophenazone Dyes Soluble in Chloroform

Transfer a suitable aliquot of the prepared solution containing 0·2 to 0·4 mg. of the phenol to a 150 ml. separator. Add 1 ml. of *4-aminophenazone solution* and wash in with sufficient *dilute ammonia buffer* to give a volume of approximately 50 ml. Add 1 ml. of *potassium ferricyanide solution* and mix. Extract the solution with 25 ml. of chloroform followed by two shakings each of 10 ml. passing each extract into a dry 50 ml. graduated flask through a small plug of cotton wool previously moistened with chloroform. Dilute to 50 ml. with chloroform, mix and read the optical density in a 1 cm. cell using an Ilford No. 602 filter and chloroform in the comparison cell. For phenol use a 0·5 cm. cell.

For Phenols giving Aminophenazone Dyes Insoluble in Chloroform

Transfer a suitable aliquot of the prepared solution containing 0·2 to 0·4 mg. of the phenol to a 50 ml. graduated flask and add 1 ml. of *4-aminophenazone solution*. Wash in with *dilute ammonia buffer* to produce a volume of about 45 ml. Mix and add 1 ml. of *potassium ferricyanide solution* and dilute to 50 ml. with the dilute buffer. Mix and read the optical density as rapidly as possible in a 1 cm. cell using an Ilford No. 603 filter and a suitable aliquot of the prepared solution, diluted with dilute ammonia buffer to 50 ml. in the comparison cell.

Reagents. 4-Aminophenazone solution. Dissolve 0·5 g. in 25 ml. of water, shake and filter. This solution is stable for 2 to 3 days. *Potassium ferricyanide solution.* Dissolve 2 g. of potassium ferricyanide in 25 ml. of water. This solution should be prepared daily. *Strong ammonia buffer.* Dissolve 67·5 g. of ammonium chloride in 570 ml. of strong solution of ammonia and dilute to 1 litre with water. *Dilute ammonia buffer.* Dilute 2 ml. of strong ammonia buffer to 1 litre with water.

Some Observations on the General Method

The method, as described above, has been based on considerable experimental work to determine the optimum conditions, particularly of pH and of quantity of ferricyanide solution added.

The effect of pH was examined by carrying out the method on a solution of chlorocresol at each of 9 pH levels between 4 and 12. Simultaneously control determinations were carried out using no chlorocresol. As can be seen from Figure 1, the blank value is very high in aqueous and quite appreciable in chloroformic solutions at pH values below 9 and at values greater than 10 the maximum colour is not obtained. In aqueous solutions the very high blank at pH values below 9 is due to the formation of "antipyrine red". Earlier workers[3,6,7] have suggested various means of adjusting the reaction solution to a pH of about 10 but unless the solution is buffered the pH may fall to a value as low as 7 or 8 on reaction, which may lead to erroneous results. For this reason an ammonia buffer solution has been recommended and in the many determinations which have been carried out by this procedure the initial pH has been about

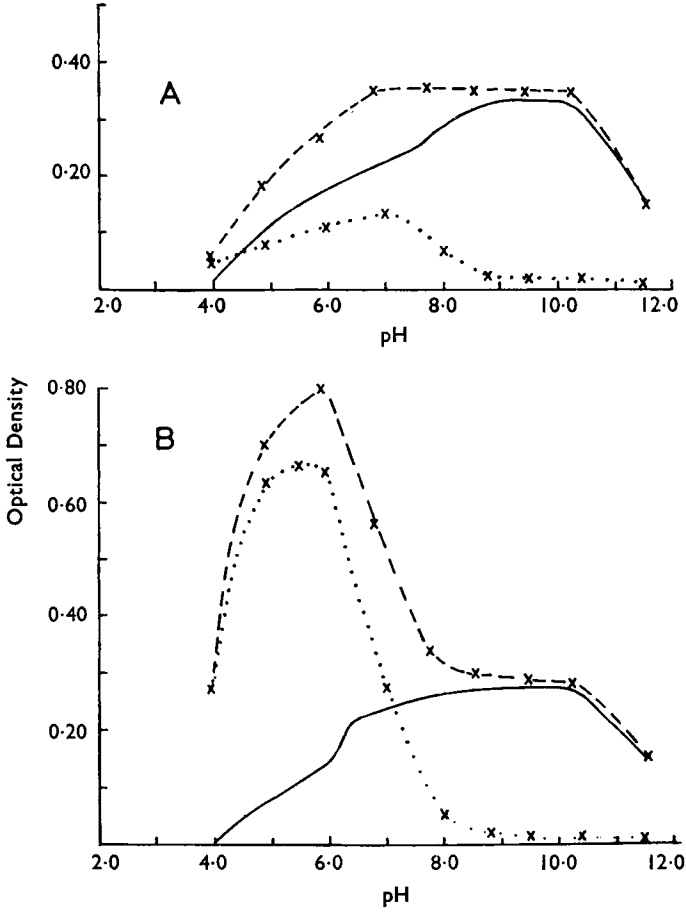


FIG. 1. The effect of pH on the colour formed with chlorocresol. A, in chloroform; B, in water; – – – – – reagents and chlorocresol; . . . . . reagents only; ——— difference due to chlorocresol.

10 and has never fallen below 9·4; this means that the pH is maintained safely within the permitted limits (Fig. 1).

The quantity of potassium ferricyanide required has also been studied in detail. The recommended amount ensures that sufficient is present without excess. The excess has little effect on the chloroform extraction procedure but it contributes to an increased blank value in the aqueous method. The choice of order of addition of reagents recommended by Ettinger[3] has been confirmed; it is important to prevent the aminophenazone reagent and the ferricyanide from coming into contact with each other except in the buffered solution. Temperature changes within the usual laboratory limits have no significant effect on the intensity of colour produced. In aqueous solutions the colour of the blank slowly increases whilst that of the sample slowly fades and the extinction value of the solution should be measured within 5 minutes if this method is being used; in chloroform the colours are stable over long periods and little or no change has been observed after 24 hours.

The absorption characteristics of each substance considered have also been examined and curves from 400 to 600 mμ have been prepared in both aqueous and, where possible, chloroformic solutions. In aqueous solutions most phenols show a broad peak in the region of 480 to 500 mμ but in chloroform there is a fairly definite maximum. This occurs at about 450 mμ for phenol itself and for most of the other substances listed in Table I giving an orange colour. With salicylamide and methyl salicylate the peak occurs at about 490 mμ and for chloroxylenol at about 510 mμ. Beer's Law is obeyed over the range 0 to 0·4 mg. for all those substances listed giving colours (with the exception of the two dihydric phenols).

Application of the Method to Pharmaceutical Preparations

The general method has been applied to the determination of the phenolic constituents in injection solutions and a considerable number of other pharmaceutical preparations. A selection from these is given below.

Injection Solutions

The phenolic preservative in many injection solutions has been determined by a direct application of the method described above. The following general procedure is applicable:

Dilute 1 ml. of injection solution to 100 ml. with *dilute ammonia buffer*, mix thoroughly, filter if necessary, and transfer a suitable aliquot to contain about 0·2 to 0·4 mg. of the phenol to a 150 ml. separator and continue as described in the general method (chloroform extraction procedure) beginning with the words "Add 1 ml. of *4-aminophenazone solution*. . . ".

Some typical recovery results are shown in Table II. In addition to the examples listed a sample of Injection of Ascorbic Acid was prepared in the laboratory to contain 5 mg. of phenol per ml. Ten determinations were made on this sample (five on each of two successive days) and a mean recovery of 101·1 per cent of the expected value was obtained (standard deviation 0·467).

One series of injection solutions, the Insulin Zinc Suspensions, contain methyl *p*-hydroxybenzoate (methylparaben) as a preservative and this does not couple with aminophenazone. In this case a preliminary hydrolysis is necessary similar to that described below for the determination of methylparaben in a suspension for oral use.

TABLE II

RECOVERY OF PHENOLIC BACTERIOSTATS FROM INJECTION SOLUTIONS

Injection solution	Content of Bacteriostat (mg. per millilitre)					
	Nominal		Found	Added	Found	Recovery per cent
Aneurine hydrochloride ..	Cresol	3 mg.	3·20	2·0	5·22	101
Bismuth	Chlorocresol	1 mg.	1·08	2·0	3·11	101·5
Bismuth oxychloride* ..	Chlorocresol	1 mg.	0·60	1·0	1·62	101
Cyanocobalamin	Phenol	5 mg.	5·20	2·5	7·80	104
Concentrated liver* ..	Chlorocresol	1 mg.	0·80	1·0	1·78	98
Heparin*	Cresol	3 mg.	2·40	2·0	4·44	102
Insulin	Phenol	2·5 mg.	2·60	2·0	4·58	99
Insulin zinc suspension ..	Methyl *p*-hydroxybenzoate	1 mg.	0·90	2·0	2·84	97
Stilboestrol dipropionate ..	Phenol	6 mg.	6·15	2·0	8·20	102·5

* These samples were several months old.

Injection of Stilboestrol Dipropionate B. Vet. C. is an example of an oily injection and in such a case the bacteriostat may be determined by the method described below for Carbolised Oil B.P.C., 1 g. of sample and a 50 ml. aliquot of the solution after dilution to 1 litre is satisfactory.

Assuming, as is usually the case, the direct application method can be used, the whole determination can be carried out within 15 or 20 minutes and this compares very favourably with the time required for a steam distillation and bromination assay. Providing attention is paid to certain details such as the adequate washing down of the reagents, the method is capable of yielding accurate results in the hands of junior personnel. In the extensive series of recovery experiments which have been made it seems evident that the method is capable of yielding results well within $\pm$ 3 per cent of the true figure.

Solution of Chloroxylenol B.P.

Dilute 1 ml. with 2 ml. of *strong ammonia buffer* and sufficient water to produce 1 litre. Take 10 ml. in a 150 ml. separator and continue as described in the general method (chloroform extraction procedure) beginning with the words "Add 1 ml. of 4-*aminophenazone solution* . . ." but using an Ilford No. 604 filter.

Ointment of Methyl Salicylate B.P.C.

The method described below has given satisfactory recoveries but if the alkaline solution is allowed to stand just before colour development a progressive hydrolysis of the ester occurs and low results are obtained. No serious loss takes place within 5 minutes, however, and this allows adequate time to carry out the necessary operations of mixing, pipetting and adding the aminophenazone and ferricyanide reagents.

To about 0·5 g., accurately weighed, in a 100 ml. round-bottomed flask add 20 ml. of ethanol (95 per cent) and two glass beads; reflux for 30 minutes on a water bath using a straight bore water condenser. Allow to cool and wash down the condenser with 10 ml. of ethanol (95 per cent); cool thoroughly and filter through a fast filter paper (Postlip Mills 11 cm.) into a 100 ml. graduated flask. Wash the flask and paper well with ethanol (95 per cent) to produce 100 ml. Transfer 10 ml. to a litre graduated flask which already contains 2 ml. of strong ammonia buffer and 800 ml. of water and dilute to 1000 ml. with water. Mix thoroughly and immediately transfer 10 ml. to a 150 ml. separator and continue by the general method (chloroform extraction procedure) beginning with the words "Add 1 ml. of 4-*aminophenazone solution* . . ." but using a 0·5 cm. cell.

This method is equally applicable to Compound Ointment of Methyl Salicylate and may be carried out much more rapidly than the official procedure.

Non-staining Ointment of Iodine with Methyl Salicylate B.P.C.

This method is similar to that described for Ointment of Methyl Salicylate except that an additional heating with ethanol has been prescribed since a somewhat intractable residue is formed from which it is difficult to extract all the methyl salicylate with a single treatment.

Proceed by the method described for Ointment of Methyl Salicylate to the words "filter through a fast filter paper (Postlip Mills 11 cm.) into a 100 ml. graduated flask". During this filtration retain the bulk of the residue in the flask. Wash flask and filter two or three times with ethanol (95 per cent), add 20 ml. of the ethanol to the residue in the flask and reflux again for 30 minutes; cool and filter through the same paper. Wash flask and filter with ethanol (95 per cent) until exactly 100 ml. of filtrate and washings have been collected. Transfer 10 ml. to a litre flask which already contains 2 ml. of strong ammonia buffer and 800 ml. of water and dilute to 1000 ml. with water. Mix thoroughly and immediately add 50 ml. to a 150 ml. separator and continue by the general method (chloroform extraction procedure) beginning with the words "Add 1 ml. of 4-*aminophenazone solution* . . ." but using a 0·5 cm. cell.

Gargle of Potassium Chlorate and Phenol B.P.C.

Again a direct application of the general method is possible without the need for the steam-distillation procedure described in the B.P.C. The method described for Solution of Chloroxylenol is applicable except that 2 ml. of sample is taken and an Ilford No. 602 filter is used.

Ointment of Zinc Oxide and Camphor B.N.F.

In this case it has been found convenient to extract the phenol into an alkaline aqueous solution and this is conveniently carried out by the following method:—

Transfer about 0·5 g., accurately weighed, to a 250 ml. round-bottomed flask and add 25 ml. N sodium hydroxide, 5 ml. of industrial methylated

spirit and two glass beads. Reflux for 1 hour under a straight bore condenser; wash down with plenty of hot water and allow to cool. Filter through a fast paper (Postlip Mills 11 cm.) into a litre graduated flask and wash well with cold water. To the contents of the graduated flask add 5 ml. of a 25 per cent solution of ammonium chloride and dilute to the mark. Mix thoroughly, transfer 10 ml. to a 150 ml. separator and continue by the general method (chloroform extraction procedure) beginning with the words "Add 1 ml. of 4-*aminophenazone solution*. . . ".

Carbolised Oil B.P.C. 1949

A similar procedure to that described for the above ointment may be applied, but using 0·5 g. of sample and 12·5 ml. of 2N alcoholic potassium hydroxide.

Determination of Hexachlorophene in a Dusting Powder

The formulation under consideration contained hexachlorophene (1 per cent), together with other organic substances and fillers. The determination is of particular interest since hexachlorophene forms with 4-aminophenazone a dye which is insoluble in chloroform and the aqueous procedure must therefore be used. The following method has given good recoveries.

To about 1 g., accurately weighed, add 20 ml. of acetone and stir well. Allow to settle and decant the supernatant liquid through a filter paper into a dry 100 ml. flask, washing the residue and filter paper with successive quantities of acetone to remove all soluble material. Adjust the volume of the filtrate to 100 ml., mix and transfer 2 ml. into a 50 ml. graduated flask. Continue by the general method (aqueous procedure) commencing with the words "Add 1 ml. of 4-*aminophenazone solution*. . . ".

Determination of Phenol in Strong Solution of Iodine with 2 per cent Phenol

Dilute 25 ml. of the sample to 100 ml. with 90 per cent ethanol. To 10 ml. add 20 ml. of water and titrate with 0·1N sodium thiosulphate until the iodine is just decolourised. This titration may be used to report the percentage of iodine present. Wash the titration solution into a litre graduated flask, add 2 ml. of *strong ammonia buffer* and dilute to the mark. Mix and transfer 5 ml. of the dilution to a 150 ml. separator and continue by the general method (chloroform extraction procedure) commencing with the words "Add 1 ml. of 4-*aminophenazone solution*. . . ".

Determination of p-*Hydroxybenzoates used as Preservatives*

Methyl *p*-hydroxybenzoate is not directly determinable, of course, but a preliminary hydrolysis converts it to the acid and the general method may then be applied. The following procedure has been found to be satisfactory for the determination of methyl and propyl *p*-hydroxybenzoates in a number of preparations.

Take 10 ml. of sample containing 0·2 per cent of methyl *p*-hydroxybenzoate in a 250 ml. conical flask and wash in with 10 ml. of water. Add 25 ml. of N sodium hydroxide solution and reflux gently for 2 hours. Wash down the condenser with water, cool and transfer to a litre graduated flask containing 5 ml. of a 25 per cent solution of ammonium chloride.

Wash the flask out with water and dilute the hydrolysate and washings to 1 litre. Mix and filter, rejecting the first few mls. Transfer 10 ml. of the filtrate to a 150 ml. separator and proceed as described in the general method (chloroform extraction procedure) commencing with the words "Add 1 ml. of 4-*aminophenazone solution*. . . ".

TABLE III

RECOVERY OF PHENOLIC COMPOUNDS FROM PHARMACEUTICAL PREPARATIONS

Preparation	Content of phenolic substance		
	Expected	Found by proposed method	Found by reference method
Solution of Chloroxylenol B.P.†	Chloroxylenol 5·0 per cent w/v	5·00	
" " " *		5·08	5·11
" " " *		5·28	5·08
" " " •		5·00	5·15 (Piria and Schiff)
Carbolised Oil B.P.C. 1949†	Phenol 5·0 per cent w/v	5·12	
" " " "		4·94	4·80 (Bromometric)
Garg. Pot. Chlor. and Phenol B.P.C.†	Phenol 1·30 per cent w/v	1·30	
" " " " " *		1·26	1·24 (B.P.C. Method)
Hexachlorophene dusting powder†	Hexachlorophene 1·0 per cent w/v	0·97	
" " " †		1·03	
" " " †		0·97	
" " " *		1·02	
Ointment of Zinc Oxide and Camphor B.N.F. †	Phenol 3·2 per cent w/v	3·16	
" " " " " *		2·95	3·39
" " " " " •		3·17	3·28 (Iodimetric)
Ointment of Methyl Salicylate B.P.C.†	Methyl salicylate 50 per cent w/v	49·2	
" " " " *		49·4	51·2
" " " " •		52·1	48·5
" " " " *		52·6	48·9 (B.P.C. Method)
Non-staining Ointment of Iodine with Methyl Salicylate †	Methyl salicylate 5·9 per cent w/v	5·83	
" " " " •		5·82	5·72
" " " " *		5·49	5·44
" " " (old sample)		5·36	5·53 (B.P.C. Method)

• Production samples.
† Laboratory prepared samples.

Table III lists some results obtained in recovery experiments on some of the examples given above. The few applications given have been chosen to demonstrate the scope of the method, since it would not be practicable to list all the applications which have been made.

DISCUSSION

The 4-aminophenazone method is considered to be superior to many other methods of determining phenols because it is simple to operate, it is rapid, the results are reproducible, the reagents are readily available and are stable, and the method is applicable over a fairly wide range of concentrations.

REFERENCES

1. Elvidge, Proctor and Baines, *Analyst*, 1957, **82**, 367.
2. Emerson, *J. org. Chem.*, 1943, **8**, 417.
3. Ettinger, Ruchhoft and Lishka, *Analyt. Chem.*, 1951, **23**, 1783.
4. Shaw, *ibid.*, 1951, **23**, 1788.
5. Mohler and Jacob, *ibid.*, 1957, **29**, 1369.
6. Gottlieb and Marsh, *Industr. Engng Chem.* (*Anal. Ed.*), 1946, **18**, 16.
7. Martin, *Analyt. Chem.*, 1949, **21**, 1419.

DISCUSSION

The paper was presented by MR. R. A. SAVIDGE.

THE CHAIRMAN. The method was not of universal application and each preparation would require individual study. Would the sesamol present in sesame oil react with 4-aminophenazone? Did Tubocurarine react?

MR. S. G. E. STEVENS (London). After what time did the change in the dye become appreciable? Was there degradation at the interface during extraction and would this interfere? What was the partition ratio? Could highly emulsified products be separated within 15 minutes by the technique described.

DR. W. MITCHELL (London). Did vanillic acid give a positive reaction?

PROFESSOR W. H. LINNELL (London). Had the authors any evidence that the sulphonic group and all the alkoxy groups were actually eliminated or was this assumed from the production of a colour? There might be another type of reaction with vanillin; the aminophenazone would react with the aldehyde group.

DR. R. E. STUCKEY (London). Had amounts of less than 0·1 per cent of phenol or particularly of hexachlorophene been determined?

DR. F. L. ROSE (Macclesfield). The mechanism of the reaction seemed to be similar to the coupling of diazonium salts which displaced sulphonic acid groups and the same would take place here. Had aniline interfered with the reaction?

MR. H. B. HEATH (Sudbury). Was it necessary to determine a standard curve on each occasion? What instrument was used? Would the results for methyl salicylate using a No. 604 filter have been as reproducible as those using a No. 602 filter?

MR. G. J. W. FERREY (Manchester). Were the results in Table III single determinations or means? Did the Authors suggest that the 7 per cent discrepancy between the methods for ointment of methyl salicylate mean that the B.P.C. method was 7 per cent in error? It would be preferable to dissolve ammonium chloride in water and add ammonia when making the buffer.

MR. C. A. JOHNSON replied. He could not explain why there was apparently no *ortho* coupling and asked assistance from organic chemists for an explanation.

MR. SAVIDGE replied. One could predict if a phenol would react by its structure. If the *para* position were free or occupied by the radicals mentioned in the paper it would react. Stilboestrol had the position blocked so phenol could be determined in its presence. He had not tried sesame oil, tubocurarine or vanillic acid, but no interference had been observed when vanillin was present. It was difficult to believe that the sulphonic acid or chlorine groups were expelled in the reaction, but it was the only theory they could put forward. The results seemed to show that the reaction must take place in the *para* and not the *ortho* position. They had not tried determining hexachlorophene at 0·1 per cent concentration, but many of the phenols in injections had been determined at this level. Aniline interfered with the reaction, but in none of the preparations examined were there any aniline derivatives. Standard curves for each phenol had been prepared. Filter instruments had been used because they were more widely available, but spectrophotometers could be used. The 604 filter had not been used for salicylates. They were investigating the determination of two phenols together by the two-point method. The results in Table III were the means of duplicates, and those who had used the B.P.C. method would understand the discrepancy.