in the x/m values from 0.25 to 1.44 mg/g produced from 0.07 to 0.09 mg % of desorbed ethindrone (Fig. 3A). In 0.05 N HCl, the amounts of ethindrone desorbed were relatively higher. When the amounts of steroid desorbed were converted to percentages desorption (with reference to the x/m values), a gradual decrease occurred as the x/m values were increased (Fig. 3B).

The results of dissolution testing of the brand of contraceptive tablets in both water and 0.5% (w/v) magnesium trisilicate are shown in Fig. 4. The presence of the antacid in the medium drastically decreased the concentration of norethindrone acetate in solution. After 3 hr, the concentration of steroid in solution was less than 2%, compared with 75.2% in water. The reduction in concentration of steroid was a direct result of the adsorption onto the antacid particles.

It is suggested that the concurrent administration of magnesium trisilicate and oral contraceptive tablets containing the steroids tested might interfere with the steroid absorption. A recent report (9) that confirmed the decreased bioavailability in humans of digoxin in the presence of magnesium trisilicate lends supporting evidence to this suggestion.

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Improved Colorimetric Determination of Aspirin and Salicylic Acid Concentrations in Human Plasma

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Abstract □ An improvement in a previously described method for the determination of plasma salicylic acid and aspirin levels in humans is described. The procedure was simplified by employing only one plasma sample for both salicylates. More accurate estimation of salicylates, particularly aspirin, was achieved by using two different calibration curves. Salicylic acid was estimated by reaction with an aqueous solution of the Folin–Ciocalteu phenol reagent. Absorbance of the blue-colored complex, which formed on addition of sodium hydroxide, was measured at 670 nm. The influence of alkalinity in the formation of the colored complex is discussed. The average recovery of aspirin added to plasma was 94.61%; it was 214.72% by the previous method.

Keyphrases □ Aspirin—colorimetric analysis in human plasma □ Salicylic acid—colorimetric analysis in human plasma □ Colorimetry—analyses, aspirin and salicylic acid in human plasma □ Analgesics—aspirin and salicylic acid, colorimetric analyses in human plasma

Aspirin is hydrolyzed rapidly *in vivo* to salicylic acid (1–3). Determination of blood levels of both salicylates is of considerable pharmaceutical and clinical interest. Several spectrometric procedures are available (4–6) for the estimation of these salicylates after aspirin ingestion.

The colorimetric method of Smith (4), utilizing Folin–Ciocalteu phenol reagent, is used commonly to quantitate salicylates in plasma (Scheme I). The absorbance density of the colored complex formed in alkaline solution is read at 670 nm, and the salicylic acid concentration is obtained from a standard calibration curve. The aspirin concentration is estimated from the difference between free (nonhydrolyzed) and total (hydrolyzed) salicylates.

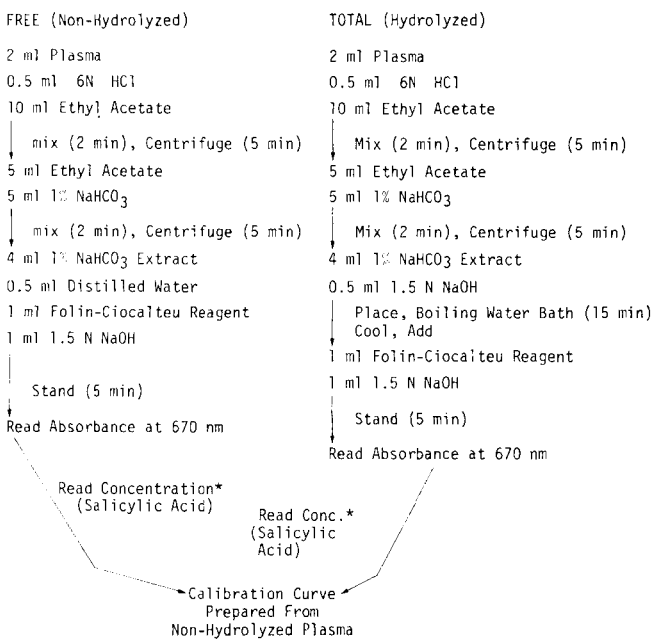
Table I—Typical Recoveries of Salicylic Acid

Added, μg[a]	Found, μg[b] Nonhydrolyzed Curve[c]	Found, μg[b] Hydrolyzed Curve[d]
5	5.25 ± 0.63	3.08 ± 0.55
10	10.50 ± 0.90	7.00 ± 0.66
20	21.67 ± 2.04	15.92 ± 1.67
25	23.90 ± 0.85	17.33 ± 1.04
40	42.33 ± 0.50	32.25 ± 0.43

[a] Amounts of salicylic acid in distilled water added per milliliter of human plasma sample. [b] n = 3. [c] Free curve, approximate pH 9.6 (Smith method, improved method). [d] Approximate pH 10.4 (improved method; used for total salicylates and aspirin determination).

When this general procedure was applied in this laboratory in bioavailability studies of various aspirin dosage forms in human volunteers, the absorbance intensity of the colored complex was extremely pH dependent. Furthermore, the Smith method (nonhydrolyzed standard curve) resulted in much higher recoveries of total salicylates and aspirin from human plasma because the final pH values of the colored solutions read at 670 nm are different for free (pH 9.6) and total (pH 10.4) samples. The average difference in pH values, about 0.8 unit higher in total (hydrolyzed) samples, is due to an additional amount of 0.5 ml of 1.5 N NaOH in the final test solution. As far as could be determined, the correlation between the alkalinity of a test solution and the absorbance of the colored complex has not been reported. Such a pH influence could be important in the estimation of salicylates, particularly aspirin.

The present report describes a modification of the Smith



*Scheme I—Flow chart of the Smith (4) method. *Blank plasma absorbance is subtracted before the concentration of salicylic acid is read.*

*Scheme II—Flow chart of the improved method. *Blank plasma absorbance is subtracted before the concentration of salicylic acid is read.*

method (4); it is simpler and gives more accurate results in the assay of salicylic acid, total salicylates, and aspirin in human plasma. Recoveries of salicylates from human plasma, as determined by the Smith method and by the modified method, are also reported.

EXPERIMENTAL

Chemicals—Pure salicylic acid[1], mp 157–159°, and aspirin[1], mp 135–137°, were used. All solvents were analytical grade. Folin–Ciocalteu phenol reagent[2] (diluted 1:3 with distilled water) was used for the colorimetric determination of salicylates.

Apparatus—A spectrophotometer[3] with an optical system providing a wavelength range from 220 to 1000 nm was used. The alkalinity of various samples was measured with a pH meter[4].

Plasma Extraction—Plasma samples, including blanks, were extracted according to Schemes I and II.

Colorimetric Analysis—All samples were read at 670 nm. A distilled water solution containing 1% $NaHCO_3$ was used as a reference. The samples were read 7–20 min after the addition of 1 ml of 1.5 *N* NaOH. In each instance, the pH of the final solution was measured before the absorbance was read.

pH Studies—The effect of varying pH on the absorbance intensity of the colored complex was studied using different concentrations of salicylic acid in 4 ml of 1% $NaHCO_3$ solutions (Scheme II). The total volume of each test sample at the time of the absorbance measurement was kept constant (6.5 ml). The pH was varied from 8.9 to 11.7 by adding different volumes of sodium hydroxide solutions of various normalities. The final volumes were adjusted to 6.5 ml by adding appropriate volumes of distilled water.

Preparation of Calibration Curves—Various concentrations of salicylic acid and aspirin in distilled water were added to plasma samples, which were processed as shown in Scheme II. The concentrations of salicylic acid *versus* their respective absorbances (aspirin was hydrolyzed to salicylic acid) were plotted on coordinate graph paper.

Recovery Studies—Several different concentrations of salicylates (mixtures of salicylic acid and aspirin in distilled water) were added to human plasma, and the samples were processed as shown in Schemes I and II. Recoveries of salicylates were calculated, after subtracting blank values, by employing only one calibration curve, free (nonhydrolyzed) or by using both the free (nonhydrolyzed) and total (hydrolyzed) calibration curves.

RESULTS AND DISCUSSION

Smith (4) used two different plasma samples (2 ml each), one for the analysis of salicylic acid and the other for aspirin analysis. The present simplified method employs only one plasma sample (2 ml); the sodium bicarbonate extract is divided into halves, one for salicylic acid analysis and the other for aspirin assay (Schemes I and II). This step reduces the

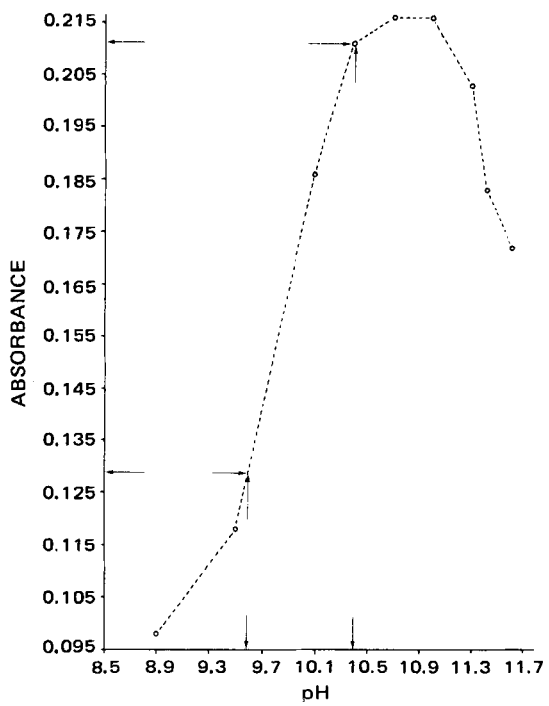


Figure 1—*Absorbance at various pH values.*

[1] Dow Chemical Co., Midland, Mich.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] DU model 2400, Beckman Instruments, Fullerton, Calif.
[4] Zeromatic II, Beckman Instruments, Fullerton, Calif.

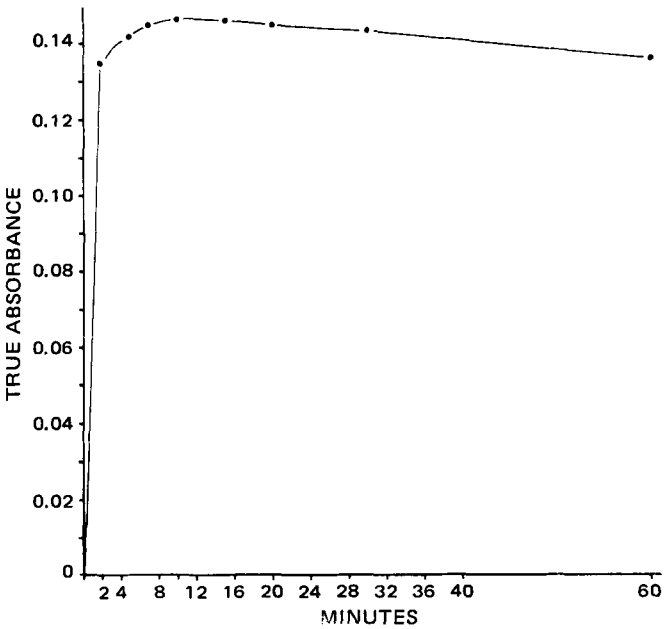


Figure 2—*Folin–Ciocalteu reagent color formation.*

volume of plasma needed for the complete analysis and eliminates two additional extraction steps. Improved accuracy was achieved by using two (nonhydrolyzed and hydrolyzed) calibration curves for the estimation of salicylic acid and total salicylate and, by difference, aspirin.

The reason for using two calibration curves is as follows. The absorbance intensity of the colored complex (a reduced form of molybdenum and tungsten) is extremely pH dependent. The final pH of the colored complex solution from nonhydrolyzed plasma (Smith referred to it as "free") was 9.61 ± 0.01 (n = 50), while the average pH of the hydrolyzed test samples (Smith's "total salicylates") was 10.41 ± 0.01 (n = 50). This result is probably due to the excess amount of 1.5 N NaOH added to the test samples for aspirin hydrolysis. Absorbance intensities of colored solutions containing similar concentrations of salicylic acid are markedly different at pH 9.6 and 10.4 (Fig. 1). Therefore, reading the values of both salicylic acid and total salicylate from a single curve prepared either at pH 9.6, as described by Smith, or at pH 10.4 gives erroneous results.

Tables I and II summarize the salicylate recovery data obtained. Average recoveries of aspirin (total salicylates − free salicylic acid/0.767) from human plasma were 214.72 ± 9.66, 169.71 ± 8.22, or 94.61 ± 11.68%

Table II—Typical Recoveries of Aspirin

Added, μg[a]	Found, μg[b]		
	Both Curves[c]	Nonhydrolyzed Curve[d]	Hydrolyzed Curve[e]
2.5	2.06 ± 1.46	5.00 ± 2.74	3.91 ± 2.17
5.0	3.26 ± 0.99	10.00 ± 1.21	7.82 ± 0.82
10.0	8.15 ± 2.54	19.99 ± 2.46	15.65 ± 2.07
12.5	14.67 ± 1.49	28.25 ± 2.33	23.22 ± 1.27
20.0	25.32 ± 0.76	49.54 ± 0.68	38.70 ± 0.77

[a] Amounts of aspirin in distilled water added per milliliter of human plasma sample. [b] n = 3. [c] Nonhydrolyzed curve and hydrolyzed curve (recommended for salicylic acid, total salicylates, and aspirin estimations). [d] Approximate pH 9.6 (recommended for salicylic acid estimation). [e] Approximate pH 10.4 (improved method; recommended for total salicylates and aspirin determination).

using a nonhydrolyzed curve (prepared as described by Smith and represented by $y = 0.0033x + 0.0017$), a hydrolyzed curve ($y = 0.0042x + 0.0073$), or both calibration curves, respectively. It is readily apparent from these data that the results obtained with two curves are more reliable than with either curve separately.

Figure 2 illustrates the rate of development and fading of the Folin–Ciocalteu blue color at room temperature. This finding was in general agreement with Smith's observation, and the time interval of 7–20 min was selected for measuring the absorbance of test samples.

In conclusion, the presently reported procedure, which is a modification of Smith's method for the estimation of plasma levels of salicylic acid and aspirin, is simpler and more accurate.

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