found did not exceed the original level. This indicates that bumetanide is excreted unconjugated in human urine.

However, the urinary excretion of bumetanide cannot be considered as a measure of orally absorbed drug since a biliary excretion of the drug cannot be excluded. Biliary excretion would make the results in accordance with the low dose dog assay, where an almost equal response to the drug was obtained after oral and intravenous administration.

The results confirm the marked saluretic and diuretic effect of bumetanide after oral administration of both 0.5 and 1 mg. to human volunteers and show clearly a parallelism between bumetanide excretion and saluretic action over the total period (Figs. 2 and 3).

SUMMARY

A GLC determination of bumetanide, using flash-heater methylation by means of a mixture of tetramethylammonium hydroxide and trimethylanilinium hydroxide, was developed. The flash-heater methylation product of bumetanide proved to be methyl 3-(N-n-butylanilino)-5-dimethylsulfamyl-4-methoxybenzoate, formed by methylation under simultaneous rearrangement. The GLC determination was found to be accurate at concentrations as low as 0.1 mcg./ml. human urine. In six healthy volunteers, the urinary excretion of sodium, potassium, and chloride, the urine volume, and the urinary recovery of bumetanide were determined after oral administration of 0.5 and 1 mg. of the drug. A parallelism between bumetanide excretion and saluretic action over the total period of response is shown.

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Influence of Solution Electrolyte Content and Dielectric Constant on Drug Adsorption by Kaolin

N. A. ARMSTRONG▲ and C. D. CLARKE*

Abstract □ The adsorption of benzoic acid and crystal violet on kaolin was investigated to elucidate the influence of the system dielectric constant and the electrolyte content on this example of drug-adjuvant interaction. The differing results obtained with the two adsorbates reflect the dissimilar adsorption sites on the kaolin platelet and the different mechanisms involved in the uptake of acids and bases on clays. The experimental data presented are satisfactorily explained by a consideration of the effect of both the dielectric constant and the electrolyte concentration and valency on adsorbent and adsorbate characteristics.

Keyphrases □ Kaolin, adsorption of benzoic acid and crystal violet—location of adsorption sites, influence of dielectric constant and electrolyte content □ Adsorption of benzoic acid and crystal violet by kaolin—influence of dielectric constant and electrolyte content □ Drug-adjuvant interactions—influence of dielectric constant and electrolyte content, drug adsorption by kaolin □ Dielectric constant, electrolyte content—effect on drug adsorption by kaolin □ Electrolyte content, dielectric constant—effect on drug adsorption by kaolin

Previous papers (1, 2) showed that the Langmuir adsorption isotherm represents the adsorption of benzoic acid and crystal violet on kaolin, and they discussed the effect of environmental pH on this equilibrium process. From the results obtained, the probable adsorption sites for acidic and basic materials on clays were suggested, namely that anionic materials were adsorbed on the edge and cationics on the cleavage surface of the clay. The significance of such an evident drug-adjuvant interaction on preservative efficiency or therapeutic potency was briefly discussed. The presence of soluble material in a suspension formulation, for example as active principle, flavoring, coloring, buffering, or peptizing agents will not only influence the formulation's pH but also contribute to the electrolyte content of the system. This increase in electrolyte concentration will also result in an increase in the dielectric constant of the system (3). Both of these factors influence the characteristics of the electrical double layer of the suspended phase, this double layer constituting the area where adsorption and/or ion exchange takes place. It was, therefore, considered desirable to determine the effect of both the system dielectric constant and the electrolyte content (with respect to concentration and valency) on the problem of drug-adjuvant interaction through investigating the influence of these factors on the uptake of benzoic acid and crystal violet on kaolin.
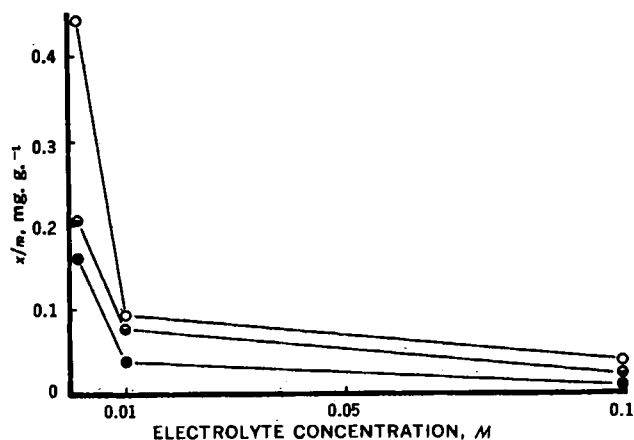
Figure 1—*Effect of electrolyte concentration and valency on the adsorption of benzoic acid on kaolin (pH 4.8). Key: O, sodium chloride; ◒, sodium sulfate; and ●, sodium citrate.*

EXPERIMENTAL

Materials—Benzoic acid (Analar grade), crystal violet, sodium salts, and ethanol (reagent grade) were used without further purification. Light kaolin (BP quality) was dried at 100° (3 hr.), blended thoroughly, and stored in sealed containers. The weight mean diameter of the kaolin was 3.3 μm. (sedimentation balance), the surface area was 11.2 m.²g.⁻¹ (nitrogen adsorption[1]), and the cation-exchange capacity was 10.3 meq./100 g. of clay (ammonium acetate method).

Influence of Electrolyte Concentration on Adsorption—*Benzoic Acid*—One hundred milliliters of a solution containing benzoic acid (40.mg.) in sodium chloride of strengths 0.001, 0.01, and 0.1 M was added to flasks containing kaolin (20 g.). Duplicate determinations were carried out in each case. After agitation at 24 ± 0.5° for 24 hr. and centrifugation at 10,000×g (2 min.), the supernate was diluted with 0.1 N hydrochloric acid and the benzoic acid concentration was determined spectrophotometrically at 275 nm. Solutions of benzoic acid were shown to obey Beer's law, and it was demonstrated that the presence of sodium chloride did not interfere with the assay procedure.

Crystal Violet—To flasks containing kaolin (2 g.) was added 100 ml. of solution containing crystal violet (50 mg.) and 1% w/v disodium ethylenediaminetetraacetate (I) (1 ml.) in sodium chloride solutions of strengths 0.001, 0.01, and 0.1 M. The sequestering agent was included to maintain the salts of soil cations in solution, which otherwise have only low water solubility. After agitation at 24 ± 0.5° for 12 hr., a portion of the suspension was centrifuged at 10,000×g (2 min.). The supernate was then examined for dye concentration by spectrophotometric assay at 590 nm. It also was examined for magnesium content by atomic absorption spectroscopy, since it was previously shown (1) that cation exchange is the major mechanism for dye uptake by kaolin. Solutions of crystal violet were previously found to obey Beer's law, and it was also shown that the presence of sodium chloride and I did not interfere with the assay methods.

Influence of Electrolyte Type on Adsorption—The effect of 0.001, 0.01, and 0.1 M concentrations of sodium chloride, sodium sulfate, and sodium citrate on the uptake of benzoic acid and crystal violet on kaolin was investigated. The procedure followed was as described above.

Influence of Dielectric Constant on Adsorption—Solutions of the adsorbate were prepared in solvents of differing water-ethanol proportions, thus providing a range of dielectric constants from 33.6 to 81.7.

Benzoic Acid—Benzoic acid (10 mg.) was dissolved in a water-ethanol mixture (100 ml.), the concentrations of ethanol being 0, 25, 50, 75, and 95% v/v. Kaolin (20 g.) was then added; and after equilibration, centrifugation, and dilution, the benzoic acid concentration of the supernate was spectroscopically determined. Duplicate determinations and appropriate blanks were used.
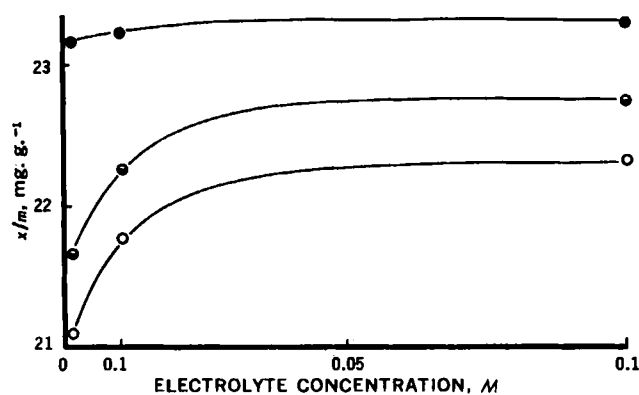
¹ Perkin-Elmer, Shell Sorptometer, model 212D.

Figure 2—*Effect of electrolyte concentration and valency on the uptake of crystal violet on kaolin (pH 5.6). Key: O, sodium chloride; ◒, sodium sulfate; and ●, sodium citrate.*

Crystal Violet—Crystal violet (50 mg.) and I (100 mg.) were dissolved in a water-ethanol mixture (100 ml.), the concentrations of ethanol being identical to those already described. Kaolin (2 g.) was then added; and after equilibration and centrifugation, the supernate was assayed for dye and magnesium content. Higher alcohol concentrations affected the determination of magnesium by atomic absorption spectroscopy, but this was corrected by use of a calibration curve of atomic absorption spectroscopic readings of known magnesium concentrations in the presence of alcohol.

RESULTS AND DISCUSSION

The molecular structure of kaolin, Al₄Si₄O₁₀(OH)₈, is composed of two basic units and is well documented (4, 5). One unit is an octahedron of oxygen atoms (or hydroxyl groups) surrounding a central aluminum atom, which forms a continuous sheet through the sharing of oxygen atoms of identical adjacent units. The second unit is composed of four oxygen atoms situated at the corners of a regular tetrahedron, with a silicon atom in the center. The atoms at the base of the tetrahedra are shared by neighboring modules, and the two basic units are joined such that the apexes of the tetrahedron and the top layer of the octahedron form a common plane (6). The origin and nature of the electrostatic charges to which the adsorptive capacity of the clay has been attributed and the variation of these charges with pH were discussed elsewhere (2, 3). It has been pointed out that the kaolin lamella has two distinct areas for adsorption: the kaolin edge surface and the larger cleavage plane surface.

Influence of Electrolyte Concentration and Valency on Adsorption—*Benzoic Acid*—The adsorption of benzoic acid on kaolin is markedly influenced by the electrolyte content of the system (Fig. 1), with sorption of the preservative decreasing with an increase in sodium chloride concentration. A valency increase of the electrolyte anion also further depresses the adsorption of benzoic acid on the clay.

Chloride, fluoride, and oxalate ions are known to be adsorbed on the edges of kaolin particles (7–9) because of their affinity for lattice aluminum. Moreover, phosphate anions are stated to be edge adsorbed on clays (10, 11), such phosphate adsorption being progressively reduced in systems of increasing saline concentrations (12). Citrate ions are also claimed to be edge adsorbed by clay minerals, the affinity of the anion for the adsorption site being greater than that of chloride ions, so that citrate is capable of displacing previously held chloride ions from the positive sites occurring on the edge of the kaolin platelet (13).

The results discussed in a previous article (2) strongly suggest that the adsorption of benzoic acid on kaolin is mediated through the attachment of the negatively charged benzoate anion to sites of positive charge occurring on the edge of the kaolin particles. Since the results of other workers, as briefly reviewed here, also indicate the edge adsorption of other anionic species, it seems reasonable to assume that in systems containing benzoic acid and other anionogenic material, competition will exist between the benzoate and other anions present for the available adsorption sites on the clay. Increasing the concentration of the added salt will favor its preferential sorption and, therefore, reduce the likelihood of vacant sites
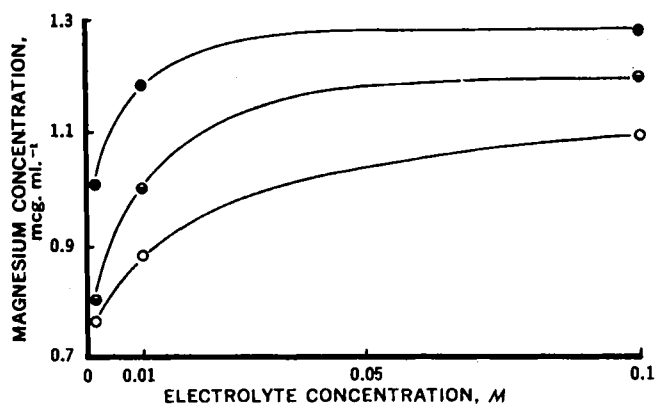
Figure 3—*Effect of electrolyte concentration and valency on the liberation of magnesium from kaolin at pH 5.6. Key:* O, *sodium chloride;* ◐, *sodium sulfate; and* ●, *sodium citrate.*

Figure 4—*Variation in the adsorption of benzoic acid on kaolin with the dielectric constant of the suspension medium.*

existing for benzoate anion sorption. The depressive effect of increasing the valency of the added anion is considered to result from its increased affinity for the adsorption sites, the affinity of binding being citrate > sulfate > chloride.

Crystal Violet—Contrary to its effect upon the adsorption of benzoic acid, an increase in the electrolyte content of the system raises the amount of dye taken up by kaolin (Fig. 2). The extent of dye removed from solution is also further increased with an increase in the valency of the added salts (Fig. 2). Blaug and Gross (14) obtained similar results for the adsorption of methantheline bromide by antacids in systems of varying electrolyte conditions.

Since it has been demonstrated that crystal violet uptake on kaolin is mediated through two distinct mechanisms, namely a cation-exchange process and physical adsorption (1), it is necessary to determine the influence of added electrolyte on each of these mechanisms to explain the overall increase in dye uptake.

The distribution of ions in the electrical double layer is a function of the electrolyte content of the bulk solution, the diffuse counterion atmosphere being compressed toward the surface with increasing bulk electrolyte concentration. The effect of the electrolyte addition on the surface potential and the surface charge of the particle depends on the type of double layer. With most hydrophobic colloids, the double layer is created by the adsorption of potential-determining ions when the magnitude of this potential is not affected by the addition of an indifferent electrolyte, providing the presence of the electrolyte does not influence the activity of these potential-determining ions. For this type of double layer, therefore, the surface charge of the particle increases with increasing indifferent electrolyte concentration, and the surface potential remains constant.

The electrical double layer on the cleavage surfaces of kaolin is of a different type, since this surface has a constant charge solely determined by the type and degree of isomorphous replacement of metal cations within the lattice. The charge density of the layer surface is, therefore, independent of the presence of electrolytes in suspension (15). However, an increase in bulk electrolyte concentration decreases the surface potential and the ζ-potential due to the compression of the double layer, which, in turn, favors a decrease in the amount of dye held by physical adsorption. This effect is small, since the added anions are of the same sign as the surface charge. On this basis, the uptake of dye should lessen with an increase in the bulk electrolyte concentration.

The results shown in Fig. 2 indicate an overall increase in dye uptake with increased electrolyte content. It is considered that the influence of the added salts on the cation-exchange process must be responsible for this, the ion-exchange mechanism being responsible for the greater part of the total amount of dye held by kaolin (1). Increasing both electrolyte concentration and valency produces an increase in ion exchange, as indicated by the presence of magnesium ions in the suspending medium (Fig. 3). This increased exchange of sodium for divalent ions produces an increasingly negative charge on the clay, leading to increased dye uptake.

Sorby *et al.* (16) noted the increase of promazine adsorption on kaolin and talc from solutions of increasing sodium chloride concentration. These authors commented that sodium ions, as well as promazine, compete for the anionic sites on the kaolin surface;
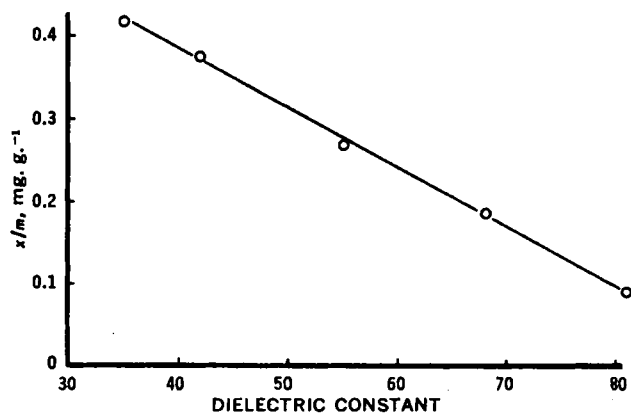
thus the addition of large amounts of sodium ion would be expected to decrease the extent of promazine adsorption if ion-exchange mechanisms were solely responsible for uptake. They concluded that since adsorption of the phenothiazine derivatives was actually increased in systems of high electrolyte concentration, ion exchange cannot be the only mechanism of adsorption. They suggested that the sodium chloride may be exerting its action through effects on the solubility of the promazine or on the physical properties of the adsorbent. This argument ignores the influence of the exchange of univalent sodium for counterions of higher valency on the imbalance of charge on the clay surface.

The observed increase in uptake on kaolin of positively charged crystal violet from solutions of increasing electrolyte concentration is, therefore, concluded to be due to the increase in the negative charge on the clay surface due to the exchange of divalent compensating cations on the clay surface for the added sodium ions.

Effect of Dielectric Constant on Adsorption—*Benzoic Acid*—The adsorption of benzoic acid on kaolin shows a linear increase in uptake with a decrease in the dielectric constant of the suspension medium (Fig. 4).

Crystal Violet—Contrary to what was observed for benzoic acid adsorption, the overall uptake of crystal violet by kaolin shows a linear decrease with decreasing dielectric constant of the suspending medium (Fig. 5). To determine whether this was due to the effect of the dielectric constant on ion exchange or physical adsorption, the extent of the magnesium replacement under these conditions was determined. The ion-exchange process, as indicated by the appearance of magnesium in the clay supernate, was markedly increased with an increase in the ethanol proportion (Fig. 6); that is, although the total uptake of dye progressively diminished in systems of lowering dielectric constant, the degree of ion exchange occurring increased under these conditions.

The use of ethanol-water mixtures in differing proportions to achieve a range of solvent dielectric constants introduces many
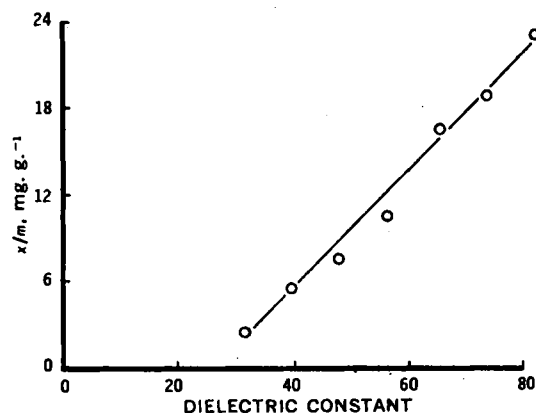

Figure 5—*Variation in the uptake of crystal violet on kaolin with the dielectric constant of the suspension medium.*
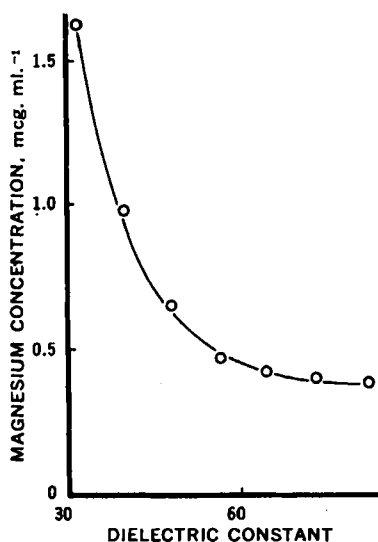
Figure 6—*Influence of the dielectric constant of the suspension medium on the liberation of magnesium from kaolin.*

variable and confusing factors, so the role of the dielectric constant becomes very difficult to identify. Such water–ethanol solutions can influence the characteristics of both adsorbent and adsorbate.

Influence of Water–Ethanol Mixtures on Adsorbent Characteristics—With a decreasing dielectric constant of the system, the diffuse counterion atmosphere surrounding the clay particle is compressed toward the particle surface, thereby reducing the value of the ζ-potential and the surface potential (15). The potential reduction might be expected to diminish the force of attraction between the surface and the adsorbate, leading to a decrease in the amount of drug held by physical adsorption. Indeed, Coulomb's law relates the force of attraction between two charged surfaces, q_1 and q_2, separated by a distance, r, to the medium dielectric constant, D, by the relationship:

$$\text{force of attraction} = \frac{q_1 q_2}{D r^2} \quad \text{(Eq. 1)}$$

A further effect on the adsorbent is the possible preferential adsorption of alcohol by the clay which may result in displacement of the zero point of charge (15).

Furthermore, increasing concentrations of ethanol alter the viscosity and surface tension of the system when the increased wetting of the solid by solutions of decreasing surface tension might favor increased sorption through enhanced adsorbent–adsorbate contact.

Influence of Water–Ethanol Mixtures on Adsorbate Characteristics—The degree of solvation of the ions in the system (i.e., their effective size) is reduced in solutions of increasing ethanol concentration, this effect favoring better adsorbent–adsorbate contact.

Ionization and dissociation of the adsorbates are depressed in conditions of low medium dielectric constant.

All of these considerations are simultaneously influencing the adsorbent–adsorbate system; hence, pending further investigations, a possible valid explanation can only be tentatively proposed for the results obtained in this study.

On the basis of previously discussed experimental evidence (2), the adsorption of benzoic acid on kaolin is considered to be mediated through the attachment of benzoate anions to the positive adsorption sites located on the edge of the clay particles. However, although dissociation of the acid to benzoate anion is depressed in solutions of low dielectric constant, adsorption is greatest under these conditions (Fig. 4). It is, therefore, to be assumed that these experimental results reflect the increased force of adsorbent–adsorbate attraction under conditions of low dielectric constant as expressed by Coulomb's law, this taking dominance over the other aqueous ethanol effects in the system.

The uptake of dye is more complex, involving as it does the mechanisms of cation exchange and physical adsorption. The total amount of dye taken up by the kaolin is seen to decrease with a decreased medium dielectric constant (Fig. 5), and this may be due to the suppression of dye ionization and dissociation in these solutions. However, a decrease in dye uptake is accompanied by a marked increase in ion exchange, a seemingly contradictory situation. Although ion-exchange mechanisms in aqueous systems have been well documented, little work appears to have been done on exchange in organic or mixed solvent systems, and the data available are often of a conflicting nature.

It is stated (17) that smaller ions will be more tightly held to the exchange surface than larger ions. In solutions of increasing ethanolic concentration, the ionic radius of the exchanging sodium and magnesium ions decrease as the ions become less hydrated. Grim (18) claimed that in alcohol, exchangeable cations are held in the order of their true (nonhydrated) ionic size; magnesium was observed to have a smaller ionic radius than sodium (19) and, therefore, was less likely to be replaced. The results obtained in the study are contrary to this observation.

Moody and Thomas (20) observed that the use of ethanol in a solvent system influences the degree of selection of a cation exchanger toward the available ions in the ambient solution. Wiegner and Jenny (21) studied ion exchange between a calcium zeolite and various alkali chlorides dissolved in aqueous ethanol, and they observed that more sodium ions passed on to the exchanger as the concentration of ethanol was increased. Other workers (22–24), using synthetic cation-exchange resins (hydrogen form) in aqueous ethanol systems, noted an increased exchange of sodium–hydrogen with an increasing ethanol content of the system.

From the results of this study and the observations of previous workers, it seems reasonable to suggest that the selectivity of the clay toward exchangeable cations in the surrounding solution is strongly influenced by the ethanol concentration of the system. The theory is advanced that with an increasing ethanol concentration, the kaolin shows a greater affinity for cation exchange involving sodium ions in preference to those of the dye species. This would produce an increasing negative charge on the clay and, therefore, promote increased crystal violet uptake by physical adsorption. However, cation exchange represents the major mechanism in dye uptake by kaolin; with increased exchange of solution sodium ions, the participation of the dye in this process is suppressed, resulting in the overall decrease in dye uptake in solutions of decreasing dielectric constant.

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Determination of Total Salicylates, Phenacetin and/or Total N-Acetyl-p-aminophenol, and Caffeine in Biological Samples and Analgesic Formulations

HIRA L. GURTOO▲ and BARRIE M. PHILLIPS

Abstract ☐ An analytical method was developed for the determination of phenacetin (apparent phenacetin), total salicylate (aspirin, salicylic acid, and its conjugates), total N-acetyl-p-aminophenol (N-acetyl-p-aminophenol and its conjugates), and caffeine in biological samples. Two 1-ml. samples are required. The first sample, after being acid hydrolyzed (2 N HCl, 2 hr., 121°) to convert total salicylate to free salicylic acid and total N-acetyl-p-aminophenol to p-aminophenol, is extracted with chloroform to remove caffeine and salicylic acid. p-Aminophenol in the aqueous phase is estimated by its reaction with phenol and sodium hypobromite. Salicylic acid extracted from the chloroform with sodium bicarbonate solution is estimated by reacting it with either Folin–Ciocalteu reagent or ferric nitrate. The caffeine, remaining in the chloroform, is estimated from its absorbance at 276 nm. In the second sample, phenacetin is hydrolyzed (8 N HCl, 6 hr., 121°) to p-aminophenol (total p-aminophenol), which is estimated as before. Time-dependent plasma and kidney levels of caffeine, phenacetin, total N-acetyl-p-aminophenol, and total salicylate, following the oral administration of a single dose (500 or 900 mg./kg. body weight) of aspirin–phenacetin–caffeine mixture to rats, were determined. The method also was adapted to the analysis of aspirin–phenacetin–caffeine powders.

Keyphrases ☐ Analgesic formulations—determination of total salicylates, phenacetin and/or total N-acetyl-p-aminophenol, and caffeine ☐ Plasma and tissue levels—determination of total salicylates, phenacetin and/or total N-acetyl-p-aminophenol, and caffeine ☐ Salicylate content, biological samples and analgesic formulations—determination, mixtures with phenacetin and caffeine ☐ Phenacetin and/or N-acetyl-p-aminophenol content, biological samples and analgesic formulations—determination, mixtures with aspirin and caffeine ☐ Caffeine content, biological samples and analgesic formulations—determination, mixtures with aspirin and phenacetin ☐ Aspirin–phenacetin–caffeine mixtures—determination of components in biological samples and analgesic formulations

As a result of continuing interest in the elucidation of the biochemical and toxicological mechanisms of "analgesic abuse nephropathy," different groups of researchers have employed single-entity or combination analgesics in their *in vivo* studies. Aspirin, phenacetin, and caffeine, the common components of analgesic mixtures, and their metabolites (1–6) have been studied

and the current consensus points to the involvement of analgesic mixtures rather than of any single component in the production of "analgesic abuse nephropathy" (7).

In the final analysis, the availability of an analytical technique suitable for the analysis of caffeine and total amounts of salicylate and phenacetin in the tissue or biological fluid is more meaningful as injury to the tissue is related to the concentration of the injurious chemical in that tissue. This report describes an analytical method developed for the estimation of phenacetin and total N-acetyl-p-aminophenol (which includes N-acetyl-p-aminophenol and its conjugates) as p-aminophenol, total salicylates (including aspirin, salicylate, and its conjugates) as free salicylate, and caffeine. The major advantages of this method are its accuracy, its applicability to small size samples, and its feasibility for the analysis of aspirin, phenacetin, and caffeine in compound analgesic tablets and powders.

EXPERIMENTAL

For the GC analysis, a gas chromatograph[1] was used under the following operating parameters: column contents, 3% OV-1 on Anakrom Q, 80–100; column size, 0.42 cm. × 1.83 m. (0.125 in. × 6 ft.); column temperature, 160°; carrier gas, helium; carrier gas flow rate, 33 ml./min.; detector, flame-ionization type; injection port and detector block temperatures, 300°; hydrogen flow rate, 3 ml./ min.; and air flow rate, 300 ml./min.

Chemicals—Sodium salicylate[2], p-aminophenol[3], phenacetin[3], and caffeine[4] of analytical grade were obtained. Analytical grade dichloroethylene, sodium bicarbonate, sodium hydroxide, dibasic potassium hydrogen phosphate, ferric chloride, and hydrochloric acid were used. Solvent grade ether and chloroform were washed successively with 1 N NaOH, 1 N HCl, and distilled water (three washings) before use. Double-distilled water was used for all purposes.

Biological Materials—The analyses were performed on rat plasma or rat kidney homogenate prepared by homogenizing (Potter-

[1] Varian 1200.
[2] Matheson, Coleman and Bell.
[3] Aldrich Chemical Co.
[4] Eastman Chemical Co., Inc.