EMPLOYMENT OF MODERN ANALYTICAL METHODS FOR ADSORPTION STUDIES AND PHARMACEUTICAL ANALYSIS

WENDY M. FOSS

Employment of Modern Analytical Methods for Adsorption Studies and Pharmaceutical Analysis

by

Wendy M. Foss

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Supervisory Committee:

Jerome E. Haky

Thesis Advisor

J. William Lantz

Chairman, Department of Chemistry and Biochemistry

Dean, College of Science

Dean of Graduate Studies and Reseach

4/2/97

Date

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ABSTRACT

Author:	Wendy M. Foss
Title:	Employment of Modern Analytical Methods for Adsorption Studies and Pharmaceutical Analysis
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Thesis Advisor:	J.E. Haky, Ph.D.
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Ultraviolet spectrophotometry was employed to investigate the adsorption of phenylphosphonic acid onto the surface of alumina from aqueous solution. It was found that an initial chemisorption occurred with monolayer coverage, reaching a maximum at a solution pH of 3.0. The results were interpreted as indicating that this and related adsorptions are controlled by ligand exchange processes involving electrostatic attraction between oppositely-charged species. In a separate project, high performance liquid chromatography was employed for the quantitative analysis of aminophylline in commercial thigh cream formulations. The analysis required derivatization of the compound by dansylation under carefully-controlled conditions. This enhanced its detection and separation from other cream components.

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PART I

MECHANISM OF PHOSPHONIC ACID ADSORPTION ONTO ALUMINA

INTRODUCTION

Adsorption is a process that occurs in the interfacial region between a gas or liquid phase and a solid. This surface phenomenon should be distinguished from processes in which molecules of one component move across the interphase and penetrate throughout the body of a second. Solute adsorption is the basis of a great number of industries including deterents, dying, pollution control, photography, and liquid-solid chromatography and is essential in many physiological processes.

The interaction between a surface and an adsorbed species may be either physical or chemical in nature, depending on the energetics of the interaction. Physical adsorption arises from electrostatic effects (such as ion association), London dispersion forces, hydrophobic interactions and hydrogen bonding. These effects account for the interaction of compounds with stationary phases in both gas and liquid chromatography. In aqueous systems, physical adsorption interactions are restricted to those whose energy is comparable to that which occurs between solvent molecules (30kJ/mole or less).[1] In contrast, chemical adsorption (also termed chemisorption) interactions possess enthalpies of adsorption comparable to those of ordinary chemical reactions (85 kJ/mole or greater for aqueous systems). This process involves the formation of chemical bonds between the adsorbent (solid surface) and the adsorbate. The net interaction of an adsorbate molecule with a surface may involve more than one type of interaction, depending on the chemical structures of both components.

Many kinetic and thermodynamic treatments have been developed to describe the adsorption process. Langmuir pioneered the kinetic approach in 1916 when he derived a mathematical model to describe vapor phase adsorption in monolayers.[2] This model only considered those interactions between adsorbate molecules and the surface. Brunauer, Emmett, and Teller later developed a more generalized form of this process which included multilayer adsorption (*i.e.*, interaction of adsorbate molecules with the monolayer).[3] In the adsorption of dissolved solids from solution, interactions other than those between solute and substrate and between solute molecules must be considered. Such a process is likely to involve solvent-substrate, solute-solvent, and solvent-solvent interactions as well, making a complete theoretical description very complex.[4] Using a thermodynamic treatment to describe the adsorption process, Heit *et al.* demonstrated that if solute-substrate and solvent-substrate interactions occur at the same sites on the adsorbent surface, Langmuir's approach is sufficient to describe adsorption between a liquid phase and a solid.[5]

Investigating the theoretical aspects of any adsorption process is most often done by studying the isotherm, in which the amount adsorbed (usually expressed in mmoles adsorbed per square meter of adsorbent) is plotted against the pressure (or concentration) of the adsorbate at constant temperature and under equilibrium conditions. The shape of such an isotherm is largely determined by the adsorption mechanism and can therefore be used to determine the nature of the adsorption. Langmuir's mathematical model correctly predicted the shape of such an isotherm for compounds which exhibit strong attractive forces to the substrate, but relatively weak intermolecular forces, and consequently adsorb on the surface in monolayer coverage.[5] Adsorption of such compounds (*e.g.*, inorganic phosphate derivatives onto metal oxides) produce isotherms which are convex with respect to the vertical axis until the amount adsorbed reaches a limiting value, beyond

which no further increase in the amount adsorbed is observed (see Figure 1).[6] These adsorption processes are said to exhibit Langmuir behavior.

Phosphate Adsorption

The adsorption of inorganic and organic phosphates and their derivatives on metal oxide surfaces has received much attention in recent work because the process is important in controlling the bioavailability of phosphorus-containing nutrients to plants in aquatic systems. Additionally, studying the interaction of phosphate and related compounds with metal oxides is a method by which the chemistry of the oxide surfaces may be better defined.

Over the past 20 years, several studies have shown that phosphate ions are strongly adsorbed onto the surface of aluminum oxide from aqueous solutions and that this process follows Langmuir behavior.[7-11] In an effort to obtain thermodynamic parameters for the adsorption process (*e.g.*, free energies of adsorption, activation energies, *etc.*) data has been fit to Langmuir equations at several temperatures. This has shown that the extent and energy of phosphate adsorption occurring on alumina is greater than that of many other anions.[8] Although it is difficult to describe precisely the principle mechanism of adsorption, indirect experimental evidence indicates that phosphate and other anions of weak acids displace hydroxyl and/or water ligands on the oxide surface to form inner-sphere surface complexes with the first coordination layer of metal ions.[7,10,12] Such a ligand exchange reaction, as shown in equations 1 and 2, is referred to as specific adsorption. Specifically-adsorbed anions are resistant to surface desorption under a wide variety of conditions, including changes in pH and ionic strength.[7]

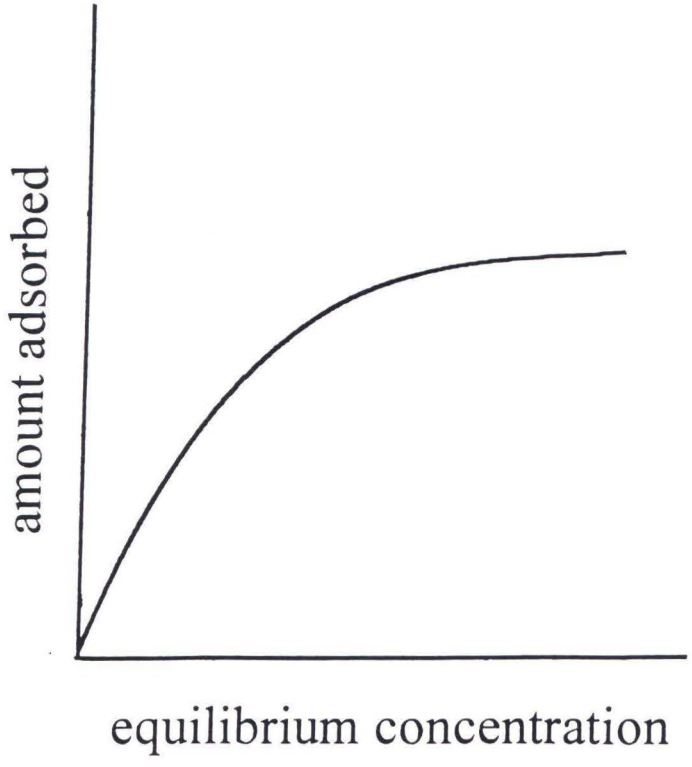


Figure 1: Hypothetical Langmuir Adsorption Isotherm

$$\text{--Al-OH} + H_2PO_4^- \rightarrow \text{--Al-}H_2PO_4 + OH^- \quad (1)$$

$$\text{--Al-}OH_2^+ + H_2PO_4^- \rightarrow \text{--Al-}H_2PO_4 + H_2O \quad (2)$$

In contrast, with non-specific adsorption there is no penetration of the inner coordination sphere. Instead, a positive surface charge is balanced by adsorption of a counterion beyond the first coordination sphere. Non-specific exchange of one anion for another may also occur. Such non-specific adsorption and/or exchange occurs for anions of strong acids such as chloride as shown in equations 3 and 4.

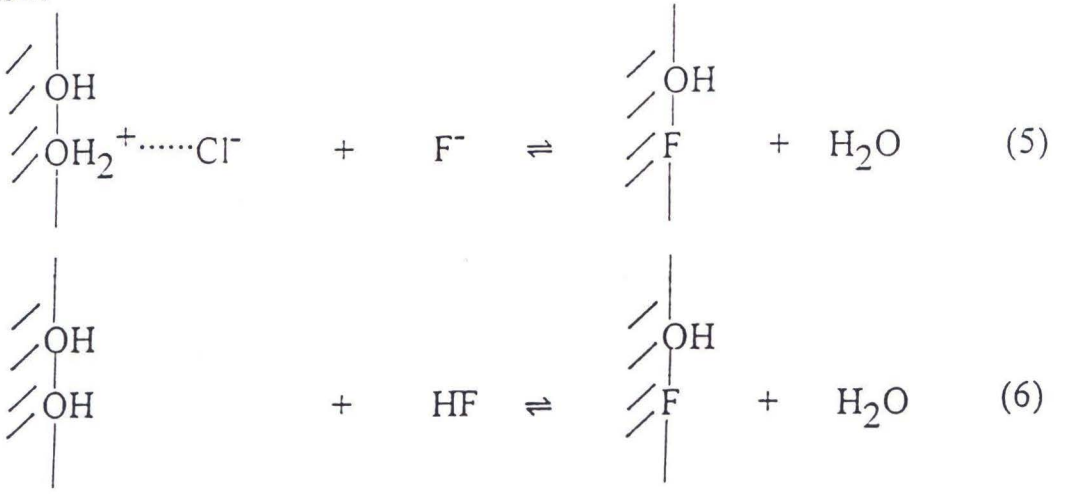
$$\text{--Al-}OH_2^+ + Cl^- \rightarrow \text{--Al-}OH_2^+Cl^- \quad (3)$$

$$\text{--Al-}OH_2^+X^- + Cl^- \rightarrow \text{--Al-}OH_2^+Cl^- + X^- \quad (4)$$

Studies of Specific Adsorption

Inner and outer sphere ligand exchange exhibit a strong dependency on solution pH.[7-9] This behavior can be illustrated graphically by constructing an adsorption envelope, a plot of the amount of anion adsorbed on a metal oxide versus solution pH at constant temperature and equilibrium solution concentration. For anions of weak monoprotic acids (*e.g.*, fluoride ions) there is an adsorption maximum at the pK_a value corresponding to the presence of both the fully associated parent acid and its corresponding anion, in equal concentrations.[7] In addition, when present alone, neither the undissociated free acid (present at low pH values with respect to the pKa) nor the fully dissociated anion (predominating at pH values greater than the pKa) are adsorbed at the oxide surface.[7] In a pioneering paper, Hingston and coworkers attributed this to the

requirement that any specifically adsorbed anion must possess enough negative charge to be attracted to positively-charged sites and to displace water ligands on the oxide surface, while simultaneously possessing enough acidity to transfer protons to the surface hyroxyl groups, facilitating their displacement, as shown for the fluoride ion in equations 5 and 6 below.



Adsorption involving such a process would produce a surface-modified oxide whose net positive charge is significantly reduced. This reduction in positive charge has been confirmed experimentally by potentiometric titrations performed in the presence and absence of specifically adsorbed anions.[7,10]

In Hingston's model, the adsorption envelope for a polyprotic acid (*e.g.*, phosphoric acid) would exhibit several maxima, at solution pH's corresponding to each of the pK_a values. Phosphate adsorption on alumina, however, has been shown to be greatest at a solution pH near 4.5 which does not correspond to any of phosphoric acid's dissociation constants. Further, no additional adsorption maxima are observed. To explain these results, Huang and Chen *et al.* developed a modified theory in which consideration was given to the effects of changes in the surface charge of alumina with pH, the degree of electrostatic attraction of the phosphate anions to the alumina surface

and the inherent amphoteric nature of protonated anions of phosphoric acid.[8,9] This theory, later refined and termed the "diffuse double layer model", is discussed below.

Metal oxides in contact with aqueous solutions develop an electrical double layer consisting of an inner region containing ions adsorbed on the surface and a diffuse region in which ions are distributed according to the influence of electrical forces and random thermal motion. The resulting net surface charge is dependent on the pH of the solution and the nature of the oxide itself. Alumina develops a net positive charge at pH values below its isoelectronic point (pH 8.5) through protonation of its surface hydroxyl groups, as shown in equation 7.[13]

$$\text{--Al-OH} + H^+ \rightarrow \text{--Al-}OH_2^+ \qquad (7)$$

At pH values above 8.5, alumina's surface hydroxyl groups become increasingly deprotonated producing a negatively-charged oxide surface as shown below.

$$\text{--Al-OH} + OH^- \rightarrow \text{--Al-}O^- + H_2O \qquad (8)$$

Both the nature of the oxide surface (*i.e.*, surface charge distribution) and the extent of ionization of the adsorbing species depend uniquely on pH. According to this model, the extent of adsorption, or adsorption density, is controlled significantly by the surface acidity and acid/base behavior of the adsorbing species. For example, as the solution pH is lowered from 8.5 (alumina's isoelectric point) down to approximately 4.5 (the pH of maximum adsorption), the adsorption density is observed to increase. This enhancement in the adsorption process is attributed to the electrostatic attraction of

phosphate anions for the positively-charged alumina surface. As the pH is lowered past the pH of maximum adsorption, electrostatic attraction of the increasingly protonated (*i.e.*, neutral) phosphate species for the positively charged surface is reduced, thus accounting for the decrease in the adsorption density that is observed as the pH is lowered below 4.5.

Although the theory presented by Huang and Chen *et al.* does not explain the occurrence of an adsorption maximum for phosphate ions onto alumina at a solution pH of 4.5, additional consideration of Hingston's model predicts such a maximum. For polyprotic acids, an anion containing hydrogen can exhibit both acidic and basic properties (Hingston's requirement for adsorption). Because of its amphoteric nature, such a species is capable of simultaneously donating and accepting a proton. Maximum amphoteric anion concentrations can be calculated by averaging the pK_a corresponding to the deprotonation of the parent acid giving that anion (pK_{a1}) and the pK_a corresponding to the deprotonation of the anion itself (pK_{a2}). For the diprotic phosphate anion, $H_2PO_4^-$, this pH is given by averaging the first two pK_a's of phosphoric acid[14], or

$$pH = (1.96 + 7.12)/2 = 4.54$$

There is also a high degree of electrostatic attraction of such diprotic phosphate species for the predominantly protonated alumina surface at pH 4.5 (Huang and Chen *et al.*'s requirement for adsorption). Thus, this mechanism predicts an adsorption maximum at a solution pH of 4.5, which is in accord with that found experimentally. Electrostatic effects explain why a second adsorption maximum at the pH corresponding to maximum concentration of the other amphoteric species, HPO_4^{2-}, is not observed. This pH occurs at 9.8, above the isoelectronic point of alumina. Phosphate adsorption remains low in

solutions with such high pH's because there is little or no electrical attraction of the monoprotic phosphate anion for the negatively charged alumina surface.

Adsorption vs. Precipitation

Besides changes in the surface-solution equilibria, other factors contribute to the reduction in phosphate adsorption at pH values both lower and higher than 4.5. These factors include changes in aluminum oxide solubility and the formation of non-adsorbing aluminum-phosphate solution complexes (*i.e.*, precipitation). It is known that phosphate is capable of both adsorbing from aqueous solution onto alumina's surface and forming complexes with components of the adsorbent. The possibility of complex formation competing with the adsorption process increases as the solution pH is lowered and the solubility of aluminum oxide increases.[9,11] Because both precipitation of aluminum-phosphate complexes and adsorption of phosphate ions onto the surface would be evidenced as a decrease in the concentration of phosphate present in solution, some researchers have been concerned that the adsorption capacity of aluminum oxide is ambiguous. As a consequence, Hsu and Rennie said that the adsorption capacity at various pH values can be compared only when the solutions are saturated with aluminum phosphate.[15] According to Bleam *et al.*, this condition is satisfied when, at slight aluminum phosphate undersaturation, repeated additions of phosphate yield no further decrease in the solution phosphate concentration.[11]

In contrast to the approaches described above, Miller and Logan distinguished between adsorption and precipitation by examining plots of the fraction of total phosphate remaining in solution at equilibrium as a function of pH.[16] If precipitation was the controlling process, it was reasoned that addition of increasing amounts of phosphate

would not change the equilibrium phosphate concentration but rather increase the amount of precipitate formed. The fraction of phosphate remaining in solution with increasing phosphate addition would therefore decrease in the presence of an aluminum phosphate precipitate. However, results obtained at several pH's indicated that the fraction of phosphate remaining in solution increased with increasing phosphate concentrations. It was therefore concluded that adsorption, not precipitation was the controlling process.[16]

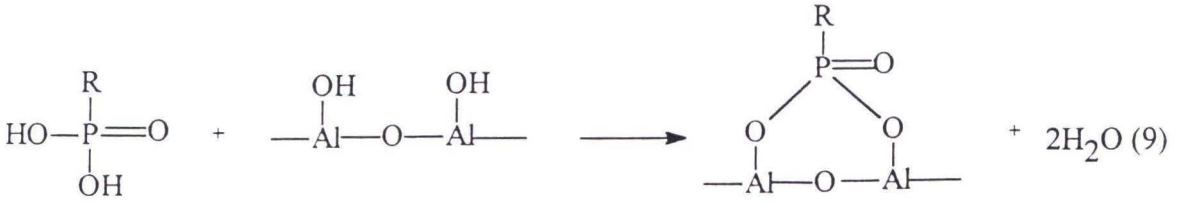
Phosphonic Acid Adsorption

Understanding the mechanism of phosphonate adsorption onto the surface of metal oxides has become increasingly important due to its implications in such diverse areas as the environment and separation technology (*i.e.*, chromatography).

Many organic phosphonate anions (*e.g.*, ethyl phosphonate) are known to inhibit both the growth and sporulation of pathogenic agents, making such compounds useful in the control of damping-off and rot in plant roots, stems, and fruit.[17] Fosetyl-aluminum (aluminum tris(ethyl phosphonate)), for example, is a systemic fungicide sold under the trade name Aliette (Rhone-Poulenc Agrochemie, Lyon, France). Typically, it is administered by incorporating it directly into the soil prior to planting.[18] The bioavailability of the ethyl phosphonate anion in the soil is strongly influenced by its interaction with specific mineral components, namely, metal oxides and clay minerals. These components are believed to be important adsorbents for phosphonate due to their positive surface charge at slightly acidic and neutral pH's and corresponding high affinities for anions.

Besides those adsorptive processes which occur naturally in the environment, similar processes have been developed and used in the laboratory to prepare surface-modified alumina stationary phases (by adsorbing organic phosphonic acids onto chromatographic-grade alumina) for application in reversed-phase HPLC. The development and evaluation of these surface-modified aluminas, however, has preceded a complete understanding of the adsorptive processes by which they are prepared.

In constrast to the extensive research performed on inorganic phosphate adsorption, there have been relatively few studies on the adsorption of organic phosphates and their derivatives from aqueous solution onto the surface of alumina. Coletti-Previero and coworkers discovered that organic phosphate esters and phosphonates are strongly adsorbed onto alumina. and could be selectively desorbed from the oxide suface by aqueous solutions of inorganic phosphate ion.[19] Based on this discovery, they employed alumina for the chromatographic separation and purification of a variety of biologically-active compounds containing the phosphate ester or phosphonate moiety by their selective displacement with aqueous phosphate solutions.[20] Other studies have focused on elucidating the structure of these surface-modified materials. Based on data obtained by sophisticated vibrational spectroscopic techniques, Kuiper *et al.* and Weinberg *et al.* proposed structures which are consistent with phosphonic acid adsorption onto alumina resulting in the formation of a bridged alumina-phosphonate complex, as shown in equation 9.[21,22]



In collaboration with researchers at Alcoa Laboratories, Haky *et al.* successfully adsorbed a number of alkyl and aryl phosphonic acids from aqueous solutions onto the surface of chromatographic-grade alumina.[23,24] The resulting materials were shown to possess properties similar to that of reversed-phase alkyl and aryl bonded silica used in high performance liquid chromatography (HPLC) except that they are stable over a wider mobile-phase pH range (*i.e.*, pH 2-11 vs. pH 2-8 for silica), and have somewhat different selectivities for compounds exhibiting hydrogen bonding.[24] These surface-modified aluminas were employed as stationary phases in a variety of HPLC applications, including the analysis of alkaloid mixtures[24,25], the determination of octanol-water partition coefficients of organic compounds[25]and the separation of complex peptide and protein mixtures[26,27].

Despite the relatively mild conditions required for removal of adsorbed phosphonic acid in excess of monolayer coverage, the remaining inner-sphere adsorbed phosphonic acid was shown to be remarkably resistant to surface desorption even under conditions of extreme acidity (pH 2) and basicity (pH 11).[24-27] Aqueous solutions of inorganic phosphate were the only solutions found to displace adsorbed phosphonic acid species.[24]

Similar resistance to desorption has been found for other anions, including phosphate and selenite.[7] Although pH adsorption envelopes for these and other anions indicate that their desorption should be thermodynamically favored in solutions of low and (especially) high pH's, the formation of partially covalently-bonded surface complexes such as that shown in equation 9 apparantly imparts high hydrolytic stability to such adsorbed compounds, resulting in extremely slow desorption rates. This is especially true for adsorbed organic phosphonates, since the resulting surface-modified materials are highly hydrophobic, thereby slowing hydrolysis even further. That such adsorbed

phosphonates are rapidly desorbed from alumina by solutions of inorganic phosphate at virtually any pH[19,20,24] suggests that the phosphate ion has a greater affinity for the alumina surface.

Zero Point of Charge as an Adsorption Indicator

The affinity of a given anion for the alumina surface is difficult to predict, as evidenced by the non-correspondence of elution orders for anions separated on alumina-based ion exchange columns to the order followed for conventional ion-exchange columns.[28] In a study of the chromatographic separation of inorganic anions on an alumina ion exchange column, Schmitt and Pietrzyk discovered that anion affinity toward alumina is governed by a number of unique factors, including the stability of the resulting adsorbed complex, the hydrolytic stability of the adsorbed anion, and the anion's ability to form inner-sphere complexes with the adsorbent surface.[28] A general indicator, however, of the affinity of an anion toward a protonated alumina surface is the extent to which the adsorbing anion reduces the positive surface charge.[7] A net reduction in surface charge is evidenced by a shift in the pH of zero net charge (*i.e.*, ZPC)[29] toward more acidic values. The greater this shift, the greater the affinity of a given anion for alumina's surface.

Electrophoretic mobility techniques, in which the movement of ions over the alumina surface is monitored as a function of an applied electric field (tangential to the charged surface)[30], have shown that the specific adsorption of phosphate onto an aluminum oxide surface results in a reduction in the pH of zero net charge from 8.5 (for the unmodified surface) to 5.7 [11], while phenylphosphonate ion reduces this value somewhat less (ZPC = 6.4)[31]. The greater reduction of the ZPC of alumina for inorganic phosphate, relative to phenylphosphonate, indicates that the former has a greater affinity

for alumina's surface and is therefore capable of displacing adsorbed phenylphosphonate ions from the surface.

Purpose of The Present Study

The similarities in the structures of the phosphorus-containing moieties of inorganic phosphate anions and organic phosphonates suggest that they adsorb onto alumina by similar mechanisms (*i.e.*, displacement of surface water and/or hydroxyl groups and subsequent inner-sphere coordination to the metal atoms). The similarities in the resistances of both adsorbed species to desorption under a wide range of conditions provides additional evidence for the applicability of the phosphate adsorption mechanism to phosphonate adsorption. Nevertheless, a controlled, systematic study of phosphonic acid adsorption onto alumina from aqueous solution has never been reported. Such a study is needed to better define the nature of phosphonate adsorption onto alumina, and to establish methods for controlling the extent of such adsorption.

The present study aims to investigate the following aspects of phosphonate adsorption onto alumina surfaces: (1) The effects of phosphonic acid solution concentration on its adsorption onto alumina; (2) The effects of solution acidity on the adsorption process. Toward this end, data is obtained to construct adsorption isotherms for a model compound, phenylphosphonic acid, onto alumina at various pH's. These isotherms are then analyzed to develop a consistent model for the mechanism of phosphonic acid adsorption onto alumina.

EXPERIMENTAL

Materials

CPN-100 alumina (92.5% aluminum oxide by weight) with a surface area of 313.0 square meters per gram was obtained from Aluminum Company of America (Vidalia, LA). Phenylphosphonic acid (PPA) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Ammonium hydroxide and hydrochloric acid were purchased from Fisher Scientific, Inc. (Fair Lawn, NJ). Water was deionized to a resistivity of 1 MΩ by means of a Culligan activated charcoal mixed-bed exchange resin.

Instrumentation

Ultraviolet absorption measurements were obtained on a Varian Cary 3E UV-Visible spectrophotometer. Adsorption experiments were performed in a New Brunswick Scientific gyrotory water bath shaker (Model G76D) and filtered through Gelman nylon acrodisc syringe filters (25mm diameter, 0.45mm pore size). A CMS Labcraft pH meter (Model pH 102) was utilized for pH measurements. Elemental analysis was performed by Atlantic Microlab, Inc. (Norcross, GA).

Adsorption Studies

Equilibrium Method:

Phenylphosphonic acid (PPA) and 5.00 grams of alumina were mixed and the pH was raised to 5.0 with dilute ammonium hydroxide. The sample was allowed to equilibrate at 25^{o}C in a water bath shaker. The pH was monitored and maintained at

specified levels by addition of dilute hydrochloric acid and/or ammonium hydroxide. When equilibrium was achieved, the resulting phosphonate concentration was determined by UV analysis of an aliquot of solution prepared as described below. To confirm that the adsorption mixture was at equilibrium, a second aliquot was analyzed the following day. This concentration (considerably less than the initial concentration) was taken as the first equilibrium concentration value.

Sufficient PPA was then added to the sample to restore the initial concentration. The pH was monitored and adjusted as necessary during the adsorption process. Equilibrium was confirmed by similar absorbance readings of aliquots removed on successive days. The resulting phosphonate concentration, as determined from the absorbance, was taken as the second equilibrium value. The amount of PPA adsorbed at this concentration was taken to be the sum of the amounts adsorbed during both equilibrium processes.

The entire process of restoring the initial phosphonate concentration and reequilibration was repeated up to five times. In this way, five equilibrium concentration values could be achieved with a single sample. Additional samples that were analyzed ranged in concentration from 0.0300 to 0.100M. The pH was adjusted to values between 1.5 and 8.0.

Concentration-Null Method:

In order to obtain specific equilibrium concentration values at pH 5.0, a method based on that developed by Nunn and Everett[32] was utilized. Three samples, each containing 50 mL of aqueous phenylphosphonic acid (0.0150, 0.0010, and 0.00750M, respectively) and 5.00 grams of alumina were mixed and the pH was raised to 5.0 with

dilute ammonium hydroxide. The samples were equilibrated at 25^{o}C in a water bath shaker. The pH was monitored periodically and adjusted to 5.0 with dilute hydrochloric acid. When equilibrium was achieved (as evidenced by a stabilization in the pH), an aliquot of solution was removed and analyzed by UV spectroscopy, as described later.

After analysis, sufficient phenylphosphonic acid was added to each sample to restore the initial phosphonate concentration. Once equilibrium had been achieved (with pH adjustment, as before), the samples were analyzed by UV spectroscopy for PPA content. The initial concentration of each solution was restored by addition of PPA. This entire process (equilibration, UV analysis, and restoration of initial conc.) was repeated until no decrease in the solution concentration was observed in two successive analyses. In this way, the initial and the final phosphonate concentration were made to be equal.

UV Analysis of Samples (Both Methods)

An aliquot of supernatant liquid (2-4 mL) was removed from the adsorption mixture and filtered through a 0.45mm syringe filter to remove any residual alumina. This aliquot was diluted with sufficient deionized water to give an absorbance value at 262 nm (λ_{max} for phenylphosphonic acid at pH 5.0) that fell within the linear range (correlation coefficient, $R > 0.98$) of a previously-obtained Beer's Law calibration curve using standards adjusted to the same pH's as the solutions to be analyzed. The phosphonate concentration was determined from the absorbance reading by reference to the calibration curve as described below.

Calculations

Equilibrium concentrations (in mol L^{-1}) were determined from absorbance measurements using the following equation:

$$c_e = A/\varepsilon b(V_2/V_1)$$

where A = absorbance, b = path length (1 cm), ε = molar absorptivity in L $cm^{-1}mol^{-1}$, V_1 = volume of aliquot before dilution in mL, V_2 = volume after dilution in mL. The molar absorptivity at 262 nm was determined from the slope of a Beer's Law calibration curve constructed at each pH value.

The amount adsorbed (in mmoles) was calculated from the equilibrium concentration as follows:

$$\text{amt. adsorbed} = c_iV - c_eV$$

where c_i = initial molar concentration and V = total solution volume (50 mL).

The amount of phenylphosphonic acid (in grams) required to restore the initial concentration was determined as follows:

$$\text{g PPA} = (c_iV - c_eV_c)MW/1000$$

where $V_c = V - V_1$ (corrected solution volume in mL) and MW = molecular weight of phenylphosphonic acid (158.09 g mol^{-1}). This relationship takes into account the addition of sufficient water to restore the initial solution volume.

RESULTS AND DISCUSSION

Phenylphosphonic acid was chosen as the adsorbate for the present study because of its reasonably high water solubility (molar solubility = 0.10mol/L) when compared to other phosphonic acid derivatives. In addition, the phenyl group imparts a high absorbance for this compound, relative to its concentration, in the ultraviolet region of the electromagnetic spectrum, enabling the adsorption process to be monitored by means of UV spectrophotometry.

Adsorption Kinetics

Rate experiments were conducted at room temperature ($25^{o}C$) and a solution pH of 5.0. Since we were not addressing the effects of temperature on the rate of phenylphosphonic acid adsorption (or on any other aspect of the adsorption process), the temperature was chosen based on its convenience. The solution was maintained at a slightly acidic pH due to our belief, from previous work with inorganic phosphate, that this was near the pH of maximum adsorption. Utilizing these parameters, the removal of phenylphosphonic acid from solution by aluminum oxide was followed for three phenylphosphonate equilibrium concentrations over a time period of several months. The rate of the adsorption reaction decreased with prolonged reaction time. The initial rate was regarded as pseudo-first order over a period of 53 days due to the linear relationship exhibited when the natural log of the amount adsorbed was plotted as a function of time (see Figure 2). Rate constants and corresponding half lives were determined for each equilibrium concentration as shown in Table 1 below.

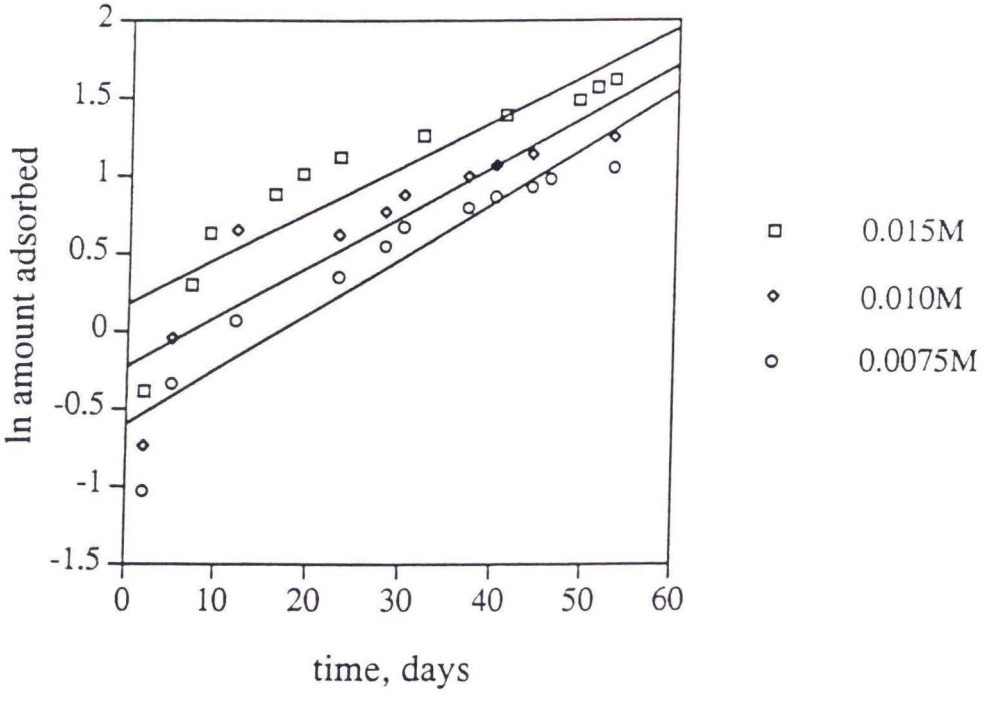


Figure 2: First Order Rate Plot at Three Equilibrium Concentrations

Table 1
Kinetic Data

Equilibrium Conc. (moles/L)	Rate Constant	Half-Life (days)
0.0150	0.036	19
0.0100	0.032	22
0.0075	0.030	23

Equilibrium, defined as 97% completion, was reached after 5 half-lives, an average of 105 days. This rate of uptake is slower than that found for adsorbates which interact with metal oxide surfaces by physical rather than chemical means.[33]

Isotherm Studies at pH 5.0

In order to determine the equilibrium phenylphosphonate concentration required to achieve complete monolayer coverage of the alumina surface, equilibrium experiments were conducted at phenylphosphonate concentrations ranging between 0.0200M and 0.100M and a solution pH of 5.0. Classically, the data required to construct an isotherm is obtained by allowing solutions at known initial concentrations to equilibrate, measuring their resulting equilibrium concentrations, and determining the corresponding amount adsorbed.[32] We have found that this method usually produces isotherms with points clustered at lower equilibrium concentrations, especially when compounds which adsorb strongly on the surface are being studied. To obtain points at equilibrium concentrations corresponding to monolayer coverage and beyond, the concentration-null method is useful.[11] This method utilizes LeChatelier's principle to shift the equilibrium concentration to higher values. By adding sufficient adsorbate to the adsorption mixture,

the equilibrium concentration is ultimately made to be equal to the initial concentration. This procedure has the disadvantage of being slow owing to the slow nature of the adsorption process itself, as well as the constant disturbance of the equilibrium concentrations. Most of our data was obtained using a novel method which combined aspects of both the classic and concentration-null methods. Rather than obtaining a single equilibrium concentration from an adsorption sample as in the classic method, the initial concentration was restored so that additional equilibrium concentrations could be obtained. Although these concentrations were not predetermined, as in the concentration-null method, higher equilibrium values were attainable. In addition, a single adsorption sample generated several data points. With the exception of three points corresponding to equilibrium concentrations at or near monolayer coverage, the adsorption isotherm shown in Figure 3 was obtained using this technique. The shape of this curve confirms that this process exhibits Langmuir behavior. Further, at a solution pH of 5.0, the formation of a monolayer on alumina's surface occurs up to an equilibrium phenylphosphonate concentration of approximately 0.0080M. At this concentration the monolayer is complete and no additional adsorption occurs until an equilibrium concentration of 0.014M at which point a second layer begins to form. This excess, weakly-bound material represents hydrophobic interactions between adsorbate molecules (on the surface and in solution). From our data, monolayer coverage corresponds to a surface density of 4.63 μmoles per square meter of alumina. This corresponds well with results obtained in previous studies.[34] To further validate our isotherm, the surface density calculated at monolayer coverage was confirmed by elemental analysis of the adsorbed alumina. This analysis indicated a carbon content of 10.9%, corresponding to a surface coverage of 4.84 $\mu moles/m^2$.

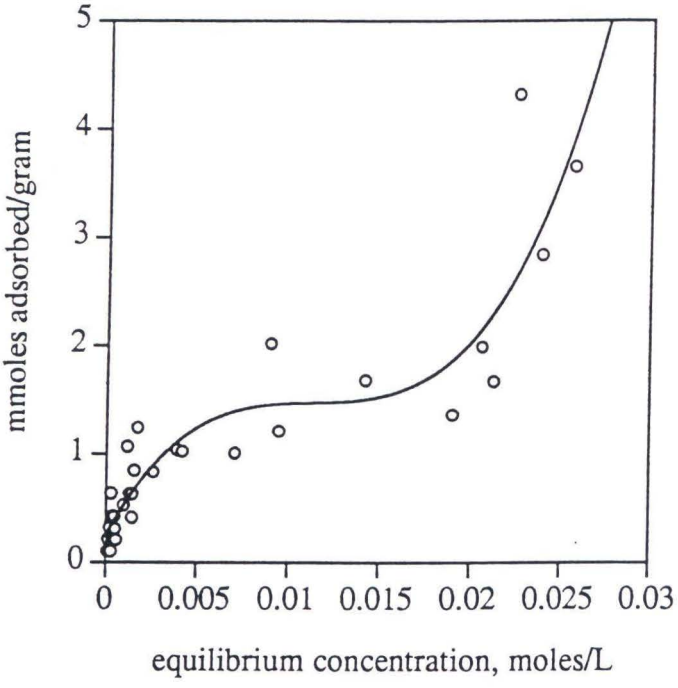


Figure 3: Adsorption Isotherm at pH 5.0

Studies at Other pH's

To examine the effect of solution pH on phenylphosphonate adsorption, isotherms were constructed at pH values ranging between 1.5 and 8.0. The pH of the adsorbate solution effects both the surface charge of an amphoteric oxide such as alumina and the extent of ionization of the adsorbate molecules. Since adsorption involves interaction between the variably-charged surface and these molecules, it is not surprising that the pH of a solution would have a profound effect on the adsorption process.

To quantitatively evaluate the extent of protonation of alumina's surface hydroxyl groups as a function of pH, Huang and Stumm compared titration curves of suspensions of alumina in an electrolyte solution (0.001 - 0.1M NaCl) with that of the electrolyte alone.[35] The data obtained was reported in terms of surface charge in μC per square centimeter. Kummert and Stumm used isotopic exchange techniques to estimate the number of exchangeable protons (*i.e.*, total surface hydroxyl groups) per unit area of alumina.[36] They reported a value of 8.5×10^{18} exchangeable protons/m^2 for hydrated γ-Al_2O_3. We used this value, coupled with the data from Huang and Stumm, to calculate the percent ionized hydroxyl groups on alumina's surface at various pH values. The following equation relates these parameters:

$$\% \text{ ionized hydroxyl groups} = \Gamma N_A / [96{,}486\ (8.5 \times 10^{18})]$$

where Γ = surface charge in $\mu C/cm^2$ and N_A = Avogadro's number

The resulting graph of the percent ionized hydroxyls as a function of pH is shown in Figure 4 for three electrolyte concentrations. It shows that as pH increases the percent of protonated hydroxyl groups decreases. This trend continues until the ZPC (point of zero

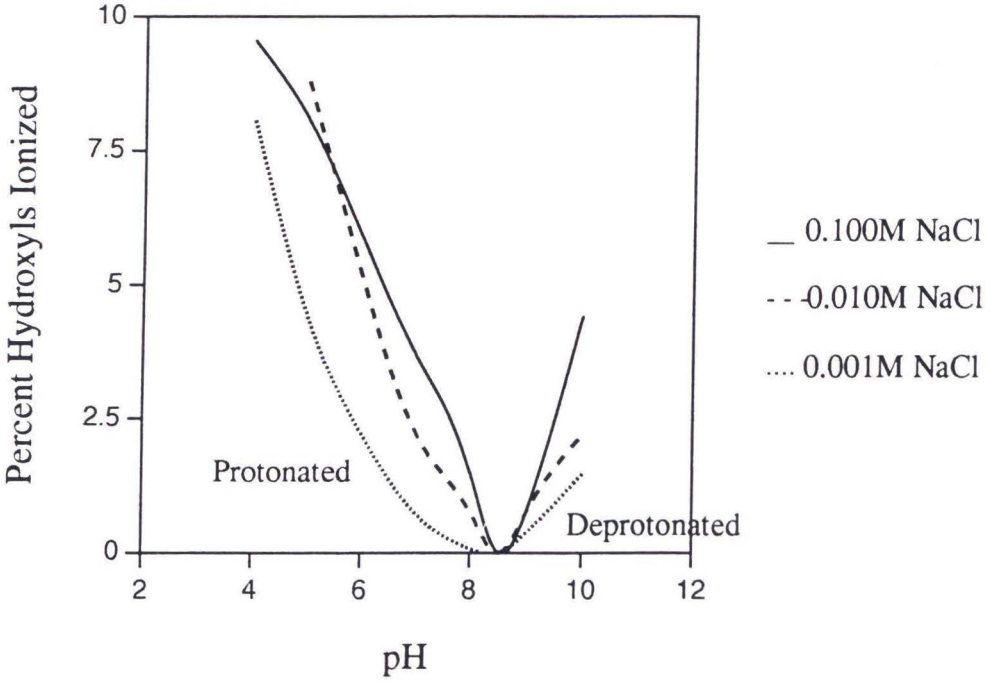


Figure 4: Ionization of Alumina's Surface as a Function of pH

charge) is reached at a pH of 8.5. Past this value, the ionization of alumina's surface begins to increase due to deprotonation of surface hydroxyl groups resulting in a negatively charged surface. It should be noted that at virtually all pH's most surface hydroxyl groups on alumina are un-ionized.

As a diprotic acid, phenylphosphonic acid is capable of existing in three states of ionization: as a completely protonated, neutral molecule, as a monoprotic, negatively charged ion, or as a completely deprotonated species. The equilibria and corresponding acid dissociation constant expressions for phenylphosphonic acid are shown below.

$$H_2PPA \rightleftharpoons H^+ + HPPA^-$$

$$K_{a1} = [H^+][HPPA^-]/[H_2PPA] = 1.48 \times 10^{-2} \quad (1)$$

$$HPPA^- \rightleftharpoons H^+ + PPA^{2-}$$

$$K_{a2} = [H^+][PPA^{2-}]/[HPPA^-] = 8.51 \times 10^{-8} \quad (2)$$

α_o, α_1 and α_2 represent the fraction of H_2PPA, $HPPA^-$ and PPA^{2-}, respectively, present in solution as shown below.

$$\alpha_o = [H_2PPA]_{eq}/\text{ total conc.} \quad (3)$$

$$\alpha_1 = [HPPA^-]_{eq}/\text{ total conc.} \quad (4)$$

$$\alpha_2 = [PPA^{2-}]_{eq}/\text{ total conc.} \quad (5)$$

where total conc. = $[H_2PPA] + [HPPA^-] + [PPA^{2-}]$

Equations 1 and 2 can be rearranged and substituted into equations 3-5 to obtain the following expressions for α_o, α_1 and α_2 based only on the hydrogen ion concentration and K_a values.

$$\alpha_o = [H^+]^2/(K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2) \quad (6)$$

$$\alpha_1 = K_{a1}[H^+]/(K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2) \quad (7)$$

$$\alpha_2 = K_{a1}K_{a2}/(K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2) \quad (8)$$

Plotting equations 6-8 as a function of pH generates the graph shown in Figure 5. At pH 4.5 the monoprotic phenylphosphonate ion is the only species present in solution. Below this pH the ion coexists with the fully protonated molecule. At pH values above 4.5, the fully deprotonated phenylphosphonate ion begins to appear in solution, and its concentration increases as the pH is increased.

In order to assess the effect that the factors described above have on the adsorption process, an adsorption envelope was constructed in which the amount adsorbed was plotted as a function of pH at a constant equilibrium concentration. It was important that the equilibrium concentration chosen corresponded to one that occured either at monolayer coverage or slightly below for each pH value. In this way, the formation of secondary layers due to the hydrophobic adsorption of phenylphosphonate ions onto the monolayer was avoided and only the specific adsorption of these ions onto alumina's surface was studied.

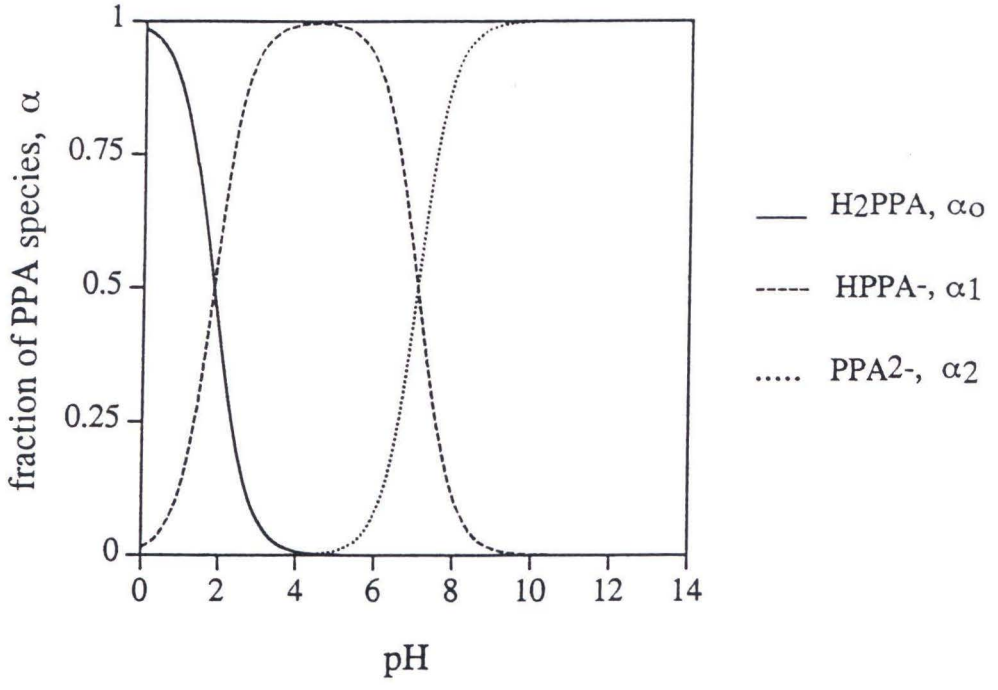


Figure 5: Fraction of Phenylphosphonic Acid Species Present in Solution as a Function of pH

Generally, the concentration-null method is used to collect data for an envelope. Since this method is based on a pre-determined equilibrium concentration, only one point at each pH value must be evaluated. However, it is difficult to verify that the equilibrium concentration chosen is below monolayer coverage for every pH value. In addition, it can take longer to collect data at one equilibrium value using the concentration-null method than to construct entire isotherms in the same manner as that described for pH 5.0. Therefore, in our study, we collected sufficient data to generate isotherms at eight pH values ranging from 1.5 to 8.0. Since the process of adsorption removes an acidic species (*i.e.*, phenylphosphonic acid) from solution, the pH rises continually as adsorption occurs. For this reason, it was important that the solution pH was monitored often and maintained at its original value. Isotherms at most pH's were generated only up to equilibrium phenylphosphonate concentrations corresponding to monolayer coverage. Figure 6 shows the resulting isotherms. The isotherms were overlaid and extrapolated to the highest equilibrium concentration value (obtained for pH 1.5) so that the relative differences in the amount of phenylphosphonic acid adsorbed at each pH could be compared. These differences are also seen in the adsorption envelope shown in Figure 7, which was generated from the isotherms by interpolation to a 0.010M equilibrium concentration. The envelope shows maximum adsorption to occur at a pH of 3.0. The extent of adsorption decreases sharply below this value while there is a more gradual decrease at higher pH's.

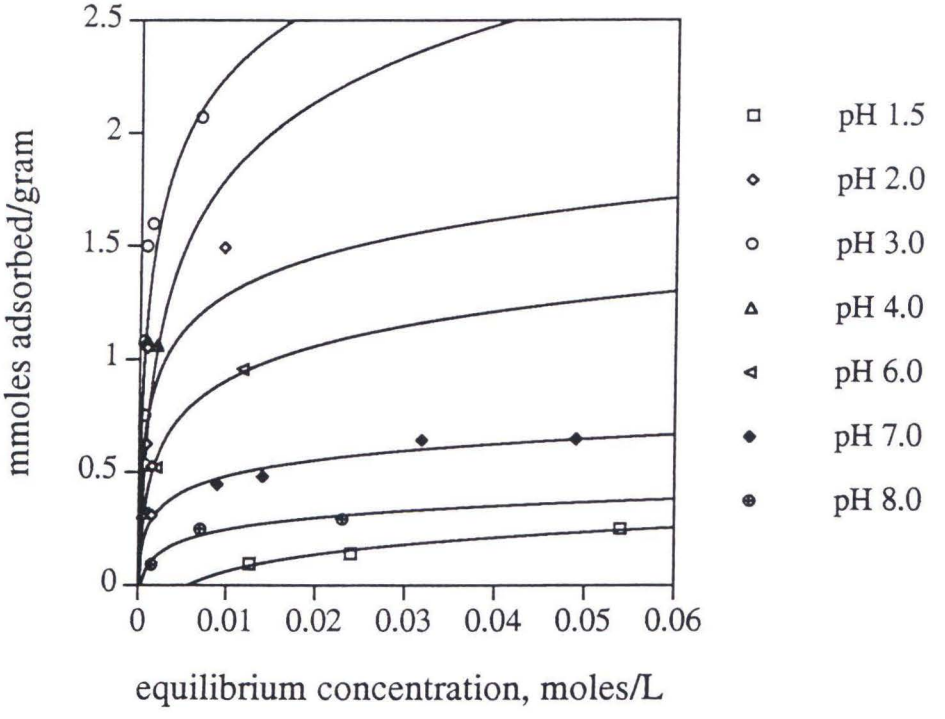


Figure 6: Adsorption Isotherms, pH 1.5 - 8.0

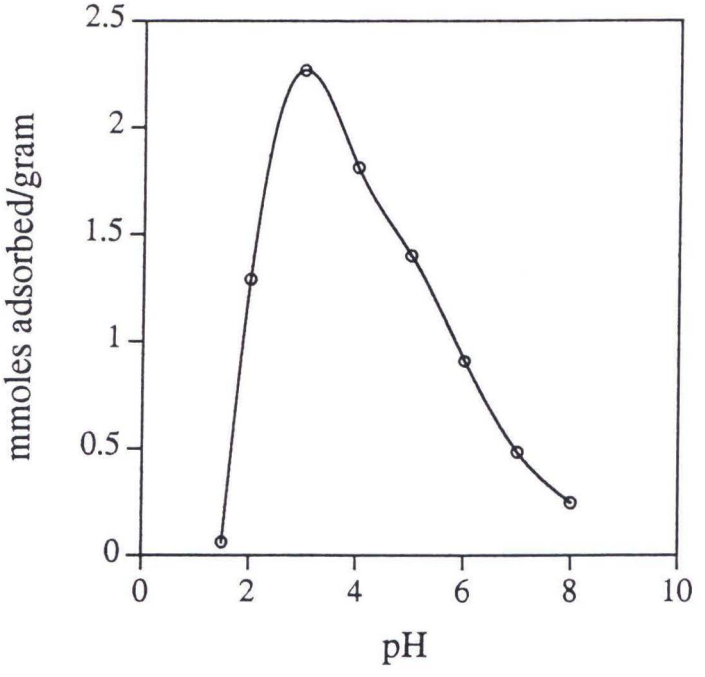


Figure 7: Adsorption Envelope at 0.010M Equilibrium Concentration

Adsorption vs. Precipitation

In modelling any adsorption process the possibility of precipitation of a mineral phase must be considered. Generally, precipitation is distinguished from adsorption by analyzing the solubility product expression of the potential interfering complex. The inability to specifically identify the mineralogy of a precipitated phase, however, often makes it difficult to draw any conclusions from this expression alone. From their work with phosphate, Miller et al reasoned that the amount of any precipitate formed (regardless of its stoichiometry) is limited by the phosphate added.[16] Based on this idea, they developed a method to verify the occurrence of adsorption only. We adapted this method for our work with phenylphosphonate and obtained similar results as described below.

Work performed previously in our lab has shown that, under the experimental conditions utilized in this study, $AlH(PPA)_2$ is the primary complex that would form if precipitation were occurring.[37] Aluminum phenylphosphonate, as any dissolved solid, has a solubility limit governed by a solubility product constant, K_{sp}, as shown below:

$$AlH(PPA)_2(s) \rightleftharpoons Al^{3+}(aq) + 2PPA^{2-}(aq) + H^{+}(aq)$$

$$K_{sp} = [Al^{3+}][PPA^{2-}]^2[H^{+}]$$

Since the aluminum and hydrogen ion concentrations are fixed by the pH, the above expression can be rewritten in terms of the phenylphosphonate concentration alone.

$$K_{sp}^{*} = K_{sp}/([Al^{3+}][H^{+}]) = [PPA^{2-}]^2$$

If precipitation is occurring, then [PPA] $= (K_{sp}^*)^{1/2}$. As additional phenylphosphonic acid is added to the solution, this excess will precipitate out, leaving the equilibrium phenylphosphonate concentration unchanged. Therefore, when precipitation is the controlling process, the fraction of phenylphosphonate remaining in solution ($[PPA]_{eq}/[PPA]_{initial}$) should be inversely proportional to the amount added. Data obtained at three initial concentrations was used to compute negative log ratios, ΔpPPA, where

$$\Delta pPPA = - \log ([PPA]_{eq}/[PPA]_{initial})$$

The calculated ΔpPPA values are plotted against pH in Figure 8. This shows that ΔpPPA decreased with increasing $[PPA]_{initial}$, the reverse of that expected if precipitation were occurring. It was therefore concluded that adsorption, not precipitation, was the process responsible for the decrease in solution PPA concentration.

The data in figure 8 also gives information regarding the pH stability limit for adsorption, defined as the pH at which no adsorption occurs. At this pH, $[PPA]_{eq} = [PPA]_{initial}$ and the value of ΔpPPA is therefore zero. This, of course, corresponds to the interception of each curve with the pH axis. Linear extrapolation gave pH stability limits of 9.4, 8.7, and 8.1 for 0.030, 0.050, and 0.100M, respectively. In each case the stability limit is near or above the pH of zero charge of the alumina substrate (*i.e.*, pH 8.5).

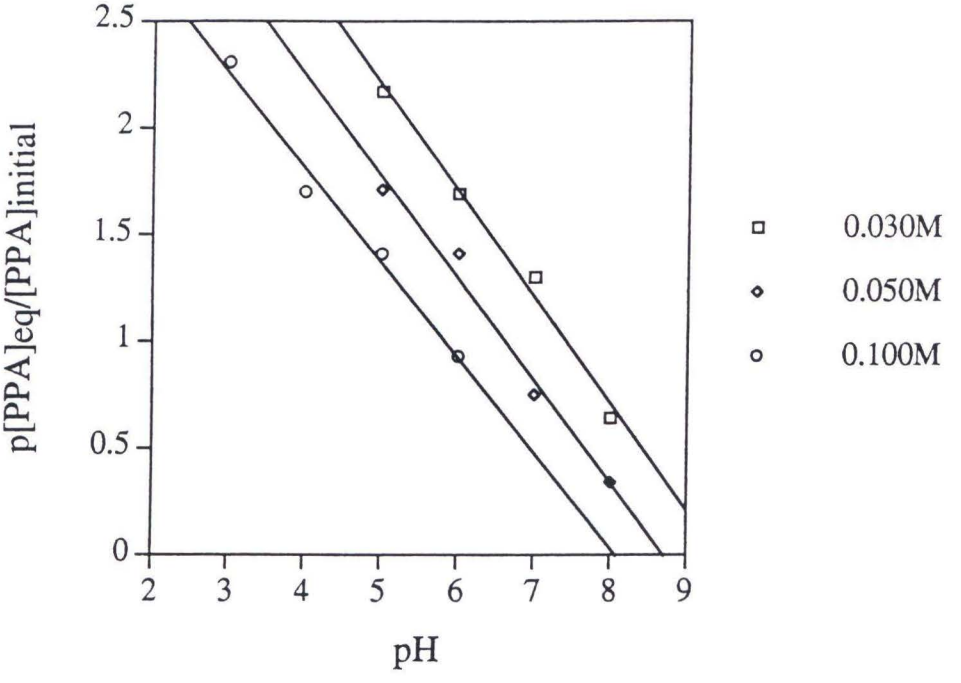


Figure 8: ΔpPPA as a Function of pH
at Three Initial Concentrations

Mechanistic Implications

The diffuse double layer model developed by Huang and Stumm[35] was used to qualitatively describe phenylphosphonate adsorption by alumina. This model depicts the interfacial region between a liquid phase and a solid surface as consisting of two planes: a surface plane for adsorption of H^+, OH^- and all specifically adsorbed solutes and a diffuse layer plane, representing the closest distance of approach for all counterions. Those ions which specifically adsorb on the surface do so by reacting with surface functional groups to form coordination complexes within the surface plane.

As discussed earlier, it has been established that phenylphosphonate ions associate with oxide surface sites via the formation of bidentate (or tridentate) inner- sphere surface complexes. The nature and degree of ionization of the species which interact (in solution and on the surface) to form these complexes varies with the pH of the solution. The possible reactions between phenylphosphonic acid and the alumina surface, therefore must vary with pH. Those reactions which are favorable will result in greater adsorption densities as compared to those reactions involving species which are less likely to interact. The adsorption envelope (Figure 7) is a quantitative measure of the extent of adsorption at different pH values.

The adsorption envelope consists of three general regions: below the pH of maximum adsorption (pH 1.5-3.0), at the maximum (pH 3.0) and above it (pH 3.0-8.0). At low pH's (relative to where maximum adsorption occurs) alumina's surface has a net positive charge through protonation of its surface hydroxyl groups. Additionally, as the pH decreases, an increasing fraction of phenylphosphonate species exist as fully protonated, neutral molecules (see Figure 5). This results in the following reaction between phenylphosphonic acid and the alumina surface:

$$\text{--Al-OH}_2^+ \quad + \quad \text{H}_2\text{PPA} \quad \rightarrow \quad \text{--Al-HPPA} \quad + \quad \text{H}_3\text{O}^+ \qquad (1)$$

This reaction, involving both a proton transfer and ligand exchange step, is unlikely for two reasons. First, this process would necessitate the release of hydronium ions into an already acidic solution. Second, there is no electrostatic attraction between the positively charged surface and the neutral adsorbate molecules.

Above the pH of maximum adsorption alumina's surface becomes increasingly deprotonated (*i.e.*, neutral) as it approaches its point of zero charge at pH 8.5. In addition, phenylphosphonic acid exists primarily in its partially ionized, monoprotic form (see Figure 5). Adsorption above pH 3.0 would therefore occur via a ligand exchange reaction between monoprotic phenylphosphonate ions in solution and hydroxyl groups on the alumina surface as shown below:

$$\text{--Al-OH} \quad + \quad \text{HPPA}^- \quad \rightarrow \quad \text{--Al-HPPA} \quad + \quad \text{OH}^- \qquad (2)$$

This exchange is also not favored for reasons similar to those given for reaction 1. First, this process involves the release of hydroxyl ions into an increasingly basic solution. Second, there is no electrostatic attraction of the negatively-charged phenylphosphonate species for the neutral substrate.

As already established, an adsorption maximum occurs at pH 3.0. At this degree of acidity, nearly 10% of alumina's surface hydroxyl groups are protonated (see Figure 4) resulting in a net positive surface charge. Further, only 6% of the phenylphosphonic acid species present in solution exist in their fully protonated form. The remaining 94% are

present as partially ionized, monoprotic species (see Figure 5). This suggests that two processes are occurring at pH 3.0. The first process involves a ligand exchange between the protonated surface and phenylphosphonate ions as shown below:

$$\text{--Al-OH}_2^+ \quad + \quad \text{HPPA}^- \quad \rightarrow \quad \text{--Al-HPPA} \quad + \quad \text{H}_2\text{O} \qquad (3)$$

This reaction is favored due to the electrostatic attraction between the two charged species. Also, no protons are released which would tend to inhibit this reaction in acidic solution.

The second process that must be occurring to a small extent involves a two-step process as follows:

1. Protons are transferred from fully protonated phenylphosphonic acid molecules (comprising approximately 6% of the total phenylphosphonate species present) to surface hydroxyl groups which, even at low pH, still predominate on alumina's surface.

$$\text{--Al-OH} \quad + \quad \text{H}_2\text{PPA} \quad \rightarrow \quad \text{--Al-OH}_2^+ \quad + \quad \text{HPPA}^- \qquad (4)$$

2. The resulting monoprotic phenylphosphonate ions replace water ligands on the surface to produce the surface-modified oxide.

$$\text{--Al-OH}_2^+ \quad + \quad \text{HPPA}^- \quad \rightarrow \quad \text{--Al-HPPA} \quad + \quad \text{H}_2\text{O} \qquad (5)$$

The net reaction is shown below:

$$\text{--Al-OH} \quad + \quad H_2PPA \quad \rightarrow \quad \text{--Al-HPPA} \quad + \quad H_2O \qquad (6)$$

This process must be occurring to a small extent at pH 3.0 in order for the adsorption density to be at a maximum at this value. Additionally, if this process did not play a role, an adsorption maximum would be expected at pH 4.5 where phenylphosphonic acid exists entirely in its partially ionized form.

Future Studies

Adsorption of phenylphosphonic acid onto alumina reaches a maximum at a pH value slightly below that corresponding to maximum amphoteric anion concentration (i.e., 4.5). To determine if this is a trend for organic polyprotic acids, data should be obtained for the construction of adsorption envelopes for the series of substituted phenylphosphonic acids shown in Table 2 below. These acids have sufficiently varied acid dissociation constants[38] such that the pH's corresponding to maximum monoprotic anion concentrations ($HRPO_3^-$) span a range of 3.78 to 4.64.

Table 2
Acid Dissociation Constants of
Substituted Phenylphosphonic Acids

Substituent	pK_{a1}	pK_{a2}	$[HRPO_3^-]_{max}$
m-NO_2	1.30	6.27	3.78
p-NO_2	1.24	6.23	3.94
m-Br	1.54	6.69	4.12
m-Cl	1.55	6.65	4.10
p-Cl	1.66	6.75	4.20
p-CH_3	1.98	7.24	4.61
p-OC_2H_5	2.06	7.28	4.64

Besides the diffuse double layer model utilized in this study, a number of different surface complexation models for describing equilibrium adsorption reactions at mineral surfaces have been developed in the last two decades.[39] Each of these models (*e.g.*, the constant capacitance model, the triple layer model, and the four layer model) assumes a different interfacial structure. The nature of the surface reactions and electrostatic interactions which are considered therefore vary from model to model. Equations have been derived for each model which mathematically describe those constraints imposed by the model's corresponding interfacial structure. Laboratory data can be fit to these equations to verify the applicability of such a model to a particular adsorption process. In this way, we can determine if, in fact, the diffuse double layer model best describes the adsorption of phenylphosphonic acid onto the surface of alumina.

CONCLUSIONS

By evaluating the effect of equilibrium adsorbate concentration on the adsorption of phenylphosphonic acid from solution onto the surface of aluminum oxide we have shown that this process follows Langmuir behavior with a monolayer density of 4.63 μmoles/m^2 at pH 5.0. Further, it has been demonstrated that this is a specific adsorption process occurring within the inner coordination sphere of the aluminum atoms. pH studies have shown maximum adsorption to occur at pH 3.0, corresponding to a predominance of monoprotonated phenylphosphonate ions. Electrostatic attraction of the negatively-charged phenylphosphonate ion by a positively-charged, protonated alumina surface facilitates ligand exchange.

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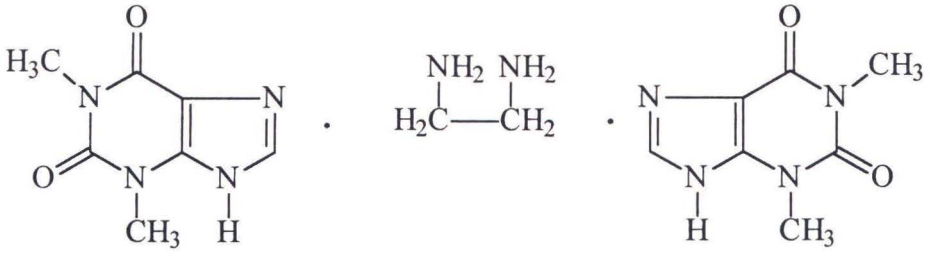
PART II

HPLC ANALYSIS OF AMINOPHYLLINE IN THIGH CREAM FORMULATIONS

INTRODUCTION

Aminophylline (1H-purine-2,6-dione-3,7-dihydro-1,3-dimethyl-1,2-ethanediamine) is a bronchorelaxant used primarily in the treatment of asthma. It consists of two components, theophylline and ethylenediamine, in a 2:1 molar ratio, as shown below. As a bronchial dilator, aminophylline is commercially available in several dosage forms. These include both single-dose and sustained-release capsules and tablets, solutions and suppositories.



Methods of Analysis

A number of methods have been reported for the quantitative determination of aminophylline in biological and pharmaceutical samples. The majority of these methods, however, measure theophylline content only. Some of the earliest methods were based on extraction of theophylline with organic solvents, precipitation of a theophylline salt, and gravimetric determination of the residue.[1] The official USP method is based on the titration of silver theophyllate with ammonium thiocyanate.[2] Other volumetric methods include the titration of theophylline with alkali to a potentiometric or colorimetric endpoint[3,4], the titration of ethylenediamine with a strong acid (*e.g.*, HCl)[5], and the complexation of theophylline with mercuric acetate and subsequent titration of excess

mercury ions with ammonium thiocyanate[6]. Alternatively, cupric acetate has been used followed by back-titration with EDTA.[7] There is also a nonaqueous titration procedure, utilizing sodium methoxide or acetous perchloric acid as the titrant, which allows for the determination of both components in a single titration procedure.[8,9]

Several spectroscopic methods have been developed in which the UV absorbance of theophylline is measured at 275 nm. Isolation of theophylline from its matrix is achieved in several ways. For pharmaceutical and many biological samples, extraction with an organic solvent is often used.[10,11] For serum samples, extraction with a mixture of ammonium sulfate, chloroform, and hexane followed by back-extraction of theophylline into aqueous borate buffer (pH 9) has been reported.[12] Charcoal extraction has also been used in which theophylline is adsorbed on charcoal and eluted with an organic solvent.[13] In another application, the theophylline present in blood samples was analyzed by oxidation with potassium dichromate in an acidic medium.[14] The oxidation product was then isolated by steam distillation and its absorbance measured at 257 nm. Finally, spectral subtraction methods were utilized to determine aminophylline content from the UV absorbances of samples containing benzyl alcohol as a preservative as well as mixtures of aminophylline and phenobarbitol.[15]

Many methods have been developed for the analysis of theophylline in biological samples based on high performance liquid chromatography (HPLC) and gas chromatography (GC).[16] Chromatographic conditions must be developed on a case-by-case basis to prevent interference from other drugs and/or theophylline metabolites. Ethylenediamine may also be analyzed by GC or HPLC although, until recently, its prior derivitization and separate analysis from theophylline was required.

Simultaneous HPLC Determination of Both Components

In 1991, Lau-Cam and Roos developed a chromatographic method which allowed for the simultaneous determination of both theophylline and ethylenediamine in solid and liquid dosage forms, specifically tablets and solutions.[17] The method is based on the HPLC separation of the two components as their dansyl derivatives (*i.e.*, dansyl-theophylline and bis-dansyl-ethylenediamine). It involves extraction of tablets into water or dilution of liquid dosage forms, reaction of the extract with dansyl chloride (5-dimethylamino-1-naphthalenesulfonyl chloride) in an alkaline medium, and analysis of the resulting mixture by reversed-phase HPLC. Separation is achieved on a standard octadecylsilica column using a quaternary mobile phase.

Derivatization of the two components prior to analysis is done for two reasons. First, by converting both theophylline and ethylenediamine to their corresponding dansyl derivatives, the two components become chromatographically resolvable and detectable under identical experimental conditions. Second, since ethylenediamine is inactive in the UV region, conversion to a UV absorbing derivative enables detection with a photometric detector.

The relative standard deviations (RSD) for five consecutive injections of a dansylated aminophylline standard was reported to be 0.45 and 0.53% (based on peak areas) for dansyl-theophylline and bis-dansyl-ethylenediamine, respectively.[17] As a statistical measure of the precision of the data, the RSD values reported show that this method demonstrates good reproducibility. Recovery was also shown to be greater than 99% for both components in both solid and liquid dosage forms.[17]

Newest Dosage Form

Recently, aminophylline has become available as an over-the-counter cream. It has been marketed by the cosmetic industry as an agent for the reduction of lower body fat. For asthma patients, aminophylline functions by dilating the bronchioles.[18] The receptors upon which it acts are also found in fat cells, with an especially high concentration occurring in the femoral region of women. It has therefore been hypothesized that, when applied topically, these creams are absorbed into the thigh where aminophylline serves to dilate the fat cells and thus facilitate the breakdown of resistant fat storage.[19]

The topic of thigh creams and the debate as to whether or not they actually work has received much media attention within recent years with articles appearing in countless women's magazines and even in a national news publication.[20] There are currently over 50 different aminophylline-based thigh cream preparations on the market. At costs ranging from $10-$30 per bottle, a year's supply of this product can cost the consumer up to $900.

There are other concerns, however, besides cost. Aminophylline is known to occasionally produce serious cardiopulmonary side effects in the dosages administered in asthma medications. Although it has been reported that, when applied topically, no trace of the drug has been found in blood chemistry tests [21], there have been no studies published to date that actually monitored cardiopulmonary functioning in response to acute and/or chronic use of such creams.

We have recently begun a study to address the issues of efficacy and cardiopulmononary effects associated with regular use. Since the actual dosing contained in these creams is not readily available to the consumer, it is conceivable that they may

contain far less than the reputed effective dose of 2% aminophylline. Quantifying the percent active ingredient in the cream preparations used in this study is therefore necessary.

Despite the extensive list of methods previously developed for the quantitative analysis of aminophylline (or at least its theophylline component), no one to date has done such an analysis on a cream formulation. The newest analytical method, that of Lau-Cam and Roos, was developed specifically for pharmaceuticals in solid and liquid dosage forms. A cream matrix presents a more formidable challenge. The purpose of this study, therefore, is to develop a means of quantitatively analyzing aminophylline in cream dosage forms through modification of Lau-Cam and Roos method.

EXPERIMENTAL

Materials

The thigh cream products were purchased from various commercial sources, as described later. Aminophylline standard was obtained from Sigma Chemical Co. (St. Louis, MO). Dansyl chloride was obtained from Lancaster Synthesis, Inc. (Windham, NH). Chromatographic-grade methanol, other solvents and sodium carbonate (used to adjust pH) were obtained from Fisher Scientific, Inc. (Fair Lawn, NJ).

Instrumentation and Conditions

All analyses were performed on an HPLC system consisting of a Perkin-Elmer Series 410 Solvent delivery system, a Rheodyne Model 7125 injector (10 μL loop), and a Perkin-Elmer Model LC-135 diode array detector set at 254 nm. Separations were achieved on a Microsorb-MV C-18 column, 15 cm x 4.6 mm i.d., 5 μm particle size (Rainin Instrument Co., Inc., Woburn, MA). Chromatograms were recorded and processed on a Perkin-Elmer Omega data system. The isocratic mobile phase consisted of 69% (by volume) methanol, 29% water, 1.5% acetic acid, and 0.5% triethylamine. The flow rate was 1.0 mL/min.

Preparation of Samples

Standard solutions were prepared from a stock solution containing 1 mg/mL aminophylline in water. 1-5 mL of the stock solution were mixed with 10 mL of dansyl chloride solution (5 mg/mL in acetone) and 10 mL of sodium carbonate solution (0.9 mg/mL in 50% aqueous acetone, by volume). The mixtures were allowed to stand at

room temperature in the dark for 12 hours, then brought to a volume of 50 mL and analyzed by HPLC under the conditions described above.

Cream sample solutions were prepared by adding 10 mL of 50% aqueous acetone to erlenmeyer flasks containing an accurately weighed quantity of cream (100-400 mg) as described in Table 10. The suspensions were then stirred for several minutes to dissolve the cream. Dansylation of the resulting solutions was performed in a similar manner as the standards under various conditions of reagent concentration, pH, and temperature as described later.

Further Preparation of Creams by Extraction

Removal of the organic, water-insoluble cream components prior to dansylation was attempted in the following manner: a 10 mL aliquot of cream solution prepared as described above was first transferred to a 50 mL centrifuge tube. 10 mL of chloroform was then added to the tube and the mixture was shaken vigorously for 2 minutes. After centrifuging at 3000 rpm for 5 minutes, the aqueous layer was removed, dansylated as described above, and finally, analyzed by HPLC.

Standard Addition Analysis

A total of 20 mL of a dansylated aminophylline standard (0.1 mg/mL) was added to an equal volume of a previously analyzed cream sample and rechromatographed.

Recovery Experiments

A mass of aminophylline weighing 13 mg was added to 1.30 g of a previously analyzed thigh cream formulation and stirred to form a homogenous mixture. 130 mg of this mixture was then prepared for analysis as outlined above.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram obtained from the analysis of a standard aminophylline solution using the method developed by Lau-Cam and Roos. The retention times for dansyl-theophylline and bis-dansyl-ethylenediamine were 7.01 min and 9.21 min, respectively. At 254 nm, the theophylline component gave a similar detector response as bis-dansyl-ethylenediamine, corresponding to the 2:1 molar ratio present in aminophylline samples.

All of our preliminary data on the cream samples was obtained using University Medical's Original Thigh Cream (University Medical Products, Newport Beach, CA). Due to the complex nature of its inactive ingredients, we perceived this cream to be the most difficult to analyze, and on this basis, chose it for all of the initial method development. The results shown in Table 1 were obtained when the cream samples were prepared and dansylated in exactly the same manner as the standards (*i.e.*, identical conditions of reagent conc., pH, and temperature). Figure 2A shows a typical chromatogram obtained from this analysis. In the presence of the other cream components, both theophylline and ethylenediamine exhibited a slight decrease in retention time. Ethylenediamine was most affected. More importantly, bis-dansyl-ethylenediamine exhibited a much lower response than did dansyl-theophylline, resulting in molar ratios of theophylline to ethylenediamine between 5 and 8 times greater than the expected 2:1 molar ratio (see Table 1).

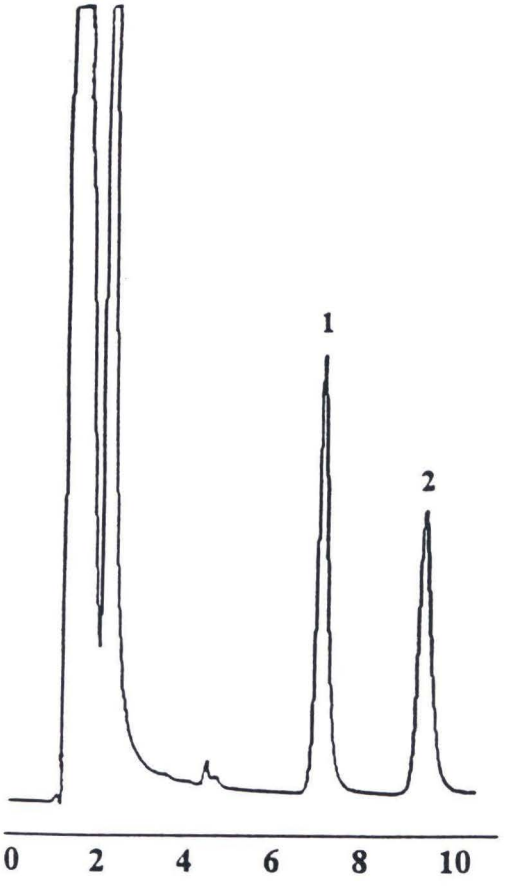


Figure 1: Chromatogram of a Standard Solution of Aminophylline after Dansylation. Identified peaks correspond to (1) dansyl-theophylline and (2) bis-dansyl-ethylenediamine.

Table 1
Initial Results on University Medical Original

Sample No.	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
1	4.97	0.128	13:1
2	3.74	0.132	9:1
3	4.87	0.147	11:1
4	5.51	0.124	15:1
5	5.64	0.143	13:1
6	5.42	0.153	12:1

Cream is a very complex matrix. Since the aminophylline in the cream was analyzed in the presence of this matrix, it appeared likely that the anamolous quantitative results were caused by interference from the cream. Two modes of interference were possible:

1. The cream matrix could be distorting the chromatographic separation and/or relative responses of the two components.
2. The cream components could be inhibiting the dansylation of one or both of the compounds in the aminophylline in an unequal manner.

Investigations were conducted to determine the source of the anamolous results and to minimize them, as discussed below.

Investigation of Possible Chromatographic Interference

As stated above, the possibility existed of cream components interfering with the quantitative data. A standard addition experiment was conducted to investigate this possibility in which an aliquot of derivatized standard was added to a previously analyzed sample and then rechromatographed. Table 2 shows the corresponding peak areas obtained. After addition of the derivatized standard, the peak areas increased by a similar amount for each component. The nearly identical increase in response indicates that the cream matrix did not affect the quantitative chromatographic results for either component. Therefore, it can be concluded that incomplete and/or unequal dansylation of the target compounds in the presence of the cream was the source of the anamolous results.

Table 2
Standard Addition Experiment

	Dansyl-theophylline Peak Area	**Bis-dansyl-ethylenediamine Peak Area**
before addition	52279984	7908328
after addition	95216896	51262802
Δ peak area	42936912	43354476

Attempted Extraction of Interfering Cream Components

The second possibility of the cause for the anomolous ratio of theophylline to ethylenediamine was interference of cream components (*e.g.*, various paraffins, herbal extracts and oils) in the dansylation process. To minimize these effects, we attempted to

extract the potential interfering compounds into chloroform and away from the reaction mixture prior to dansylation. Table 3 shows data obtained on four duplicate cream samples. As the number of extractions increased, the molar ratio was observed to decrease, approaching the theoretical value of 2:1. These results, however, were apparently caused by a decrease in the amount of theophylline detected (see Table 3) rather than an overall improvement in the dansylation. Theophylline was most likely lost by back-extraction into the chloroform layer.

To confirm the occurrence of this theophylline loss, a 0.025 mg/mL solution of aminophylline was analyzed, once with, and once without extraction into chloroform. The data presented in Table 4 shows that after four extractions only one-fifth of the original theophylline content remained in the aqueous layer removed for analysis. This confirmed the occurrence of back-extraction of the theophylline into the organic phase.

Table 3
Extraction of Cream Samples With Chloroform

Number of Extractions	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
1	3.71	0.35	3.5:1
2	3.13	0.30	3.4:1
3	2.95	0.38	2.6:1
4	1.89	0.37	1.7:1

Table 4
Extraction of Aminophylline Standard With Chloroform

Number of Extractions	Theophylline (mg/mL)	Ethylenediamine (mg/mL)	Molar Ratio
none	0.0200	0.0034	2.0:1
4	0.0039	0.0034	0.4:1

Optimization of Reaction Conditions

The demonstrated loss of theophylline during the attempted extraction of cream components prior to dansylation necessitated our reverting to performing the derivatization and analysis in the presence of the cream. Since the apparent problem with the analysis appeared to be interference by cream components in the dansylation process, we attempted to optimize the derivatization by a systematic variation of reaction conditions. Table 5 describes these variations and the resulting analytical data. As shown there, each variation produced an increase in the amount of both components detected compared to that obtained in initial experiments (Table 1). However, in most cases, the molar ratios were still higher than the expected 2:1 molar ratio, indicating incomplete dansylation of the target compounds. Only under conditions of vigorous stirring of the reaction mixture was the expected 2:1 molar ratio achieved.

Table 5
Optimization of Dansylation

Condition Varied	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
reagent conc. (increased 3-fold)	13.26	0.55	8:1
pH (raised to 3.5)	13.32	0.36	12:1
temperature (heated to 60°C)	9.87	0.39	8:1
vigorous stirring (magnetic stirrer at 300 rpm)	8.92	1.35	2:1

Method Validation

Table 6 shows the optimized derivatization conditions used in the remainder of this study. Figure 2B shows a chromatogram of a cream formulation derivatized under these optimized conditions. It exhibits the expected relative response for the two aminophylline components. System precision data, shown in Table 7, were obtained from successive injections of a single cream solution after derivatization. The RSD of results obtained for the ethylenediamine component is somewhat greater than that obtained for theophylline but still is under 3%. The precision of the entire method was evaluated by derivatizing and analyzing six separate samples of the same cream formulation. The results shown in Table 8 demonstrate good analytical reproducibility.

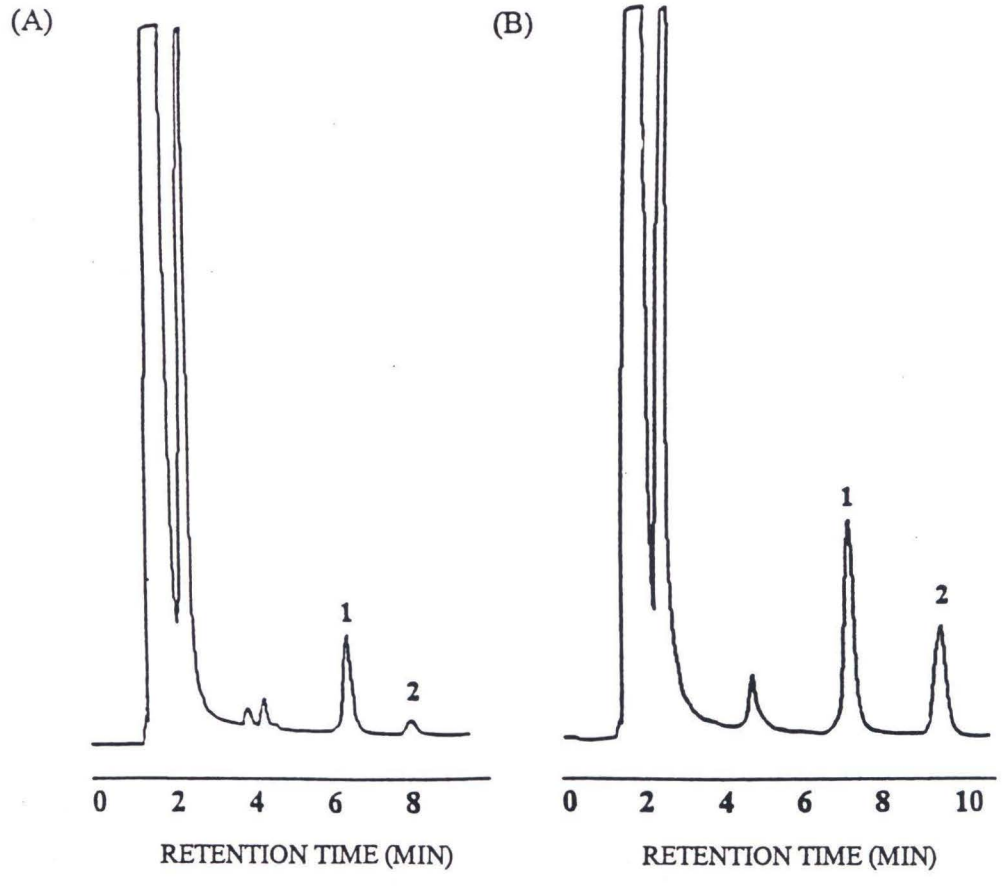


Figure 2. Chromatogram of a Cream Formulation under (A) Initial Dansylation Conditions and (B) Optimized Dansylation Conditions. Peak identities are the same as that described in Figure 1.

Table 6
Optimized Reaction Conditions

Dansyl Chloride Concentration	pH	Temperature	Special Conditions
2 mg/mL	2.2	ambient	in the dark stirred vigorously

Table 7
System Precision

Injection	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
1	8.69	1.32	2.19:1
2	8.59	1.28	2.24:1
3	8.60	1.36	2.11:1
mean	8.63	1.32	2.18:1
RSD	0.52%	2.47%	2.46%

Table 8
Method Precision

Sample	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
1	8.52	1.32	2.15:1
2	8.88	1.26	2.34:1
3	8.72	1.23	2.36:1
4	9.20	1.30	2.36:1
5	9.10	1.28	2.37:1
6	8.69	1.32	2.19:1
mean	8.85	1.28	2.29:1
RSD	2.9%	2.8%	4.3%

A recovery experiment was also conducted in which a weighed amount of aminophylline was added to a cream formulation prior to extraction, derivatization and analysis. Subtracting the amounts of each component which were initially found in the unspiked formulation gave the results shown in Table 9. However, the percent recovery determined in this way underestimates the actual recovery of the drug from a single analysis of such a formulation, since these results are based on extraction and analyses of two cream formulation samples, one spiked and one unspiked. Employing a propagation-of-errors approach, a better estimate of recovery from a single extraction and analysis was obtained by calculating a "corrected recovery", which is essentially the square root of the recovery based on the two analyses. The results, also shown in Table 9, indicate that at least 94% of each of the drug components were extracted and analyzed by this method.

Table 9
Recovery Experiment

Component	Amount Added (mg)	Amount Recovered (mg)	Percent Recovery	Corrected Recovery
theophylline	1.07	1.02	95%	97%
ethylenediamine	0.178	0.157	88%	94%

Additional Analyses of Commercial Creams

Three commercial creams were analyzed by this method. Results are shown in Table 10. Amounts of creams used for the analysis needed to be varied to obtain similar chromatographic responses. These results indicate that the percent active ingredient (calculated by adding the percentages of the two aminophylline components) varies widely among the commercial formulations.

Table 10
Aminophylline Content in Several Cream Preparations

	Amount of Cream Used for Analysis (mg)	Theophylline Detected (mg)	Ethylene-diamine Detected (mg)	Percent Aminophylline
University Medical Original (University Medical Products Newport Beach, California)	260	2.3	0.33	1.00%
Thinny Thighs (Winning Solutions, Inc., Westport, CT)	400	1.05	0.18	0.31%
Thigh High (Faneuil Companies, Scottsdale, AZ)	130	2.31	0.39	2.08%

CONCLUSIONS

In this study we have shown that the dansylation of both theophylline and ethylenediamine in thigh cream formulations is adversely affected by other components of these creams. This interference, which complicates the analysis of these components by reversed-phase HPLC, has been minimized through optimization of dansylation conditions. With this new method we have demonstrated excellent system and method precision as well as acceptable recovery. It appears that analyzing such complicated matrices requires a careful investigation and optimization of reaction and analytical conditions, especially when derivatization is involved. The analytical results show that there is a wide variation in the percent aminophylline present in various brands of thigh creams. This may have important consequences in conducting clinical studies of the safety and efficacy of these formulations.

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