

**Table III—Recovery of Steroids from a Synthetic Preparation**

Steroid	Added, μg	Recovery at 210 nm <sup>a</sup>			Recovery at 280 nm <sup>a</sup>		
		μg	%	CV	μg	%	CV
Ethinyl estradiol	51.86	50.98	98.3	1.3	51.96	100.2	1.1
Mestranol	100.23	98.97	99.0	1.0	99.78	99.8	1.1
Ethinodiol diacetate	1015.5	1008.6	99.3	0.7	—	—	—

<sup>a</sup> Average of five determinations.

**Table IV—Assay of Commercial Tablets<sup>a</sup>**

Steroid	Wavelength Setting, nm	Product A		Product B		Product C	
		% Label Claimed	CV	% Label Claimed	CV	% Label Claimed	CV
Ethinyl estradiol	210	99.9	1.2	101.3	1.3	—	—
	280	101.3	1.9	102.5	1.8	—	—
Mestranol	210	—	—	—	—	103.2	1.3
	280	—	—	—	—	100.8	0.8
Ethinodiol diacetate	210	96.5	0.9	95.2	0.9	92.4	0.5

<sup>a</sup> Average of 10 determinations.

analyses between the two wavelengths used, whereas analysis of the synthetic formulation revealed a variation of 0.8%, which is relatively minor. Variations reported for ethinyl estradiol (~1.5%) are the same for tablets and the synthetic formulation.

### CONCLUSIONS

This HPLC procedure, using dual wavelength detection, is fast and accurate. It has been specially designed for single dosage form analysis as required in the USP–NF content uniformity test, and would advantageously replace the long, open-column chromatography procedure.

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## Effect of the pH–Zero Point of Charge Relationship on the Interaction of Ionic Compounds and Polyols with Aluminum Hydroxide Gel

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**Abstract** □ The adsorption of magnesium nitrate, docusate sodium, and mannitol by chloride-containing aluminum hydroxide gel or aluminum hydroxycarbonate gel can be directly related to the surface charge characteristics of the aluminum hydroxide gel as determined by the pH–zero point of charge (ZPC) relationship. Magnesium cation is completely adsorbed under pH conditions where the gel has a negative surface charge, i.e., when the pH is above the ZPC. Docusate sodium is more strongly adsorbed when the pH–ZPC relationship causes the surface charge of aluminum hydroxycarbonate gel to be positive indicating adsorption of the docusate anion. However, adsorption also occurred when the pH was above the ZPC suggesting that adsorption of the hydrophobic portion of docusate anion by van der Waals forces also contributes to the

overall adsorption mechanism. Mannitol is adsorbed under all pH conditions. However, greater adsorption occurs when the pH is above the ZPC. Maximum hydrogen bonding is believed to occur when mannitol acts as the proton donor and the negative aluminum hydroxycarbonate gel surface serves as the proton acceptor.

**Keyphrases** □ Aluminum hydroxide—gel, adsorption of ionic compounds and polyols, effect of pH–zero point of charge □ Aluminum hydroxycarbonate—gel, adsorption of ionic compounds and polyols, effect of pH–zero point of charge □ Adsorption—ionic compounds and polyols to aluminum hydroxide and aluminum hydroxycarbonate, effect of pH–zero point of charge

The zero point of charge (ZPC) is an important property of colloidal systems possessing a pH-dependent surface charge. The ZPC is the pH at which the net surface charge is zero; at this pH the densities of the positive and negative

charges are equal. The apparent surface charge can be controlled by adjusting the pH to be either below or above the ZPC to produce a positive or negative surface charge, respectively (1). A recent study demonstrated the impor-

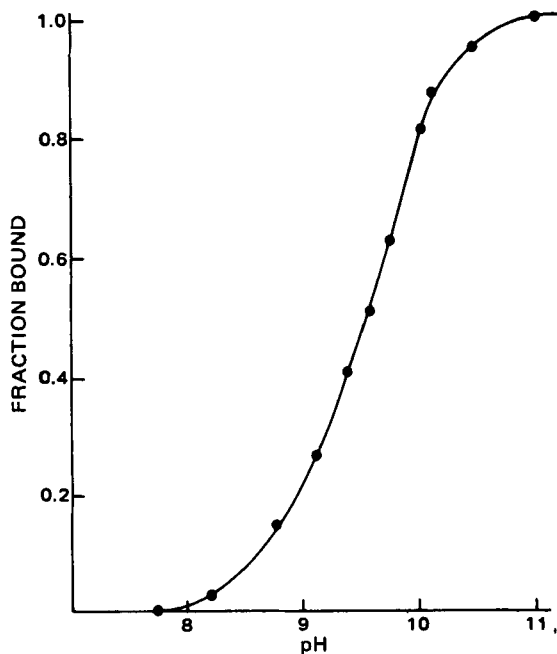


Figure 1—Effect of pH on the fraction of magnesium cation bound by a chloride-containing aluminum hydroxide gel.

tant effect which the relationship between pH and ZPC exerts on the physical properties of aluminum hydroxide gel (2). The present study investigates the influence that the surface charge characteristics of aluminum hydroxide and aluminum hydroxycarbonate gel have on the adsorption of ionic solutes such as magnesium nitrate or docusate sodium as well as mannitol, a neutral polyol.

#### EXPERIMENTAL<sup>1</sup>

The chloride-containing aluminum hydroxide gel was prepared by reacting aluminum chloride and potassium hydroxide as described previously (2). The ZPC of this gel was determined by a titration procedure (2) and was found to be 9.65. The bulk pH was 8.00 when diluted with distilled water to 3% equivalent aluminum oxide. Two aluminum hydroxycarbonate gels were obtained commercially. Aluminum hydroxycarbonate gel number 1 had a ZPC of 6.95 and a bulk pH of 6.5 at 3%

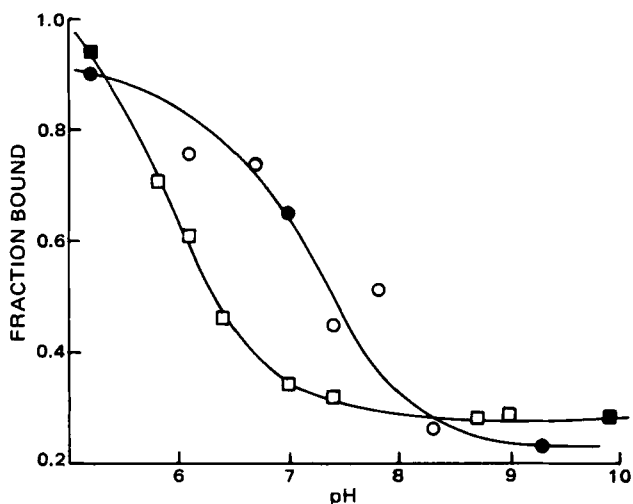


Figure 2—Effect of pH on the fraction of docusate anion bound by gel 1 (O, ●) and gel 2 (□, ■). The open data points were determined without adjusting the ionic strength and the closed data points were determined at a constant ionic strength of  $5 \times 10^{-3}$ .

<sup>1</sup> All chemicals used were either official or reagent grade.

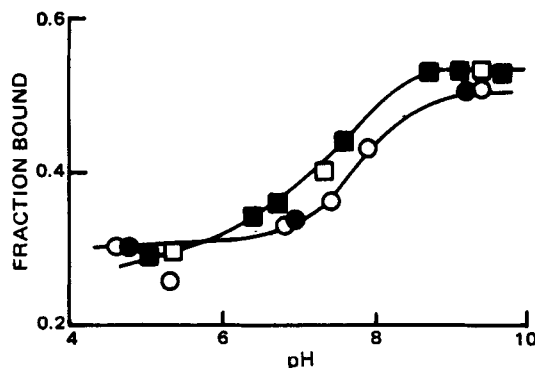


Figure 3—Effect of pH on the fraction of mannitol bound by gel 1 (O, ●) and gel 2 (□, ■). The open data points were determined without adjusting the ionic strength and the closed data points were determined at a constant ionic strength of  $5 \times 10^{-3}$ .

equivalent aluminum oxide, while gel 2 had a ZPC of 6.32 and a bulk pH of 6.25 under the same conditions.

The fraction of magnesium nitrate, docusate sodium, or mannitol adsorbed by the aluminum hydroxide gel at different pH conditions was determined by first adjusting portions of the gel to the desired pH by addition of 0.1 *N* HCl or 0.1 *N* KOH. The maximum change in ionic strength due to the pH adjustment was  $5 \times 10^{-3}$ . A second series of samples was prepared at an ionic strength of  $5 \times 10^{-3}$  by adding an appropriate quantity of potassium chloride to each sample following pH adjustment. No significant difference in adsorption was observed between the first series in which the ionic strength varied up to  $5 \times 10^{-3}$  and the second series in which all samples had an ionic strength of  $5 \times 10^{-3}$ . Adsorption is normally related to ionic strength, but the small quantities of acid or base needed to adjust the pH did not cause a large enough ionic strength differential to significantly affect adsorption. Thus, the data at constant and variable ionic strength were pooled.

Following pH adjustment, an adsorbate solution was added to produce a mixture containing  $9.8 \times 10^{-2}$  *M* equivalent aluminum oxide and either  $6 \times 10^{-4}$  *M*  $\text{Mg}(\text{NO}_3)_2$ ,  $3.2 \times 10^{-4}$  *M* docusate sodium, or  $3 \times 10^{-2}$  *M* mannitol. The docusate sodium concentration was selected to be below the critical micelle concentration which was reported to be  $6.8 \times 10^{-4}$  *M* in water (3). The mixtures were equilibrated by shaking at 25° for 2 hr, the pH was determined, the sample was centrifuged at 15,000 rpm (27,000  $\times$  *g*) for 30 min, and the supernate was analyzed. Magnesium was determined by chelatometric titration (4), docusate sodium was determined by the official assay (5), and mannitol was determined by a microcolorimetric method (6).

#### RESULTS AND DISCUSSION

The fraction of magnesium cation adsorbed by the chloride-containing aluminum hydroxide gel was strongly pH dependent (Fig. 1). Virtually no adsorption occurred below pH 8, while complete adsorption of magnesium cation occurred at pH 11. It is interesting to note that 50% of the magnesium cation was adsorbed at pH 9.65 which corresponds to the ZPC of the chloride-containing aluminum hydroxide gel. Thus adsorption of magnesium cation can be directly related to the surface charge as predicted from the pH-ZPC relationship. At pH conditions below the ZPC, the positively-charged aluminum hydroxide surface will not interact with the magnesium cation, while a strong attraction occurs when the pH is above the ZPC, producing a negative surface charge. A sigmoidal adsorption curve is commonly observed for the adsorption of transition metal ions on various metal oxide surfaces (7, 8).

The adsorption of the anionic surface active agent, docusate sodium, is somewhat more complex, but a strong dependence on the pH-ZPC relationship is evident in Fig. 2. Gel 1 had a ZPC of 6.95, while the ZPC of gel 2 was 6.32. In both cases, the ZPC corresponded approximately with the midpoint of the fraction bound curve with greater adsorption occurring when the pH was below the ZPC. However, a minimum of ~20% of the docusate anion was adsorbed even under pH conditions where the apparent surface charge of the aluminum hydroxycarbonate gel was negative.

Although docusate sodium is known to hydrolyze in alkaline solutions (9), the amount of degradation occurring at pH 10 during 4 hr (the time required for the fraction bound study) was negligible. It is believed that adsorption of the hydrophobic portion of docusate anion occurred even though repulsive forces exist between the negatively-charged hydrophilic

portion of docusate anion and the negatively-charged aluminum hydroxycarbonate surface.

The adsorption of polyols, although occurring by hydrogen bonding rather than electrostatic attraction (10, 11), was also affected by the pH-ZPC relationship (Fig. 3). The extent of mannitol adsorption by either gel 1 or 2 is much less than was observed for the adsorption of the magnesium cation or the docusate anion, reflecting the weaker adsorption mechanism of hydrogen bonding in comparison to electrostatic attraction. However, the fraction of mannitol adsorbed increased from ~30% at pH conditions below the ZPC to 50% when the pH was above the ZPC. Hydrogen bonding occurs more readily when mannitol serves as the proton donor and the negatively-charged oxygen at the aluminum hydroxycarbonate gel surface serves as the proton acceptor. This condition exists when the pH is above the ZPC and coincides with the region of maximum adsorption of mannitol.

The results of this study suggest that the pH-ZPC relationship will provide a useful guideline for predicting adsorption reactions in the formulation of antacid dosage forms and may also be useful in predicting drug interactions arising from the coadministration of drugs and aluminum hydroxide-containing antacids.

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## COMMUNICATIONS

### TLC and GLC Determination of Aromatic Amine Impurities in Bulk *p*-Aminobenzoic Acid and in Its Potassium and Sodium Salts

**Keyphrases** □ *p*-Aminobenzoic acid—aromatic amine impurities determined by GLC and TLC □ TLC—determination of aromatic amine impurities in *p*-aminobenzoic acid □ GLC—determination of aromatic amine impurities in *p*-aminobenzoic acid

To the Editor:

The current USP monograph (1) for *p*-aminobenzoic acid lacks a requirement for limiting the amount of aromatic amine impurities in the finished bulk drug. The carcinogenicity of these possible amine impurities prompted this laboratory to adapt the derivatization and GLC technique of Bruce and Maynard (2) to identify and quantitate these compounds. A TLC confirmatory test using a portion of the underivatized sample solution was also developed.

A finely ground 5-g sample of bulk *p*-aminobenzoic acid or its salts, in a 50-ml stoppered centrifuge tube, was extracted by shaking with 25 ml of benzene for 5 min. After centrifuging, the benzene layer was transferred to a separator and extracted with two 15-ml portions of 2% aqueous sodium bicarbonate. The aqueous layers were discarded, and the benzene layer was dried through anhydrous sodium sulfate.

A 2-ml portion of the extract was derivatized in a separator with 25  $\mu$ l of heptafluorobutyric anhydride. After 30 min at room temperature, 10 ml of benzene was added, the organic layer was washed with three 10-ml portions of water, and the water was discarded. The benzene layer was diluted to 50 ml with benzene and 5  $\mu$ l was injected into a

gas chromatograph equipped with a 15- $\mu$ Ci  $^{63}$ Ni-electron-capture detector, a 1.8 m  $\times$  4-mm i.d. spiral glass tube packed with 6% OV-101 and 9% OV-210 (1:1) coated on acid-washed silanized high-performance flux calcined diatomite support (100-120 mesh). Inlet, column, and detector temperatures were 225, 140, and 325 $^{\circ}$ , respectively. Argon-methane (95:5) at a flow rate of 50 ml/min was used as the carrier gas.

For TLC, 5 ml of the dried benzene sample extract was evaporated to 200  $\mu$ l, and 20  $\mu$ l was applied to a silica gel GF plate. After 10-cm development with benzene-ethyl acetate-acetic acid (90:5:5), the plate was sprayed with 1% *p*-dimethylaminobenzaldehyde in ethanol containing 5% HCl.

Table I lists the TLC  $R_f$  values for the 10 amines used in this study. The GLC retention times for the corre-

Table I—TLC and GLC Data for 10 Aromatic Amines

Amine	TLC <sup>a</sup> $R_f$ Free Amine	GLC <sup>b</sup> Retention Time, min <sup>c</sup>	Approximate Nanograms for HSD <sup>d</sup>
Aniline <sup>e</sup>	0.07	1.2	0.05
<i>o</i> -Toluidine	0.28	1.7	0.10
<i>p</i> -Toluidine	0.19	2.0	0.10
<i>p</i> -Chloroaniline	0.42	3.1	0.10
2-Methyl-5-chloro-aniline	0.67	4.4	0.10
Diphenylamine	0.85	11.9	0.80
2-Chloro-5-nitro-aniline	0.74	15.0	0.40
Benzocaine <sup>e</sup>	0.45	16.8	0.40
<i>p</i> -Nitroaniline	0.42	17.9	0.40
2-Methyl-5-nitro-aniline	0.55	26.0	0.40

<sup>a</sup> 20  $\times$  20 cm 0.25-mm thick silica gel GF plates, Mallinckrodt Chemical Works, St. Louis, Mo. <sup>b</sup> Tracor 560, Tracor Inc., Austin, Tex. <sup>c</sup> Heptafluorobutyric anhydride derivative. <sup>d</sup> Nanograms for half-scale deflection (HSD) at GLC detector setting of 7 namp, and attenuation at 5. <sup>e</sup> Found in commercial samples.