THE HYDROLYSIS OF ASPIRIN IN PHARMACEUTICAL PREPARATIONS. A LIMIT TEST FOR FREE SALICYLIC ACID

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Aspirin hydrolyses readily in the presence of water, therefore its pharmaceutical preparations may contain free salicylic acid unless produced and stored in an environment free from moisture.

Since salicylic acid has irritant properties it is desirable to limit the hydrolysis of aspirin before presentation to the consumer, and this is reflected by the B.P. Limit Tests for free salicylic acid in Aspirin and Tablets of Aspirin.

Difficulties met in applying the B.P. colorimetric test prompted an investigation of the kinetics of aspirin hydrolysis and of conditions affecting the formation and stability of the ferric-salicylate complex. A number of factors such as time, temperature, *p*H, concentration, ionic strength and the presence of certain ions, are all influential in determining the accuracy of the colorimetric test. A method of general applicability is described, with provision for a preliminary isolation of the salicylic acid if interference from citrate, phosphate or sulphate is expected. A simplified modification of the proposed general method is also described.

In the course of the present investigation, proprietary tablets and powders containing aspirin, were examined. Some of the tablets also contained codeine, phenacetin and caffeine and were of similar composition to those described in the Pharmacopœia.

Kinetics of Aspirin Hydrolysis

Hydrolysis of apirin proceeds by an acid-base catalysis mechanism[1–6]. Edwards[6] showed that if six simultaneous reactions involving dissociated and undissociated aspirin, water, hydrogen ions, and hydroxyl ions be assumed then a quantitative account could be given.

The rate of hydrolysis can be expressed by the equation

$$\frac{dm}{dt} = CKe^{-kt} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where m is the amount hydrolysed in a time t, C is the initial concentration of aspirin, and K the net velocity constant.

The rate of hydrolysis is dependent on *p*H, since an acid-base catalysis mechanism is involved. In certain *p*H ranges only one or two of the six reactions predominate. Thus, in strongly acid solutions the main reaction is $H_3CCOO{\cdot}C_6H_4{\cdot}COOH + H_3O^+ \rightarrow HO{\cdot}C_6H_4{\cdot}COOH + CH_3{\cdot}COOH + H^+$ In strongly alkaline solution $H_3CCOO{\cdot}C_6H_4{\cdot}COO^- + OH^- \rightarrow HO{\cdot}C_6H_4{\cdot}COO^- + CH_3COO^-$ is the principal reaction. At about *p*H 7 the reaction is primarily $H_3CCOO{\cdot}C_6H_4{\cdot}COO^- + H_2O \rightarrow HO{\cdot}C_6H_4{\cdot}COOH + CH_3{\cdot}COO^-$; and in the neighbourhood of *p*H 2·5 three reactions take

place at similar velocities. All these reactions are dependent on temperature. The variation of the net velocity constant K with *p*H and temperature is seen in Figure 1. Under conditions of hydrolysis where K is small the reaction approximates to a zero order reaction, and is of constant rate for the first few hours. If equation (1) is converted to parts per million per hour the initial rate is given by the expression

$$7{\cdot}667 \times 10^5 \text{Kp.p.m./hr.} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Thus for a solution containing 4 g/l. of aspirin at 25° C. the rate becomes 1850 p.p.m./hr.

Factors Affecting the Ferric-Salicylate Test

Time. Time is an important factor in a quantitative test for free salicylic acid in aspirin. Errors due to this cause are minimised in the limit test described in the B.P. monograph on Aspirin, but are less controllable in the case of the test on Aspirin Tablets where a preliminary filtration

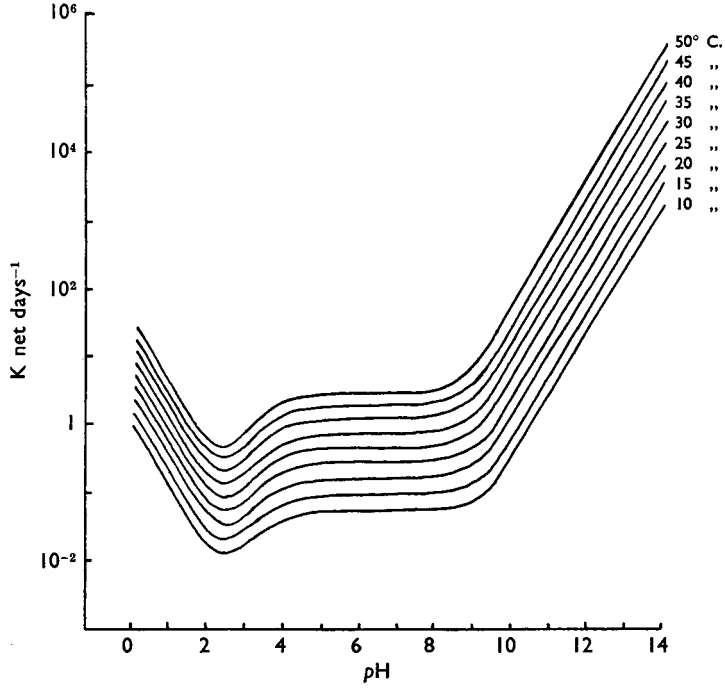

Fig. 1. The effect of variation of *p*H and temperature on the net velocity constant of aspirin hydrolysis.

is required. A larger error may arise with compound tablets of aspirin, because of the variable time taken to arrive at the point of colour matching.

Temperature. Edwards[6] described the influence of temperature on the rate of hydrolysis of aspirin and the relation is shown in Figure 1.

*p*H *Ionic Species and Ionic Strength*

Figure 1 shows the need to make quantitative tests at a constant *p*H. In estimating free salicylic acid, an additional factor was encountered in the sensitivity of the ferric-salicylate colour to changes in *p*H. Also the intensity of the ferric-salicylate complex was found to be influenced by the concentration of certain ions.

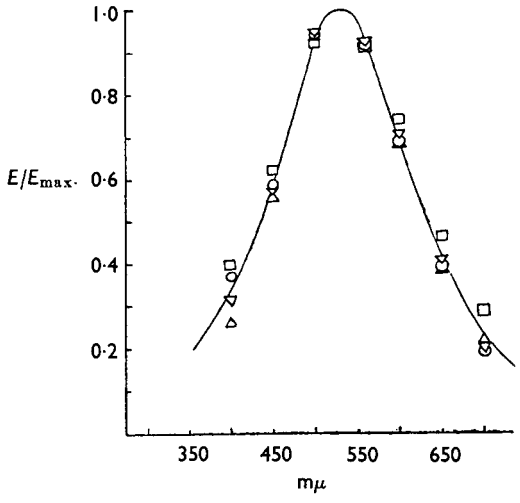

FIG. 2. Absorption spectrum of the ferric-salicylate complex in solutions of different *p*H and ionic strength (I).

Comparison cell solution. 2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + appropriate acid and base all made up to 50 ml.

Test cell solution. Ditto + 600 μg. salicylic acid. *p*H and I adjusted by addition of the correct proportions of appropriate acid and base.

○ Potassium chloride + hydrochloric acid. *p*H = 2·75, I = 0·1.

△ Ammonium hydroxide + sulphuric acid. *p*H = 2·75, I = 0·1.

▽ Ammonium hydroxide + sulphuric acid. *p*H = 2·75, I = 0·03.

□ Ammonium hydroxide + sulphuric acid. *p*H = 2·0, I = 0·1.

Spectrophotometric measurements. Some of the results of absorption measurements of the ferric-salicylate complex expressed as $E/E_{max.}$, are shown in Figure 2. Maximum absorption at 530 mμ was in good agreement with previous workers[7–10] and the extinction coefficient at this wavelength (E 530) was used in all subsequent measurements.

Choice of buffer solution is complicated by the formation of iron-ion complexes, or insoluble compounds with many of the conventional buffer systems. The variation of $E_{4\,cm.}$ 530 with *p*H in a number of these systems for a constant concentration of salicylic acid and ferric alum is shown in Figure 3. All the test solutions in this series of experiments were prepared with a constant calculated ionic strength (I) of 0·1 ($I = \frac{1}{2}\Sigma cz^2$ where c and z are respectively the molar concentration and charge of each ion present.)

Although ferric hydroxide is precipitated at or above a *p*H of approximately 3·0, some buffers, notably those containing acetates, inhibit precipitation; thus it is possible to see a maximum in the E 530 — *p*H curves in Figure 3.

Figure 4 shows the buffering effect of the various systems under the experimental conditions likely to be met in estimations of free salicylic acid in aspirin. The variation of E 530 with salicylate concentration, shown in Figure 3 is greatest in the region *p*H 2·75 to 3·75. For measurements

in this region the acetic acid—ammonium chloroacetate or glycine-acetic acid buffer systems appear the most suitable. The low buffer capacity of the nitric acid system in this region should be noted. The solution obtained in the B.P. method is essentially similar, though less concentrated. The *p*H of this solution may vary between 2·5 and 3·5 depending on the accidental presence of traces of acid or base, consequently errors of up to 20 per cent. can be expected.

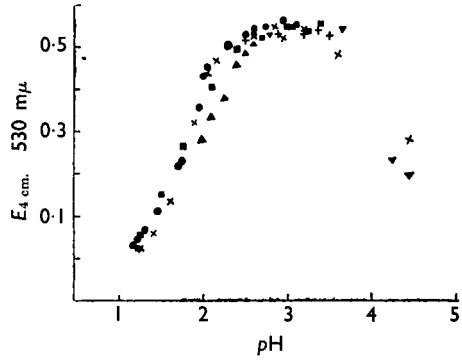

FIG. 3. The change in absorption of the ferric-salicylate complex with variation of *p*H in different buffer solutions of ionic strength 0·1.

Comparison cell (4·0 *cm.*) *solution.* 2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + buffer all made up to 50 ml.

Test cell (4·0 *cm.*) *solution.* Ditto + 600 μg. salicylic acid.

- ● Nitric acid–potassium hydroxide.
- × Hydrochloric acid–potassium acetate.
- ■ Hydrochloric acid–glycine.
- ▲ Hydrochloric acid–ammonium monochloroacetate.
- ▼ Acetic acid–ammonium acetate.
- \+ Acetic acid–ammonium monochloroacetate.

Addition of acids, bases, salts, and other materials were made to solutions containing fixed amounts of salicylic acid, ferric alum, and buffer to test the stability of the monochloroacetate system. The *p*H and $E_{4\,cm.}$ 530 values were then measured and are shown in Table II. The system appears to buffer sufficiently to deal with a number of possible constituents of aspirin preparations, as well as the slight contamination of acids and bases which might be accidentally encountered in the laboratory.

The influence of the ionic strength and species was assessed, and the change in $E_{4\,cm.}$ 530 per unit ionic strength of solution, at constant *p*H, is given in Table I. The effects of sulphate and phosphate ions are notable.

Despite the wide variation in *p*H, ionic strength and species in experiments, little change was observed in the wavelength of the absorption maximum, or the approximately symmetrical form of the absorption band, as illustrated in Figure 2. This confirms the assumption that it is the concentration and not the composition of the ferric-salicylate complex which changes with environment.

Solubility

The B.P. Test specifies an amount of aspirin equivalent to 4 g. per litre of 4 per cent. ethanol. The solubility of aspirin in this solvent has been determined spectrophotometrically[6] and the results are reproduced in Table III. They indicate that the current B.P. limit tests for salicylic acid are suspect at temperatures below 21°C.

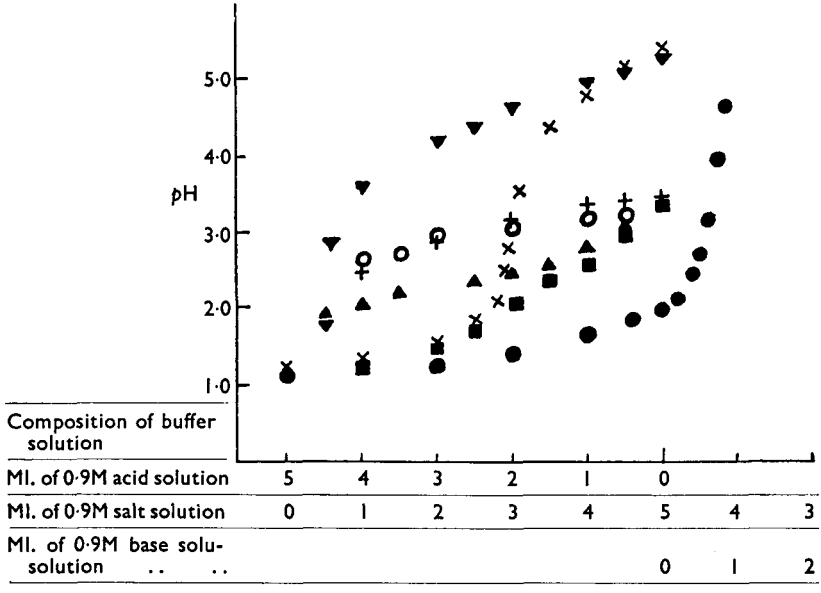

FIG. 4. The buffering effect of various buffer systems in the presence of the ferric-salicylate complex.

Solution. 2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + 5 ml. 0·9M buffer solution + 600 μg. salicylic acid all made up to 50 ml.

- ● Nitric acid—potassium hydroxide.
- × Hydrochloric acid—potassium acetate.
- ■ Hydrochloric acid—glycine.
- ▲ Hydrochloric acid—ammonium monochloroacetate.
- ▼ Acetic acid—ammonium acetate.
- \+ Acetic acid—ammonium monochloroacetate.
- ○ Acetic acid—glycine.

Pharmaceutical Materials Commonly Associated with Aspirin

Materials commonly occurring with aspirin in pharmaceutical preparations have been examined for possible interference in the formation of the ferric-salicylate complex. Phenacetin, codeine phosphate, caffeine and quinine sulphate were added separately to solutions containing a fixed concentration of ferric alum and varying concentrations of salicylic acid. The values of E 530 obtained were compared with those from control solutions and are shown in Table IV. The materials were added in such a way as to simulate the concentrations expected in the examination of a compound preparation.

With the exception of the codeine phosphate, and possibly quinine sulphate, the results showed, within the limits of experimental error, that no interference occurs.

DISCUSSION

A number of factors affect the accuracy of estimations of free salicylic acid in aspirin.

The kinetics of the hydrolysis of aspirin shows the necessity to correct for the degree of hydrolysis occurring in the course of a test for free salicylic acid. Consequently, it is essential to fix the temperature and *p*H of the test solution.

The experimental evidence on the formation and stability of the ferric-salicylate complex emphasises the importance of controlling the *p*H and the concentration of various substances in the test solution. Interference of certain ions, particularly phosphate and sulphate is noted. The inhibiting effect of citrate is, of course, well known[11,12]. Neither phenacetin, codeine nor quinine interfere, but phosphate or sulphate ions do.

A spectrophotometric method, designed to take into account these various factors, is described below and a special procedure is recommended where phosphates, sulphates or citrates would be expected to interfere.

TABLE I

CHANGE IN ABSORPTION AT 530 mμ OF THE FERRIC-SALICYLATE COMPLEX WITH VARIATION IN CALCULATED IONIC STRENGTH (I)*

System examined	Change in absorption at 530 mμ ($E_{4\text{ cm.}}$ 530) per unit ionic strength of solution at constant *p*H	
	*p*H 2·75	*p*H 2·0
$NH^+ - NO_3^-$	+0·35	+0·55
$NH_4^+ - SO_4^{2-}$	+1·50	+3·3†
NH_4^+ — Acetate	+0·25	
$K^+ - NO_3^-$	+0·20	+0·65
$K^+ - SO_4^{2-}$	+1·85	+3·8†
$K^+ - PO_4^{3-}$	+5·20†	
$K^+ - Cl^-$	+0·20	
K^+ — Acetate	+0·25	+0·90
Mg^{2-} — Acetate	+0·30	
Glycine — Cl^-	+0·25	
Glycine — Acetate	+0·30	
$Al^{3+} - NH_4^+ - Cl^-$	+0·9†	
$Ca^{2+} - NH_4^+ - NO_3^-$	−0·3†	

* *Comparison cell* (4 *cm.*) *solution:*—2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + appropriate acid and base all made up to 50 ml.

Test cell (4 *cm.*) *solution:*—Ditto + 600 μg. salicylic acid.

*p*H + I adjusted by addition of the correct proportions of the appropriate acid and base.

† Very approximate due to non-linearty of $E_{4\text{cm.}}$ 530 — I relationship.

THE PROPOSED PROCEDURE FOR ESTIMATING SALICYLIC ACID IN ASPIRIN PREPARATIONS

TABLE II

THE EFFECT OF VARIOUS ADDITIONS TO BUFFERED FERRIC ALUM–SALICYLATE SOLUTIONS*

Addition	*p*H	$E_{4\text{ cm.}}$ 530
No additions	3·0	0·540
1 ml. 0·9M Monochloroacetic acid	2·75	0·528
0·5 ml. 0·9M Hydrochloric acid	2·50	0·495
1·0 ml. 0·9M Acetic acid	3·0	0·540
0·5 ml. 0·9M Ammonium acetate	4·0	0·570
1·0 ml. 0·9M Glycine	3·3	0·564
0·5 ml. 0·9M Potassium acetate	3·7	0·572
1·0 ml. 0·3M Calcium nitrate	3·0	0·547
1·0 ml. 0·9M Potassium nitrate	3·0	0·552
1·0 ml. 0·2M Potassium hydroxide	3·3	0·589
1·0 ml. 0·3M Ammonium sulphate	3·1	0·510
1·0 ml. 0·9M Ammonium nitrate	3·1	0·544
1·0 ml. 0·3M Magnesium acetate	3·9	0·576
1·0 ml. 0·3M Aluminium chloride	3·1	0·521
0·5 ml. 0·9M Nitric acid	2·6	0·487
1·0 ml. 0·2M Ammonium hydroxide	3·3	0·556

* *Comparison cell* (4 *cm.*) *solution.* 0·032M ammonium chloroacetate and 0·008M acetic acid. 0·0008 per cent. ferric ammonium sulphate. 4·0 per cent. ethanol.

Test cell (4 *cm.*) *solution.* Ditto + 12 μg./ml. salicylic acid (equivalent to 3000 p.p.m. on the basis of the B.P. test).

The procedure may be subdivided into (*a*) one of general applicability for aspirin or preparations containing aspirin in the absence of interfering materials, and (*b*) a modification of (*a*) for use when citrates, sulphates, or phosphates are present in the preparation.

Procedure (a)

Reagents. 0·2 per cent. Ferric ammonium sulphate. Buffer

solution 0·08M acetic acid and 0·32M ammonium monochloroacetate. Absolute ethanol.

Preparation of ferric ammonium sulphate solution. To 0·2 g. of ferric ammonium sulphate crystals are added 6 ml. of 10 per cent. nitric acid and about an equal amount of water. This is boiled until dissolved (about ½ minute). After cooling, the solution is made up to 100 ml. in a graduated flask.

TABLE III

THE SOLUBILITY OF ASPIRIN IN 4 PER CENT. ETHANOL

Temperature ° C.	Solubility of aspirin g./litre
10	2·65
15	3·26
20	3·93
20·4	4·00
25	4·76
30	5·72

Preparation of buffer solution. After dissolving the appropriate quantities of ammonium monochloroacetic acid and acetic acid (A.R. grades) the *p*H should be adjusted by addition of either acetic acid or ammonia, so that a solution of *p*H 2·95 ± 0·05 is obtained on mixing 5 ml. of buffer solution, 5 ml. of ferric alum solution, 2 ml. of ethanol and making up to 50 ml.

TABLE IV

THE INFLUENCE OF PHARMACEUTICAL MATERIALS ON THE FERRIC-SALICYLATE COMPLEX

Salicylic acid		Absorption of test solution $E_{4\ cm.}$ 530				
µg./ml.	Equivalent in B.P. test	No addition	+ Phenacetin	+ Caffeine	+ Quinine sulphate	+ Codeine phosphate
2·0	500	0·105	0·106	0·104	0·100	0·100
6·0	1500	0·284	0·280	0·289	0·286	0·277
12·0	3000	0·545	0·545	0·555	0·535	0·530
20·0	5000	0·895	—	—	—	0·840

METHOD

The powdered sample, 0·2 g., is weighed and dissolved (or partially dissolved in the case of certain preparations) in 2 ml. of ethanol in a 50 ml. graduated flask. The flask is placed in a thermostat at 25° C., and about 35 ml. of distilled water at 25° C. is added. Simultaneously the time (T_0) is noted. As soon as possible afterwards, 5 ml. of buffer solution and 5 ml. of ferric ammonium sulphate solution is added, and the volume of the solution made up to the mark. The whole is then well shaken to ensure adequate mixing and solution of any precipitated aspirin. After about 10 minutes slightly more sample than is required to fill a 4 cm. cell is then removed with a pipette and, if necessary, filtered as quickly as possible through a No. 1 Whatman paper into the absorption cell. The time (T_1) is noted when the extinction coefficient measurement at 530 mµ is made, and a comparison cell containing an identical solution without the aspirin is used. Speed of filtration is important, particularly if the laboratory temperature differs greatly from 25° C. In order to hasten filtration, sintered glass filter sticks or plugs of cotton wool in 0·5 cm. polythene tubes attached to pipettes, with or without the aid of a filter pump, have been used to advantage.

At least 3 samples should be withdrawn at intervals of not less than

10 minutes. A value for the extinction coefficient at zero time (T_0) is then obtained by extrapolation.

The salicylic acid concentration in μg./50 ml. is then read from the salicylic acid $E_{4\text{ cm.}}$ 530 mμ calibration curve, the data for which is given in Table V. If the aspirin content of the preparation is known, the result is readily converted to p.p.m. of aspirin.

TABLE V

RELATIONSHIP BETWEEN SALICYLIC ACID CONCENTRATION AND EXTINCTION COEFFICIENT AT 530 mμ*

Salicylic acid μg./50 ml.	$E_{4\text{ cm.}}$ 530
200	0·188
400	0·358
600	0·545
800	0·720
1000	0·895
1200	1·078

* *Comparison cell* (4 *cm.*) *solution*:—2 ml. absolute ethanol + 5 ml. 0·2 per cent. ferric ammonium sulphate solution + 5 ml. buffer solution all made up to 50 ml.
Test cell (4 *cm.*) *solution*:—Ditto + salicylic acid.

Corrections for Irrelevant Absorption

In order to correct for irrelevant absorption, measurements can be made using a similar solution and control solution but omitting the ferric alum solution. If the correction is significant it is advisable to make extinction measurements over a period of time so that an appropriate correction can be made for each of the readings at T_0, T_1, T_2, T_3. The correction for aspirin is very small and inside the normal experimental error of the extinction coefficient determination.

It is essential that the absorption cells themselves be matched, or that their absorption differences at 530 mμ be known.

Procedure (b)

Reagents. Benzene A.R. Tested for absence of reaction with ferric alum solution and dried over anhydrous sodium sulphate. 0·2 per cent. ferric ammonium sulphate solution. Absolute ethanol. Buffer solution 0·08M acetic acid and 0·32M ammonium monochloroacetate.

Method

0·2 g. of dry powdered sample is placed in a clean dry 100 ml. separating funnel plugged just above the tap with a small piece of cotton wool. The sample is extracted with four 10 ml. portions of benzene, the benzene extracts being collected in another dry separating funnel. The bulked benzene extracts are then extracted with 5 ml. of a solution containing 5 ml. 0·2 per cent. ferric ammonium sulphate solution and 5 ml. of buffer solution made up to 45 ml. with distilled water. The aqueous layer is run off into a 50 ml. graduated flask containing 2 ml. of absolute ethanol. Extractions are repeated until there is no further pink colouration in the aqueous layer.

When the salicylic acid has been completely extracted from the benzene, the rest of the buffered ferric alum solution, if any, is poured into the graduated flask and the solution is made up to the mark and shaken so that adequate mixing is ensured. If necessary, traces of benzene in this solution can be removed by filtration through a wetted filter paper, the first portion of the filtrate being discarded. A further portion is collected

and the absorption of this solution is measured in a 4 cm. cell at 530 mμ, and the free salicylic acid in the sample is calculated as before.

Comments on the Proposed Procedure

A simplification of procedure (*a*) may be expedient in certain routine work. Once the hydrolysis rate at a fixed temperature and given weight of sample has been determined, only one extinction coefficient reading need be taken at a known time.

No hydrolysis correction need, in most cases, be made when procedure (*b*) is used, provided the time for extraction and colour measurement is relatively small (about 10 minutes). This is because the partition of aspirin between benzene and ferric ammonium sulphate solution is such that the final aspirin concentration in the ferric alum solution can be neglected.

The limits of reproducibility of procedure (*a*) are within plus or minus 10 per cent., or plus or minus 50 parts per million for samples of free salicylic acid content of less than 200 parts per million.

In the case of procedure (*b*) control experiments showed that the recovery of salicylic acid was never less than 95 per cent.

Examination of Aspirin Preparations

A number of proprietary compound aspirin preparations were examined for free salicylic acid using the proposed procedure (*a*). The results were compared with those obtained using the B.P. method (Table VI). To obtain a proper comparison a range of salicylic acid standards (intervals equivalent to 100 p.p.m.) was used in the case of the B.P. method.

In both instances it was necessary to filter the solution before estimation of the extinction coefficient or a comparison of colour could be made. With the B.P. method the solutions sometimes developed a turbidity after filtration due to crystallisation or coagulation of a component. With 1 sample (No. 13) the colour developed was so far removed in hue from the standard that comparison was impossible in the case of the B.P. method. The solution of the particular sample was markedly alkaline and the error in this instance could be attributed to a *p*H effect.

The difficulties with turbidity in test solutions in the B.P. method were not experienced with the suggested procedure, probably because the monochloroacetic acid buffer hastens and completes coagulation of colloidal matter before filtration. Crystallisation was eliminated by virtue of the constant raised temperature used (25° C.).

The use of a simple colorimetric method is unsatisfactory with many compound aspirin preparations. If an adverse significance is to be attached to the presence of small quantities of salicylic acid in such preparations it is obviously desirable to have a presumptive standard, which must necessarily depend upon a reliable method of estimation. With this purpose in view it is suggested that attention might be given to establishing a limit for salicylic acid in the compound aspirin preparations in the Pharmacopœia, and that the procedure proposed in the present communication might serve as a method for consideration.

TABLE VI

EXAMINATION OF COMPOUND ASPIRIN PREPARATIONS FOR SALICYLIC ACID USING THE PROPOSED PROCEDURE (1) AND B.P. METHOD (2)

Sample No.	Active ingredients	Form	Free salicylic acid content		
			μg./g. of preparation	p.p.m. of aspirin in preparation	
				1	2
1	Aspirin	Tablet	750	850	500
2	Aspirin Phenacetin Caffeine	Tablet	1000	700	1100 Filtration lengthy
3	Aspirin Phenacetin Caffeine	Powder	425	460	300 Slightly turbid
4	Aspirin Phenacetin	Powder	200	360	300 Slightly turbid
5	Aspirin Phenacetin Caffeine	Powder	1100	2200	1800 Turbid
6	Aspirin Phenacetin Caffeine	Tablet	475	1100	800
7	Aspirin Phenacetin Caffeine	Tablet	1150	2330	2200 Filtration lengthy
8	Aspirin Phenacetin Codeine phosphate	Tablet	400	1000	400 Turbid
9	Aspirin Phenacetin Codeine phosphate	Tablet	700	1740	700 Turbid
10	Aspirin Phenacetin Caffeine Codeine phosphate	Tablet	3900	8950	2300 Turbid
11	Aspirin Phenacetin Caffeine Quinine sulphate	Tablet	1170	1940	900 Fluorescence due to quinine
12	Cacium Aspirin	Powder	3800	4500	3000
13	Aspirin Sodium bicarbonate	Powder	4200	35,000	Unobtainable. Solution was orange-brown in colour
14	Aspirin Phenacetin Caffeine	Tablet	400	830	400 Slightly turbid
15	Aspirin Citric acid Calcium carbonate	Tablet	1270*	2560*	Unobtainable. No colour owing to presence of citrates
16	Aspirin Citric acid Sodium bicarbonate	Powder	570*	7200*	Unobtainable. No colour owing to presence of citrates

* Procedure (*b*) used.

SUMMARY

1. The kinetics of the hydrolysis of aspirin and the conditions for formation and stability of the ferric-salicylate complex have been studied.

2. The resulting information is discussed in relation to the B.P. method for assay of free salicylic acid in aspirin preparations.

3. A spectrophotometric method based on the experimental data is described.

4. Results of assays, using both methods, on currently available preparations are compared.

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DISCUSSION

The paper was presented by MR. D. N. GORE.

DR. D. C. GARRATT (Nottingham) said that as far as he knew there was no pharmacological evidence that the amount of free salicylic acid in aspirin or aspirin tablets complying with the limit test of the Pharmacopœia was of any significance. While not disparaging the work of the authors he appealed to analysts to use a sense of proportion with regard to limit tests. In the case of some sulphate and chloride limit tests the effect of other ions present might introduce errors of the order of 50 per cent., but the tests in his opinion were nevertheless sufficient.

He asked whether the optical density of the colour developed in the test was not very low and would it not be better if the test were modified so that a greater depth of colour was obtained.

DR. W. MITCHELL (London) suggested that in the Pharmacopœial test, it was usual to end up by trying to match entirely different colours! If the author had succeeded in eliminating this difficulty it would be very valuable.

DR. G. E. FOSTER (Dartford) suggested that in the determination of the free salicylic acid in compound tablets of aspirin numerous factors were involved. For instance, while the solutions were being prepared for the test, the aspirin might hydrolyse. In that connection it would be interesting to know whether there was evidence of any increase in free salicylic acid while extraction was in progress. Again, was there any information concerning the time required for the ferric salicylate to give maximum colour? It was common knowledge that in compound tablets

of aspirin there was more free salicylic acid present than in simple aspirin tablets when calculated on the basis of the aspirin actually present. In his experience a reasonable limit for the compound tablets would be about three times the amount permitted in the B.P. aspirin tablet.

MR. H. E. BROOKES (Nottingham) said it was refreshing to find that the authors had included in the paper the mechanism of the reaction. For a calculation of the hydrolysis of aspirin in equation 2, if K were taken for 35° C. the rate was approximately 7 to 8 per cent. per hour, presumably at the *p*H of the gut. The authors stated "since salicylic acid has irritant properties," but surely aspirin must have irritant properties if that were the rate of hydrolysis in the gut? Did the authors consider that it was really necessary to prescribe a narrow limit for salicylic acid and to use a rather more complicated method? Any considerable decomposition would be readily detectable by the odour of acetic acid in the tablets.

MR. C. A. JOHNSON (Nottingham) said he had found difficulty in completing an extraction within ten minutes, which was the suggested time, and the solution he obtained increased in optical density quite rapidly.

MR. R. L. STEPHENS (Brighton) said that care should be taken not to set such a low limit for the salicylic acid content of tablets that they could not be kept for a reasonable time under normal conditions of storage.

DR. J. G. DARE (Leeds) said that in fixing limits for any drug consideration must be given to the upper limits as determined by the adverse pharmacological effects and the lower limits determined by manufacturing economics. There was little evidence at present that aspirin tablets could not be made with an amount of free salicylic acid below the present limits, and he did not support any suggestion that the limits should be amended.

The CHAIRMAN of the Session (Dr. H. Davis) pointed out that while it was very easy for analysts, pharmacists, pharmacologists and doctors to maintain a sense of proportion with regard to the amount of free salicylic acid, if some persons could produce tablets of a lower limit than that of the Pharmacopœia, they had the finest advertisement they could possibly have.

DR. D. C. GARRATT (Nottingham) said he did not intend to suggest that there should be greater tolerance in the permissible amount of free salicylic acid but that a sense of proportion should be maintained.

MR. GORE prefaced Mr. Rapson's reply by expressing the view that two separate issues were involved in setting standards for free salicylic acid in aspirin or in compounded preparations containing aspirin. The physiological significance of traces of salicylic acid was a first consideration, and secondly there was the accuracy of the analytical procedure. The present contribution was primarily concerned with the latter, and suggested a method which was more adequate for stringent standards than the simple colorimetric procedure described in the B.P. monograph on aspirin and tablets of aspirin.

MR. RAPSON, in reply, agreed that the pharmacological evidence for the

deleterious effects of free salicylic acid is very slender. The colour developed in the test is weak. It could be increased by raising the concentration, which would require on solubility grounds an alteration in the alcohol content of the solvent. However, to depart too greatly from the B.P. test was felt undesirable at this early stage. He agreed with the comment on the difficulties of matching the colours in the B.P. test. He said there is evidence for hydrolysis during the extraction procedure B. Calculation indicated that errors due to hydrolysis are much less than one-tenth of those obtaining in procedure A. and was in agreement with experiments. He explained that errors can arise, if for example, finely divided aspirin passed through the cotton wool plug and in some cases it has been found necessary to use a filter paper. The experimental details in the paper are not precise enough in this respect. The 10 minutes allowed for extraction time may not be enough. The time taken for the ferric salicylate colour to develop is very short—less than one second. He said that the calculation of 7 to 8 per cent. per hour hydrolysis in the stomach—perhaps a little low due to the presence of enzymes—was very pertinent in connection with a limit for free salicylic acid. In conclusion, he agreed that a due sense of proportion must be maintained in setting the limits for salicylic acid content.