THE ION PAIR EXTRACTION OF SOME AROMATIC SULFONATES

by

Eugene Joseph Roubal

(Under the supervision of Professor Takeru Higuchi)

Aromatic sulfonates, in the "acid dye" method (1), are employed as indicators in this procedure for the analysis of compounds containing a basic amino functional group. Formation of the ion pair and its extraction is considered to occur on shaking aqueous solutions of the sulfonate and cations, inorganic and organic, with an immiscible organic solvent where the cation-sulfonate concentration is then determined photometrically.

Factors such as dye or anion to be used, pH of the aqueous phase, effect of inorganic salts commonly present in the unknown sample, extracting solvent, etc., have been considered on a rather empirical basis in the past. Only recently has some investigations begun to appear in the literature that enable these parameters to be chosen with some degree of certainty.

The present research has been concerned with experimental studies on some model sulfonate compounds that allow the use of the very sensitive fluorescence assay method. These investigations included:

a) The influence of isomeric structural changes on the selective extractability of a limited range of ion pairs.

b) The partition coefficient - pH profile of the model compounds.

c) Effect of inorganic and organic cations as measured by their extraction constants.

Among the four hydroxynaphthalene sulfonate isomers used and the 2-naphthalenesulfonate the partition coefficient-pH profile shows that lower aqueous phase pH values may be used with the 1-naphthol-3-sulfonate and 1-naphthol-4-sulfonate isomers before extraction of the protonated form of the sulfonate begins to contribute to the organic sulfonate level. These isomers may be used at a pH as low as pH 3.5 whereas protonated forms of the 1-naphthol-2-sulfonate and 2-naphthol-1-sulfonate isomers begin to appear at about pH 4.8. The hydroxynaphthalene sulfonates with the functional groups separated by at least one carbon atom in the naphthalene ring are also less sensitive to inorganic cations. Isomers having the functional groups located adjacent to each other are ten to thirty times more sensitive to excess sodium ion than those with the functional groups separated from each other. Sensitivity to inorganic cation was found to be in the following order: $Cs^+ > Li^+ > K^+ > Na^+$ with excess magnesium and calcium salts having very little influence on the extraction of these sulfonate ion pairs. This observation suggests that blanks in the acid dye method would be minimized if extraneous salts in the unknown sample and in the aqueous buffer were limited to, for example, calcium or magnesium salts; sodium salts would

be the next choice. Extraction constants for the above systems are tabulated. The effect of the inorganic cations is in agreement with their state of hydration or degree of "negative hydration" as discussed by Samoilov (2).

As in the case of inorganic cations, the hydroxy-naphthalene sulfonates with the functional groups adjacent to each other are also most sensitive to amphetamine and its N-methylated analogs. Extraction constants with these organic cations were four to five orders of magnitude greater than with the sodium cation.

Tabulated values of the alcohol dependencies of the extraction of the organic and inorganic sulfonate ion pairs indicates that the sulfonate isomers with the functional groups separated from each other in the naphthalene ring are more dependent on the solvating agent.

A method for the preparative paper chromatographic isolation and purification of naphthalene sulfonates using powdered cellulose column chromatography is described.

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(1) Higuchi, T. and Bodin, J. I., in "Pharmaceutical Analysis," Higuchi, T. and Brochman-Hanssen, E., eds., Interscience Publishers, New York, N.Y., 1961, Chapter VIII.

(2) Samoilov, O. Y., "Struktura Vodnykh Rastvorov Electrolitov i Gidratatsiya Ionov," Moscow, 1957; ["Structure of Aqueous Electrolyte Solutions and the Hydration of Ions"], Consultants Bureau, New York, N.Y., 1965, Chapter 3.

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EUGENE JOSEPH ROUBAL

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To My Wife,

my translator, without whom

those German references would

never have meant so much.

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INTRODUCTION

The "acid dye" method (1) for the estimation of basic nitrogen compounds employs relatively large molecular weight "dye" or "indicator" compounds that commonly contain one or more sulfonate functional groups attached to aromatic portions of the compound. Ion pairs are formed upon shaking aqueous solutions of these sulfonates and cations, organic and inorganic, with immiscible organic solvents and appear in the organic phase where their concentration may be determined photometrically.

Factors such as dye to be used, pH for the aqueous phase, effect of inorganic salts commonly present in the unknown sample, extracting solvent, etc., have been chosen on a rather empirical basis. Only recently have some investigations begun to appear in the literature (3-18) that enable these parameters to be chosen with some degree of certainty.

The present research has been concerned with experimental studies on some model sulfonate compounds that allow the use of the very sensitive fluorescence assay method. These investigations included:

a) The influence of isomeric structural changes on the selective extractability of a limited range of ion pairs.

b) The partition coefficient -- pH profile of the model compounds.

c) Effect of inorganic and organic cations as measured by their extraction constants.

PART I

DISTRIBUTION STUDIES WITH INORGANIC CATIONS AND THE EFFECT OF SOME ANIONS

INTRODUCTION

In the widely used acid dye method (1) for the estimation of basic nitrogen compounds, the "acid dye" commonly refers to a sulfonic acid salt such as bromcresol purple, bromthymol blue, chlorophenol red, bromcresol green, tropaeolin, bromphenol blue and others. These are mostly colored pH indicators which form readily detectable ion pairs that are extractable into the organic phase, their concentration being easily determined colorimetrically. This information is then used to evaluate the concentration of the nitrogen compound initially present in the water phase.

The effect of inorganic salts on the extraction of these ion pairs was first noted by Ballard and Isaacs (2) who observed that sodium ion added to the aqueous phase increased both the extraction of the sulfonate salt alone and also that of the amine sulfonate ion pair. Schill, et al. (3,4), observed the "interfering effect" from Na^+, K^+ and NH_4^+. A thorough, documented discussion of the optimum pH for the method was presented (1) where it was reasoned that if the protonated form of the dye is soluble in the organic solvent the pH region to be explored is the pH at which the particular dye may be used to keep the blank value

reasonable. The pH used then depends on the pK of the base in question and must be such that the base is protonated. If the base is only weakly basic, a dye with a lower pT may have to be used so a lower pH may be employed.

Schill, et al. (5-12), have published a series of papers on the determination of amines and quaternary ions as "complexes" in which he dealt extensively with the analytical aspects of the method. Work on the physical chemical aspects of the procedure associated with the molecular weight and chain branching of the aliphatic amine have been presented by Biles, et al. (13), and on the effect of a proton donating agent added to the organic phase to enhance the extraction of the amine salts of dyes (14). Higuchi, et al., have studied the physical chemistry of the system pertaining to various anions and solvating agents (15-17). The effect of anion, alkyl chain length, the kinetics and thermodynamics of ion pair extraction have been reported (18).

In the acid dye method the choice of dye or anion, extracting solvent and pH for the aqueous phase has, until recently, been largely empirically or semi-empirically chosen parameters. The blank in the system must be considered and the possibility of nonspecificity among the basic nitrogen compounds should be recognized. As noted above some work has been directed to these areas, but many problems have not yet been studied.

The indicators commonly used in the acid dye method are rather complicated structures, but they all contain one or more sulfonate groups attached to an aromatic molecule. Being the salt of a strong acid, the sulfonate remains in the anion form to a much lower pH than, for example, the usual carboxylate or other acidic groups attached to an aromatic molecule. The availability of such an anion at low pH values allows even weakly basic amino functional groups to be converted to the cation form in the aqueous phase, a requirement for the amine to partition in the form of an ion pair and not as the free base which would be undetected while following the sulfonate photometrically.

The present report concerns results of an investigation designed to determine the influence of isomeric structural changes on the selective extractability of a limited range of ion pairs. To permit some insight into the phase transfer process a series of sulfonate salts formed with inorganic and organic cations were equilibrated between aqueous and organic phases. Among the factors studied were the effects on the relative extractability of the model compound produced by some common functional groups in the "acid dye," positional isomers, inorganic cations, anions, the pH and degree of substitution of the amino functional group in the basic nitrogen compound. Particular emphasis was put on the

ion pair partitioning of the sulfonate with inorganic cations, i.e., the blank in the acid dye method.

Hydroxyl naphthalenesulfonates were chosen as model compounds to satisfy the above characteristics; the hydroxyl group lends the naphthalenesulfonate to the very sensitive fluorescence assay method and provides intramolecular masking of the electrical field of the sulfonate group, the masking effect being a part of this study. Also, the simplicity of this molecule allows manipulation of the position of the functional groups while maintaining the same molecular weight.

THEORETICAL CONSIDERATIONS

The basic principle on which the above procedures depend is the distribution of an ion association compound into the bulk of the organic phase in a solvated form such that the participating ions are somewhat soluble in an organic solvent or solvent mixture. This type of association is sometimes referred to as an ion pair, a Bjerrum-type ion pair (19), an ion association compound, a pseudo-molecular species, coordinately solvated salts, etc. In this work the associated ions will be referred to as an ion pair and the association may be considered as:

$$M^+_{H_2O} + S^-_{H_2O} \rightleftharpoons (M,S)_{organic}$$

with an association constant,

$$K = \frac{(M,S)_{organic}}{(M^+)_{H_2O}(S^-)_{H_2O}}$$

M^+ represents the cation, S^- the sulfonate and (M,S) the ion pair.

Inorganic cations are relatively small cations with a high charge density located in their electronic cloud that enables them to have far reaching effects on the water dipole and water structure. Some inorganic cations are highly hydrated by orienting the water dipole around

their electronic sphere of influence in an ion-dipole interaction. However, this sphere is not to be considered as a combination of a certain number of specific water molecules with the ions but as the action of the ions on restricting the thermal motions, and principally the translational motion of neighboring water molecules. Recent treatment of the state of ion hydration in aqueous solutions indicates that the hydration shell of a hydrated ion is in a state of flux such that the restricted, polarized and bound water molecules change equilibrium positions from the hydration shell to the water medium very frequently. This rate of change of equilibrium position is considerably diminished in the highly hydrated calcium and magnesium ions. At greater distances from the center of electric field the water structure is merely disturbed by a hydrated ion-dipole interaction and this "structure breaking" region is such that the molecules must accommodate themselves to the different configurations between which they are sandwiched, the last of which being the tetrahedral structure of water.

On the other hand, some cations of the alkali metal family have what Samoilov (20) has introduced as "negative hydration" by analogy with their negative water viscosity effect. According to present knowledge the thermal motion of a bulk water molecule causes it to undergo frequent exchange between the tetrahedral water structure and existing neighboring cavities. This filling of cavities

and the change in equilibrium positions of the water molecule represents the most important peculiarity in the molecular translational motion in water (21). Water molecules surrounding the ions considered in this paragraph are more mobile than those in pure water, i.e., they are loosened from those in the water structure, their translational energy is actually increased, the water dipole undergoes exchange with its nearest neighbor more frequently, no binding of the previous sense occurs and the viscosity of the water is lowered. These are relatively large ions, and water accepts them by an envelopment into the water structure, with the smallest possible change of that structure. This recent treatment of the ion-dipole interaction is based on N.M.R., X-ray diffraction, infrared spectroscopy and Raman spectra studies.

In the alkali metal family, lithium is highly hydrated, sodium is much less so; but potassium, rubidium and cesium are "negatively hydrated" cations (20). The charge density of the small lithium cation is sufficiently high to polarize and form a solvation shell with lower alcohols as well which is easily understood since alcohols possess the basic functional group associated with many of the physical and chemical properties of water. Furthermore, the -OH group is in a position to exhibit both its donor and acceptor character, so these solvents, like water, can solvate both cationic and anionic species.

Ion pairs are considered to be either of the "intimate" or the "non-intimate" type depending on whether there is no solvent or there is a solvent molecule or molecules interposed between the two ions. It is preferable to attach a rather wide meaning to the term "ion-pair," regarding this species as one in which the bonding is almost entirely due to long-range electrostatic forces between the oppositely charged ions (22). The more specific term of "metal complex," for which a satisfactory definition is difficult to give, has been suggested as an association in which the two ions are adjacent to and interact directly with each other, but whether covalent or ionic bonding exists is still open to question (23). Nancolas (22) views the forces of attraction in the ion pair as physical in contrast to the chemical forces involved in coordination complexes. Reported values of association constants for a series of alkali and alkaline earth metal ions with the small ionic ligands, the highly charged unidentate anion ligands and the multidentate ligands, fall into the expected sequences of Li > Na > K and Mg > Ca > Sr > Ba (22).

When an aqueous phase of a suitable buffer and water soluble salt of an aromatic sulfonate is shaken with chloroform containing, for example, isoamyl alcohol, extraction of some sulfonate into the organic layer occurs. This may be represented by the following relationship (13,17):

$$(S^-)_w + (M^+)_w \underset{-nR}{\overset{+nR}{\rightleftharpoons}} (M,S\ R_n)_o$$

where $(S^-)_w$ represents the sulfonate concentration in the aqueous phase, $(M^+)_w$ the aqueous metal ion concentration, $(M,S\ R_n)_o$ the solvated ion pair in the chloroform solvated by n molecules of the alcohol, R.

The apparent partition coefficient may be developed by considering all the species in solution:

$$P.C._{app.} = \frac{S_{To}}{S_{Tw}} = \frac{(HS)_o + (M,S)_o + (M,S\ R_n)_o}{(S^-)_w + (HS)_w + (M,S)_w + (M,S\ R_n)_w}$$

The (HS) terms may be eliminated because of the pH of the aqueous phase, the $(M,S)_o$ term may be eliminated because no partitioning occurs using pure chloroform for the organic phase, and the existence of $(M,S)_w$ $(M,S\ R_n)_w$ is not likely in the dilute solutions used in this work. The resulting relationship becomes

$$P.C._{app.} = \frac{(M,S\ R_n)_o}{(S^-)_w} = \frac{C_o}{C_w}$$

The equilibrium constant for the above reaction is defined as

$$K_o = \frac{(M,S\ R_n)_o}{(S^-)_w(M^+)_w(R)_o^n}$$

If this term is converted to its logarithm it may be seen that the slope of a plot of log $P.C._{app.}$ versus log R at constant $(M^+)_w$ gives the stoichiometry of the alcohol in the solvated ion pair. Another useful term for the above equilibrium is the extraction constant (15) as defined by

$$K_e = \frac{(M,S\ R_n)_o}{(S^-)_w(M^+)_w}$$

Using this constant, the effects of various cations on the partitioning of the sulfonate isomers may be observed.

RESULTS AND OBSERVATIONS

Temperature Dependence of Extraction of the Ion Pair

Early work being done at ambient temperatures suggested the need to control the temperature of the phases while partitioning was carried out. Figure 1 was obtained with the aqueous phase 0.04 molar in total potassium ion, 5.68×10^{-3} molar in 1-naphthol-2-sulfonic acid potassium salt in a potassium citrate buffer at pH 4.65. The extractant was chloroform containing 15% v/v iso-amyl alcohol. The temperature at which the extractions were carried out was maintained within 0.3°C of the desired temperature.

Obviously the temperature must be controlled, for the data could easily vary by as much as 25% during warm weather, decreasing as the room gets warmer. Although Weissburger (24) notes that distribution of neutral organic species between immiscible aqueous and lipoidal phases is relatively insensitive to temperature variations, partitioning of ion pairs appears to be much more sensitive to temperature.

pH Profiles and Related Sodium Dependencies

Aqueous solutions of organic sulfonates including the sodium salts of 1-naphthol-2-sulfonic acid,

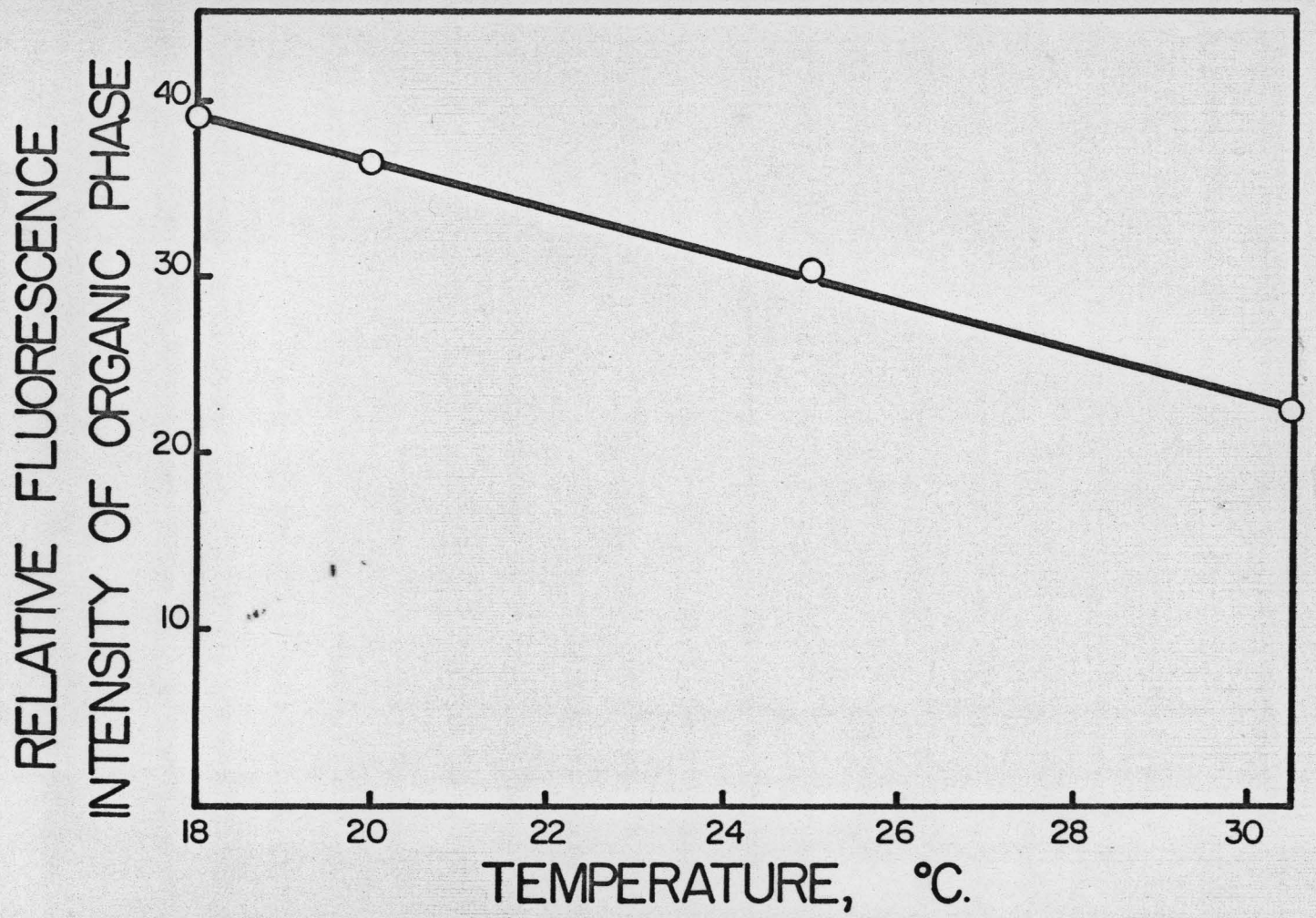


Fig. 1 The extraction of sodium 1-naphthol-2-sulfonate between the aqueous phase and a chloroform-isoamyl alcohol (15% v/v) phase as a function of temperature.

2-naphthol-1-sulfonic acid, 1-naphthol-3-sulfonic acid, 1-naphthol-4-sulfonic acid and 2-naphthalenesulfonic acid appear to be extractable into a chloroform phase both in the free acid and sodium ion-pair forms. This is evident in Figure 2 which shows the observed apparent partition coefficient, the ratio of the concentration of the sulfonate in the organic phase to that in the water phase, as a function of pH of the aqueous phase at 25°. In these studies the aqueous phase was always 7.28×10^{-3} molar in total sodium ion, 5.68×10^{-3} molar in the sodium sulfonate, 5.33×10^{-4} molar in citrate buffer and adjusted to the desired pH with dilute sulfuric acid. The organic phase was ethanol-free chloroform containing iso-amyl alcohol 20% v/v. From the profiles it would appear that below pH of 4 to 5 increasing amounts of the free acidic species seem to be extracted. The isomers with adjacent functional groups appear to be weaker acids since the $P.C._{app.}$-pH plot shows an early increase in the slope of the curve with two such isomers. The stronger acidic nature of the compounds with functional groups separated from each other is observed in the delay of extraction of the protonated form until the aqueous phase is made more acidic.

Because of the strongly acidic nature of the sulfonic acid group a valid pK value is not available; aryl-sulfonic acids are, however, generally credited with a pK

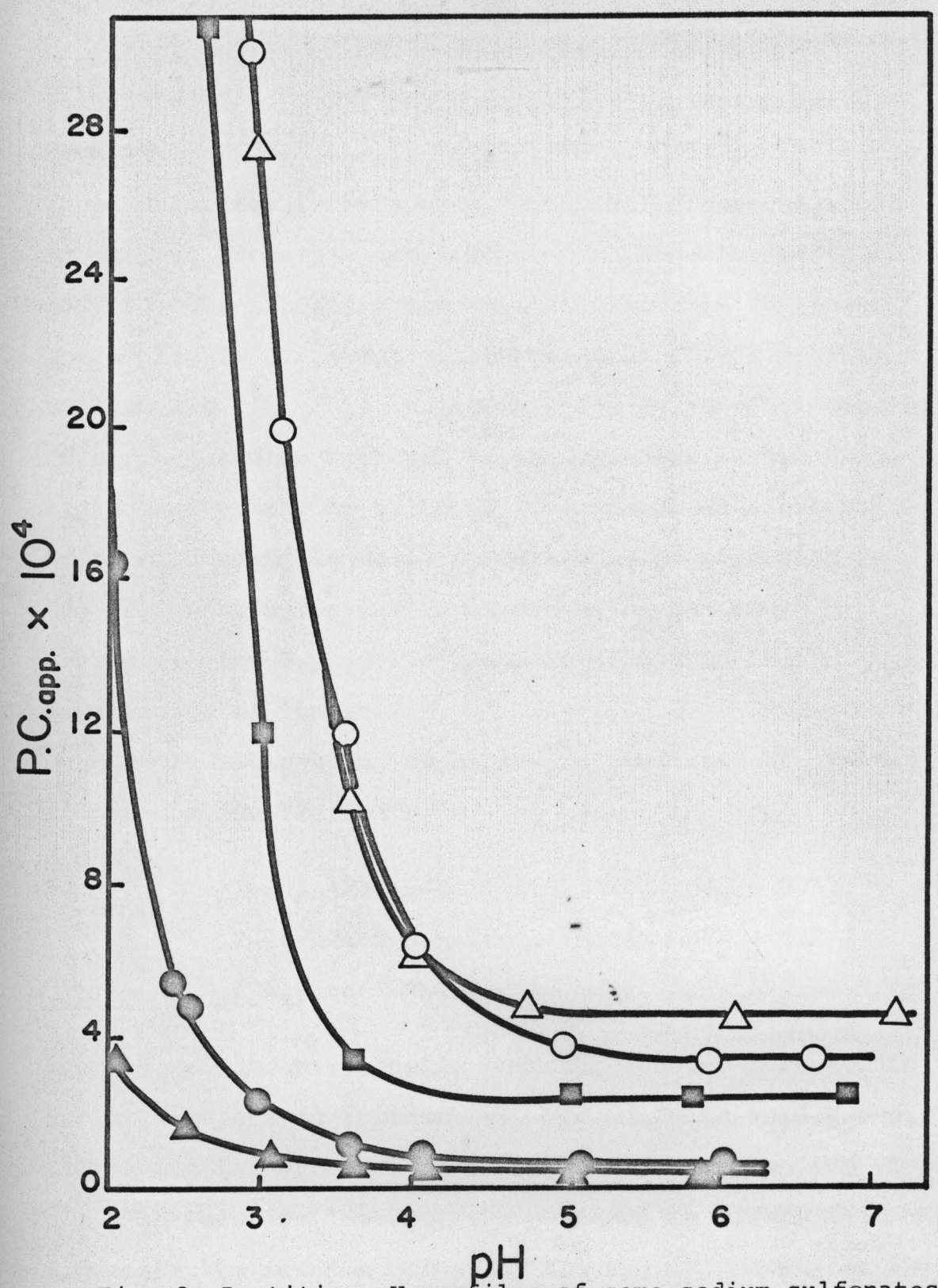


Fig. 2 Partition-pH profiles of some sodium sulfonates.
○ 1-naphthol-2-sulfonic acid, sodium salt.
● 1-naphthol-3-sulfonic acid, sodium salt.
▲ 1-naphthol-4-sulfonic acid, sodium salt.
△ 2-naphthol-1-sulfonic acid, sodium salt.
■ 2-naphthalenesulfonic acid, sodium salt.

value of about 0.6 (25). Potentiometric determinations of the pK values of the somewhat weaker appearing 1-naphthol-2-sulfonic and 2-naphthol-1-sulfonic acids gave pK 2.60 and pK 2.55 respectively. The lower acidic strength of the isomers with functional groups adjacent is curious since the appearance of a phenolic hydrogen next to the carboxyl group in salicylic acid increases the ionization constant of benzoic acid from 6.30×10^{-5} to 1.06×10^{-3}. This is probably due to internal bonding from the phenolic hydrogen to the carboxyl oxygen (26). In the case with the sulfonic acid group, the internal hydrogen bonding is still present but the planarity in the sulfonate anion (27) is hindered by the close proximity of the phenolic group resulting in the suppression of ionization.

It is interesting to note some published pK_2 values of these acids (28):

1-naphthol-2-sulfonic acid $pK_2 = 9.3$
1-naphthol-3-sulfonic acid $pK_2 = 8.2$
1-naphthol-4-sulfonic acid $pK_2 = 7.9$.

In this series the phenolic function becomes more acidic as it is separated further from the sulfonic acid group, indicating the decrease in internal hydrogen bonding (28).

The relative level of the plateaus in Figure 2 indicates the freedom gained by the hydroxyl group as the sulfonate group is moved away from it; less intramolecular

hydrogen bonding makes the molecule more water loving and less acceptable to the organic phase. The 2-naphthalene-sulfonic acid, sodium salt, with a pK of 0.25 (29) has an intermediate plateau value and the sharp rise due to the protonated species is found in an intermediate position which further demonstrates the hydroxyl water-affinity effect evident in the other compounds. For practical purposes, the pH profiles show that when an aromatic sulfonate type of compound contains a hydroxyl group and when these functional groups are separated, lower blanks can be expected in the use of the acid dye method.

A further indication of the extraction of the free acid at lower pH values may be given by looking at the $\Delta P.C._{app.}$ as a function of pH. The assumptions in the Henderson-Hasselbach equation make it inapplicable to strong acids but give a mathematical relationship of the ratio C_a/C_s to pH. From the Henderson-Hasselbach equation

$$\log \frac{C_a}{C_s} = pK_a - pH = \text{constant} - pH.$$

Now assume that the protonated acid is virtually all in the organic phase and let C_a be represented by C_o, therefore

$$\log \frac{C_o}{C_w} = \log \Delta P.C._{app.} = -pH + \text{constant}.$$

Figure 3 is obtained by plotting log $\Delta P.C._{app.}$ versus pH. The $\Delta P.C._{app.}$ is from the $\Delta P.C._{app.}$ above the plateau of the pH profile curve of Figure 2 for 1-naphthol-2-sulfonic acid, sodium salt. Experimental data is in good agreement with theory and confirms the partitioning of the protonated species.

Salts of various kinds are always present when compounds of this type are used in practice, and the extraction of the sulfonate, commonly referred to as the "blank" in the acid dye method, is quite sensitive to some of these salts. In association with the pH profiles of these isomers their partitioning dependency on the sodium ion concentration was observed at several pH values so chosen as to be at a pH on the plateau, at a pH where the protonated form was the main species being extracted and at an intermediate pH. Invariably the dependency was greatest at pH values in the plateau region, much less at the intermediate pH and far less, if any, at the pH where the protonated form was being extracted. Figures 4-8 represent these data. The lowest aqueous sodium ion concentration in all experiments was 7.28×10^{-3} molar total sodium ion and the aqueous solutions were identical except for the sulfonate used which was always 5.68×10^{-3} molar throughout. The citrate buffer was always 5.33×10^{-4} molar in citrate which contributed sodium ion to the extent of 1.6×10^{-3} molar. Stock solutions were used so the sodium ion

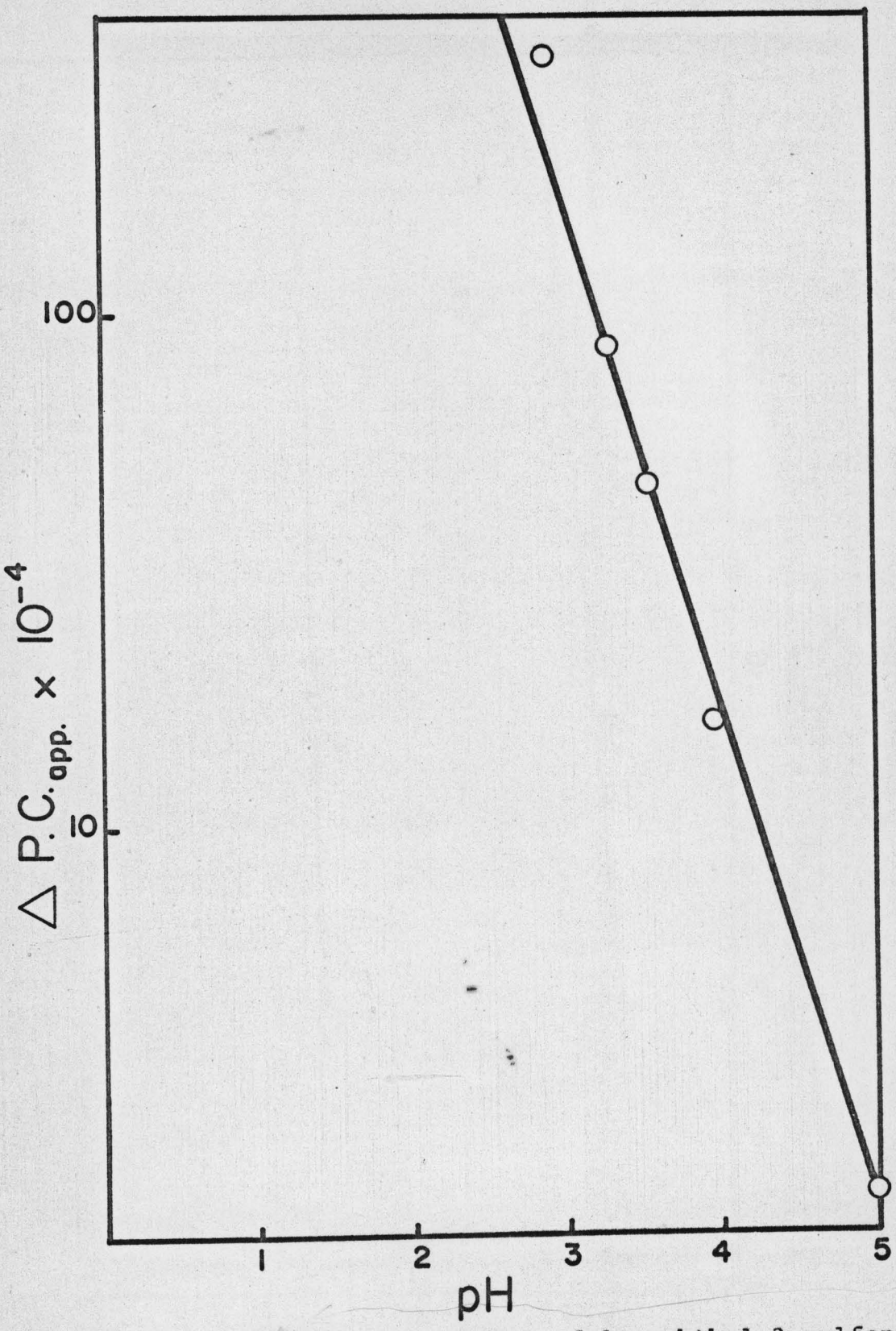


Fig. 3 The ΔP.C.$_{app.}$-pH plot of 1-naphthol-2-sulfonic acid, sodium salt.
O Experimental points.
— Theoretical line.

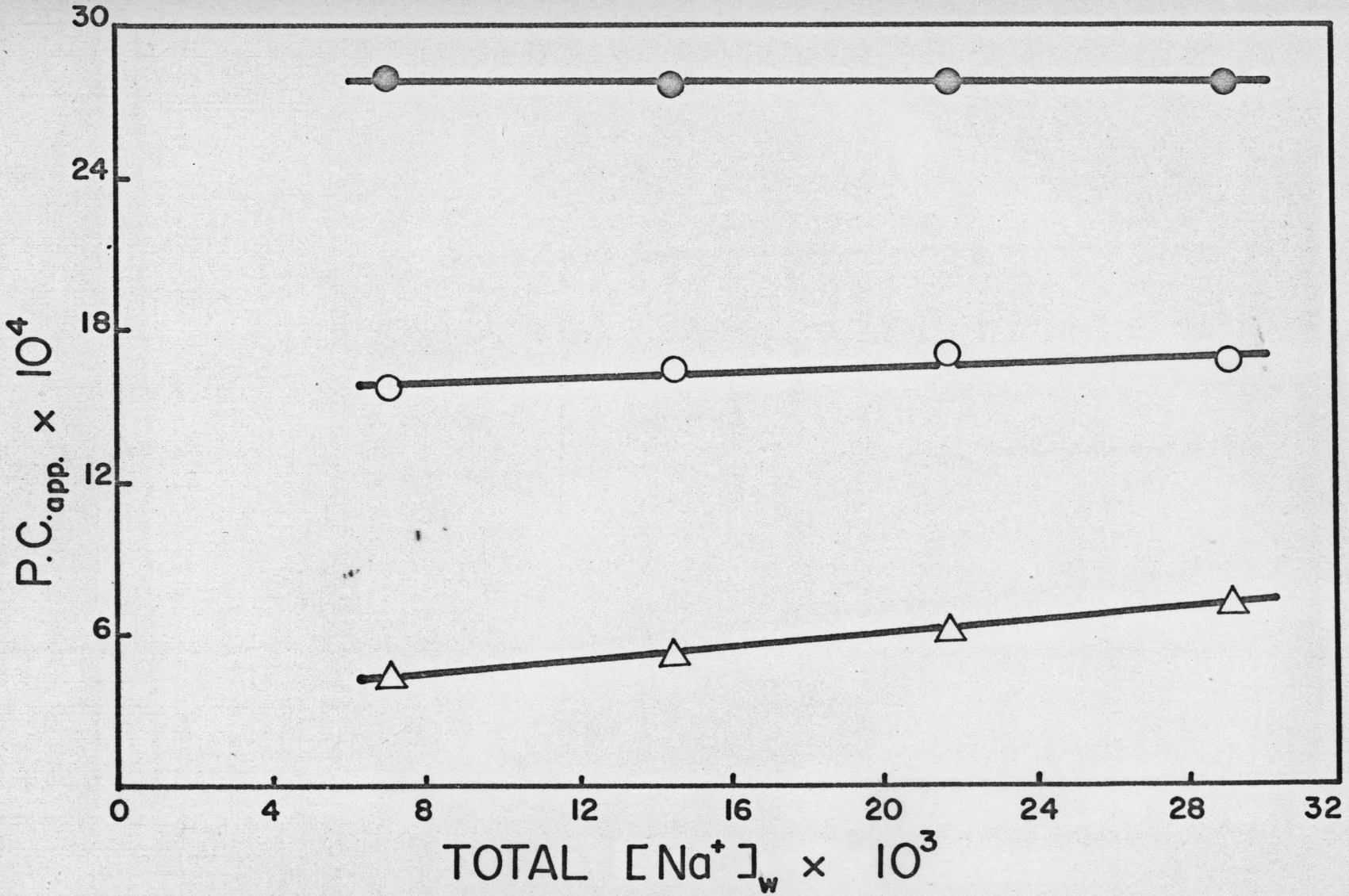


Fig. 4 The pH-sodium ion dependency of the partitioning of 1-naphthol-2-sulfonic acid, sodium salt. △ pH 6.2; ○ pH 3.5; ● pH 3.1.

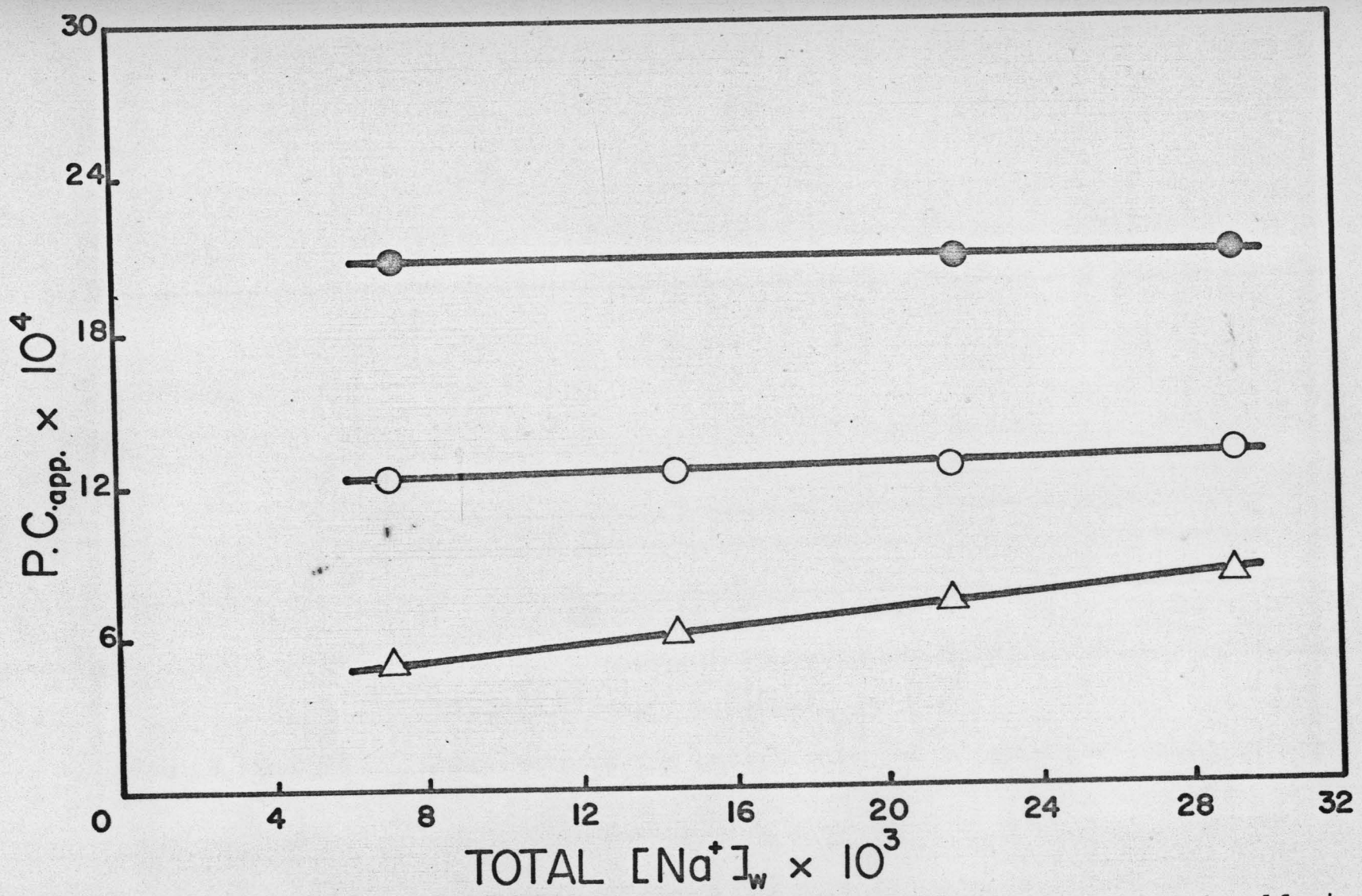


Fig. 5 The pH-sodium ion dependency of the partitioning of 2-naphthol-1-sulfonic acid, sodium salt. △ pH 6.2; ○ pH 3.5; ● pH 3.1.

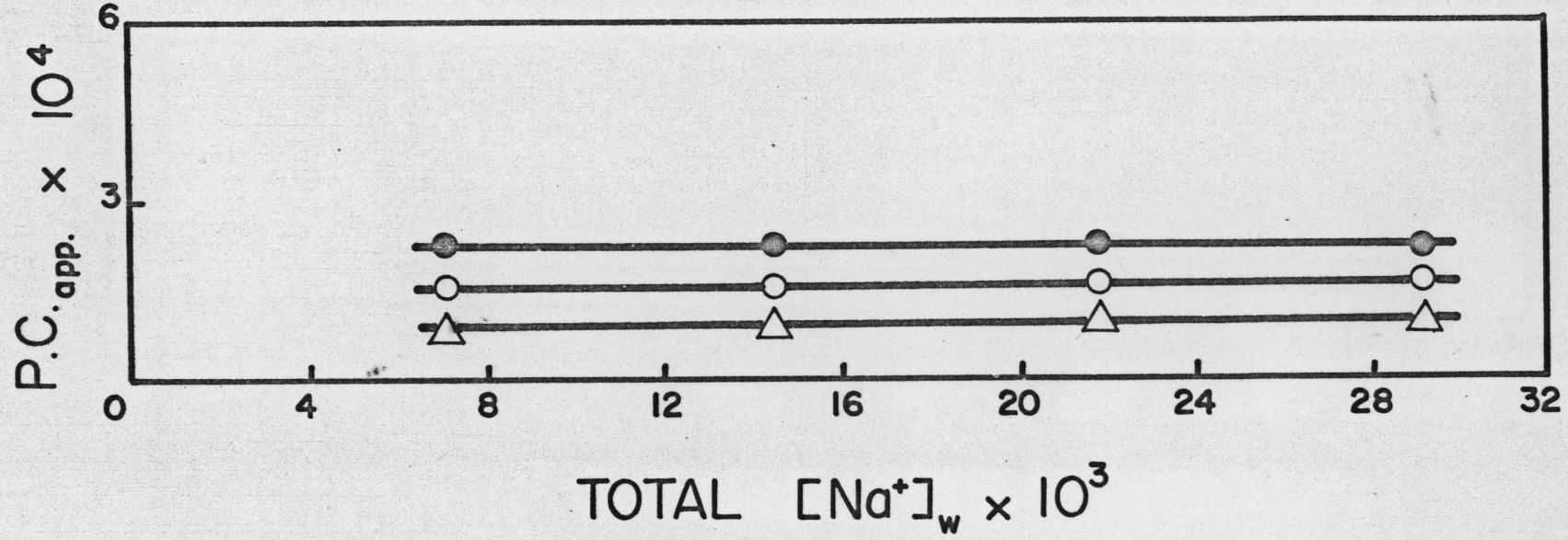


Fig. 6 The pH-Na^+ dependency of the partitioning of 1-naphthol-3-sulfonic acid, sodium salt. △ pH 6.2; ○ pH 3.5; ● pH 3.1.

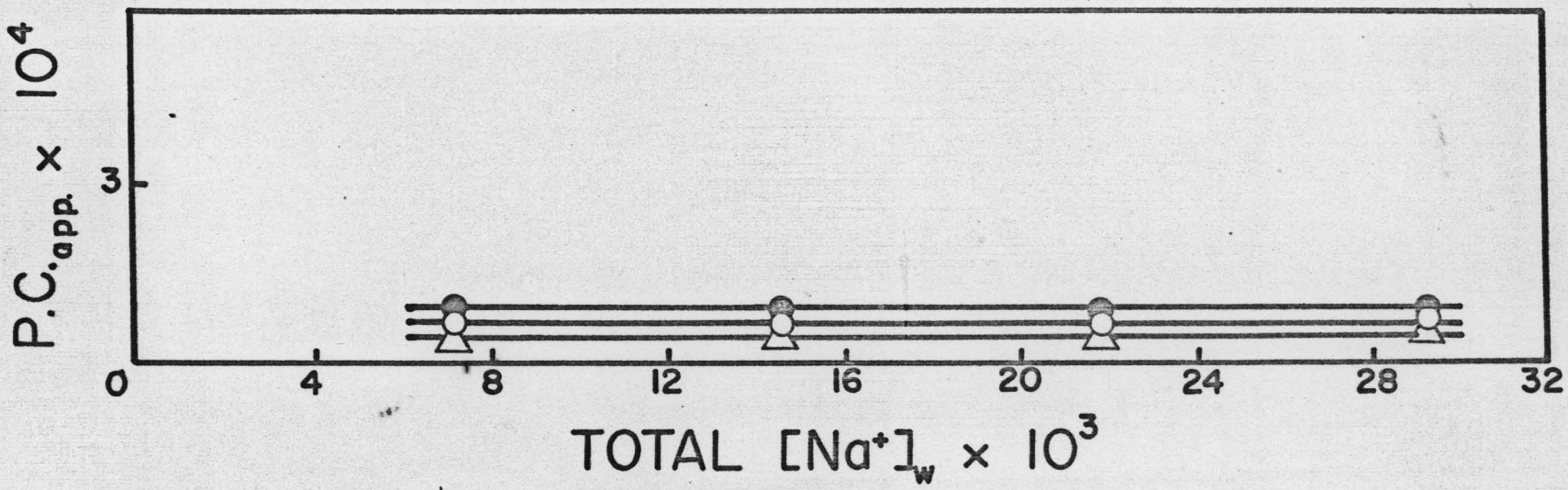


Fig. 7 The pH-Na^+ dependency of the partitioning of 1-naphthol-4-sulfonic acid, sodium salt. △ pH 6.2; ○ pH 3.5; ● pH 3.1.

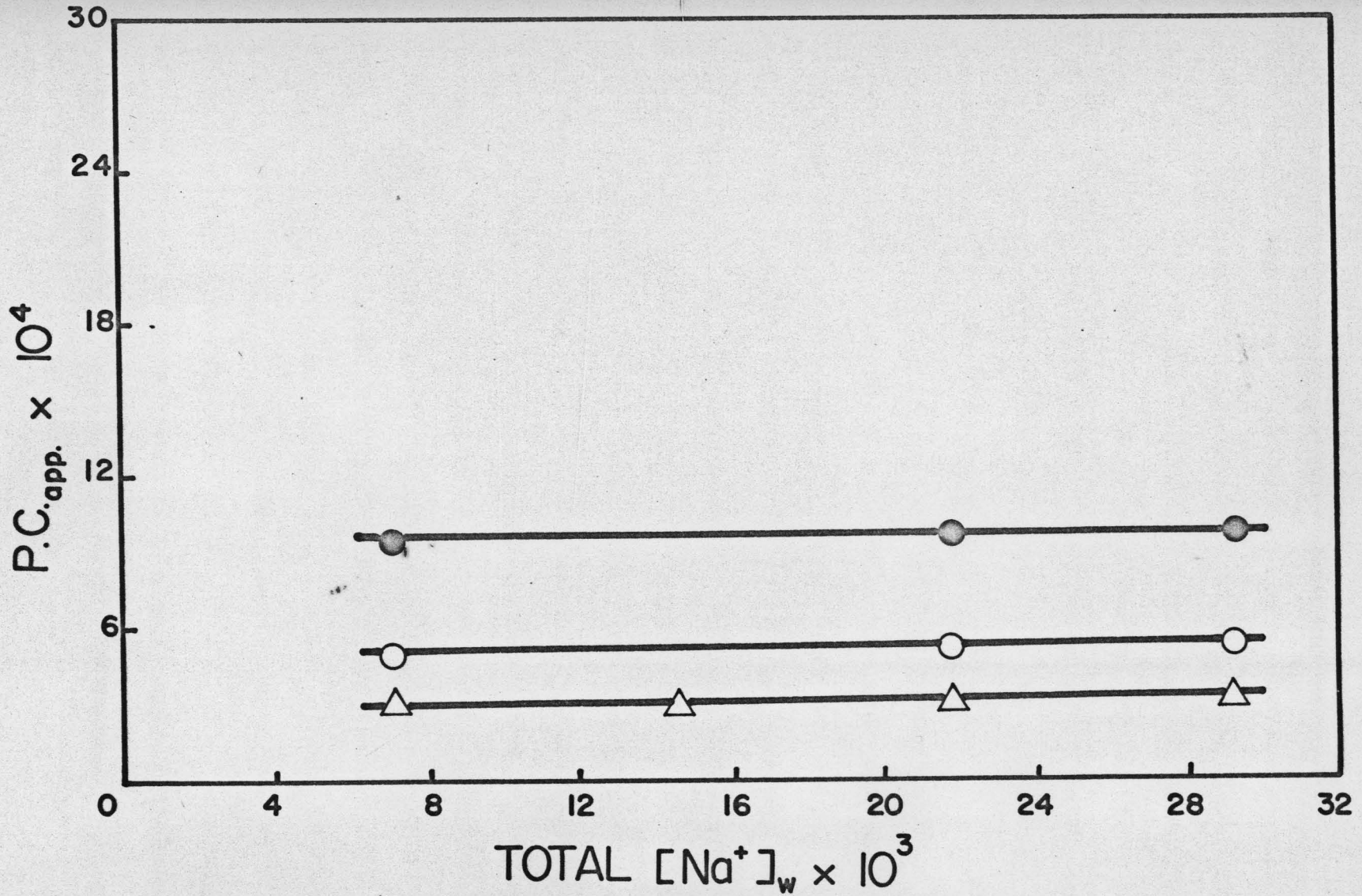


Fig. 8 The pH-Na^+ dependency of the partitioning of 2-naphthalene sulfonic acid, sodium salt. △ pH 6.2; ○ pH 3.5; ● pH 3.1.

concentration could be adjusted with sodium sulfate, the pH could be adjusted with sulfuric acid and the resulting citrate buffered solution brought to volume with water. Only the effect of the sodium ion was observed here, the more detailed effect of this and other inorganic ions are reported later in this work. Also to be noted later is that the partitioning is independent of the anions and in their concentrations used here.

The partitioning dependency of the isomers with functional groups adjacent to each other was much more than when they were separated by at least one carbon atom and this dependency decreases as the pH is lowered. This indicated that the ion association compound was the species sensitive to sodium ion, and the formation constant, while not determined here, was much higher for the isomers with adjacent functional groups. The non-hydroxyl sulfonate and compounds with functional groups separated appear not to be particularly sensitive to sodium ion (see Figures 6-8). It is shown later on, however, that there is some dependency at much higher sodium ion concentrations in the pH plateau region even though it does not appear in the latter three figures. It is interesting to note the effect of the hydroxyl group in Figures 6 and 7 compared to the absence of this group in the compound studied in Figure 8. This effect is quite evident in the $\Delta P.C._{app.}$ as a function of pH as well as the $P.C._{app.}$ of the compound.

General Cation and Anion Dependency

As noted earlier the partitioning of these compounds is quite sensitive to some inorganic cations (2-4), and the presence of such entities is common in the use of indicators chemically similar to the sulfonates studied here.

The comparative general partitioning behaviors of the 1-naphthol-2-sulfonic acid, potassium salt with Li^+, Na^+, K^+, Cs^+, Ca^{++} and Mg^{++} are shown in Figure 9. This was a qualitative study to establish some pattern of behavior during the early work on the project. The fluorometer was standardized before and the standardization checked while obtaining the fluorescence of the samples. Fluorometer standardization was done with a standard solution of Quinine Sulfate U.S.P., 1 microgram/ml. 0.1 N sulfuric acid, by adjusting the sensitivity control to obtain a transmittance of 70 with the microphotometer meter multiplier setting at 0.03 using the activation wavelength of 360 mμ and the fluorescence wavelength of 450 mμ. This gave a "relative fluorescence intensity" of 2.10 as described in the Aminco-Bowman operation manual (30). The aqueous phase was always 5.68×10^{-3} molar in 1-naphthol-2-sulfonic acid, potassium salt at pH 4.7 with the appropriate buffer as described below and the organic phase was water saturated ethanol-free chloroform containing 10% iso-amyl alcohol.

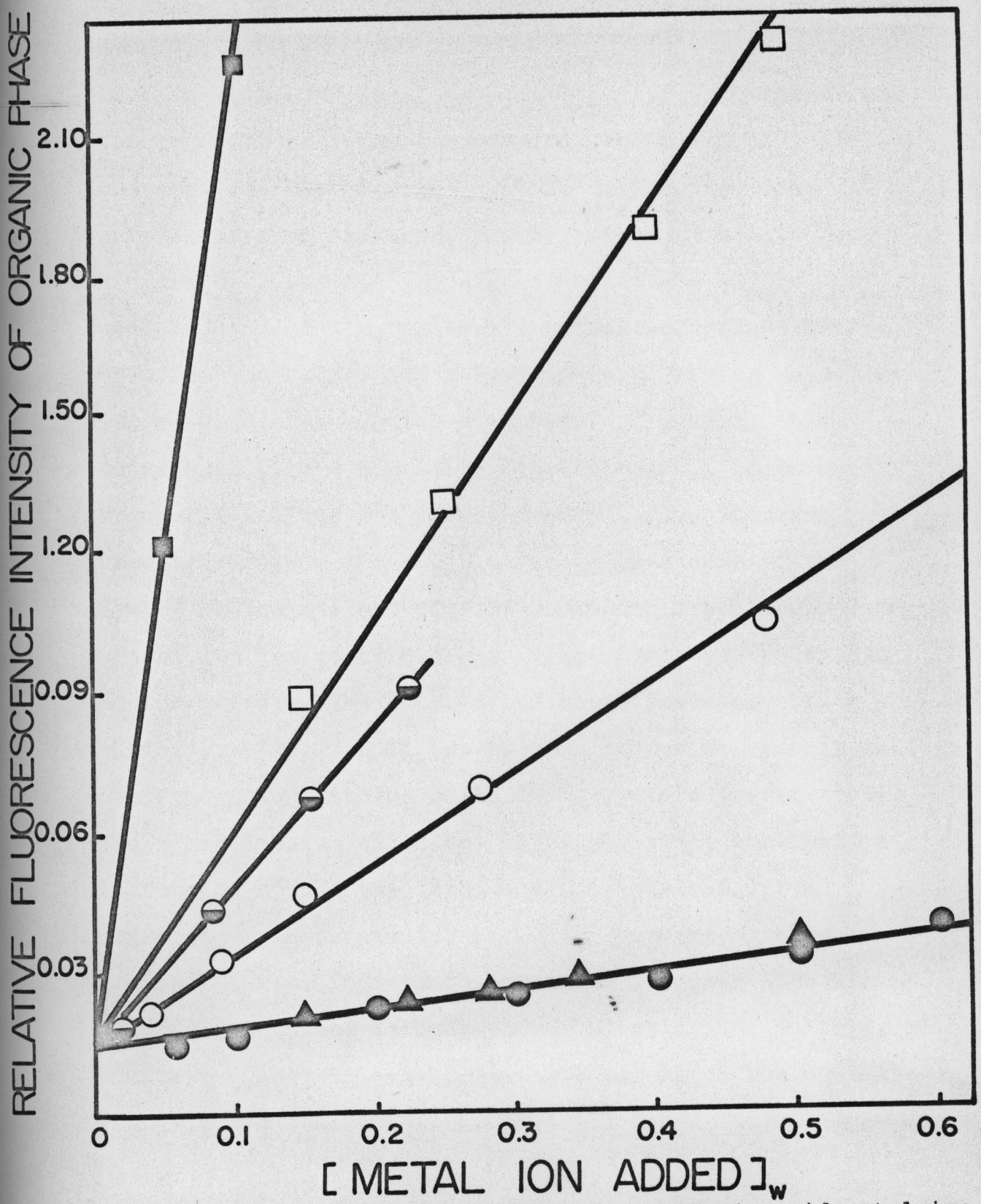


Fig. 9 Extraction of the 1-naphthol-2-sulfonic acid metal ion pair into a chloroform-isoamyl alcohol (10% v/v) phase as a function of metal ion added to aqueous phase.
○ Na^+; ⊖ K^+; □ Li^+; ■ Cs^+; ▲ Ca^{++}; ● Mg^{++}.

In obtaining these data, the buffer salt solubility characteristics, when substituting the salts of different cations, for example, Mg^{++} and Ca^{++}, required the substitution of different buffer anions as well. For studies with the Mg^{++}, a magnesium acetate buffer was used instead of the McIlvain's citrate-phosphate buffer used for the sodium and potassium work, excess magnesium ion was added as magnesium sulfate. Repeating this experiment with a magnesium citrate buffer, again using magnesium sulfate for cation control, gave the same results. Figure 10 displays the independence of the system on acetate and citrate. Similar experiments with the sodium ion confirmed the independence of the system on the acetate, the citrate and the citrate-phosphate buffer systems. In Figure 11 the lithium dependency was observed using lithium salts in McIlvain's buffer system and using lithium chloride, lithium acetate and lithium sulfate to adjust the lithium ion concentration; no difference was observed. Evidently extraction of the 1-naphthol-2-sulfonic acid potassium ion pair into the chloroformic phase is independent of chloride, sulfate, bicitrate, acetate and biphosphate anions in the aqueous phase in the concentrations employed here.

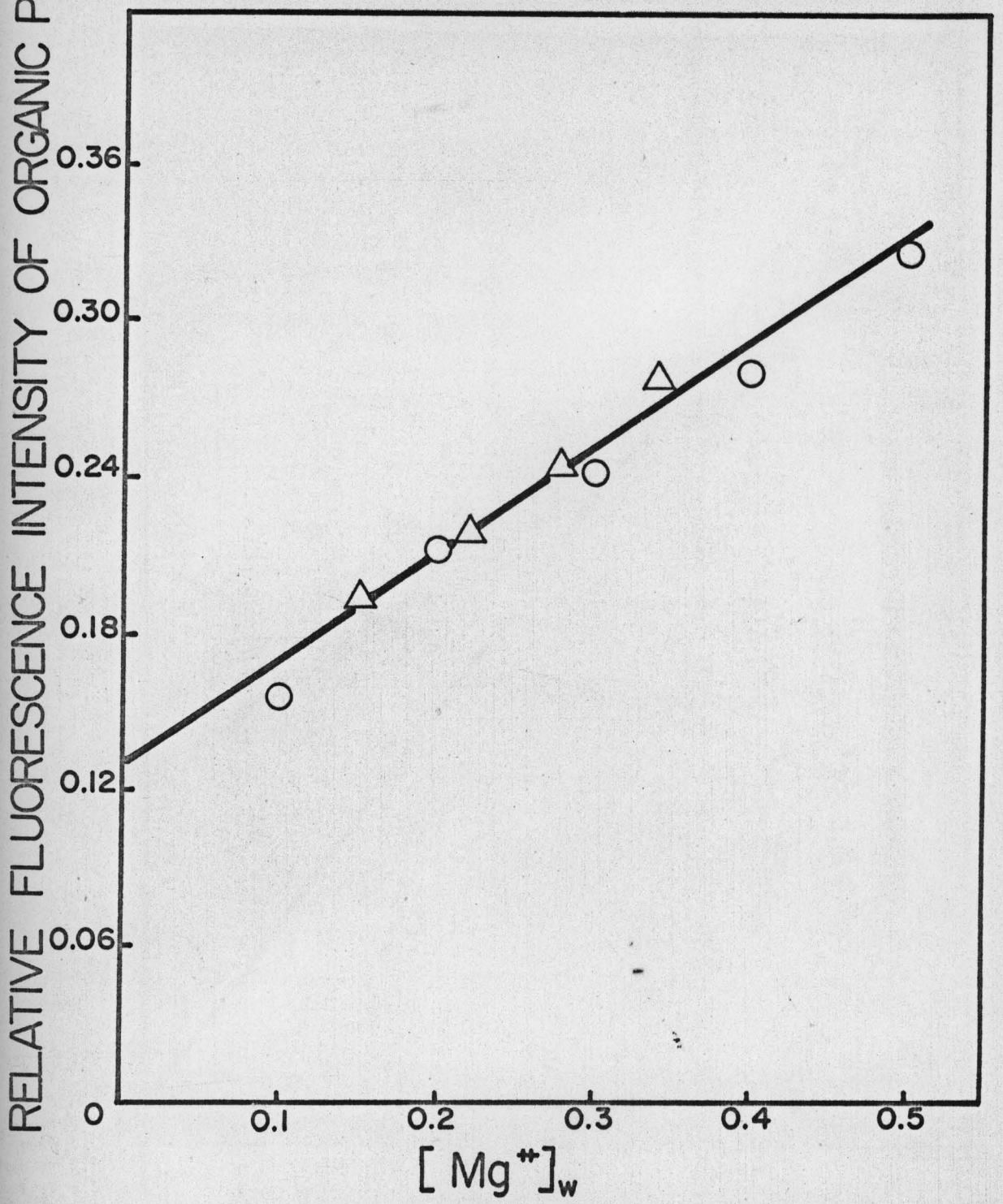


Fig. 10 Extraction of the 1-naphthol-2-sulfonic acid potassium ion pair into the chloroformic phase using a magnesium acetate buffer△and the magnesium citrate buffer○used in Fig. 9.

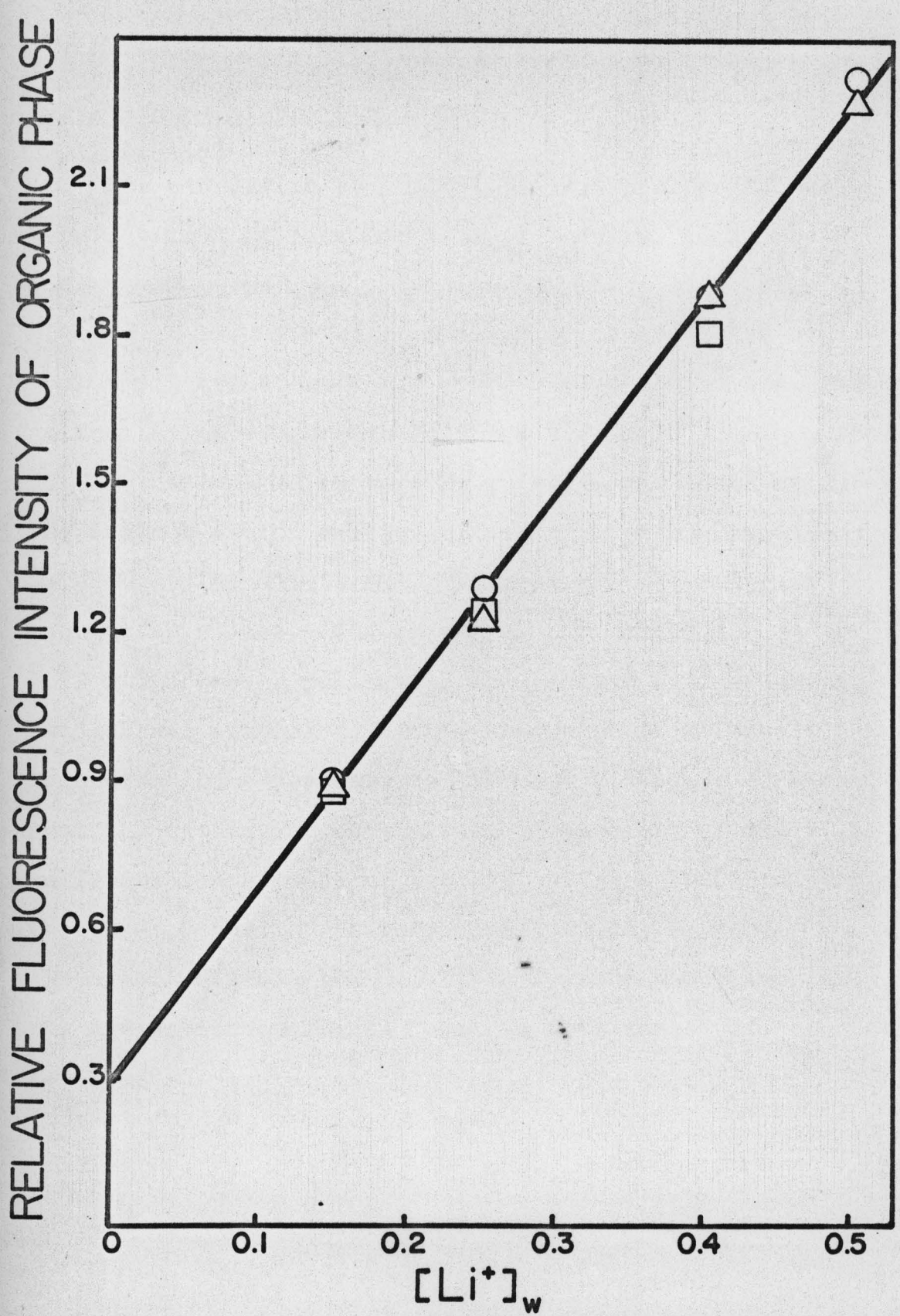


Fig. 11 Extraction of the 1-naphthol-2-sulfonic acid, potassium ion pair into the chloroformic phase using McIlvain's buffer made with lithium salts and adjusting the $[Li^+]_w$ with lithium sulfate○, lithium acetate△, and lithium chloride□.

Extended Cation Dependency of
Partitioning of the Sulfonates

The extraction constant (15) is the slope of the line obtained by plotting $P.C._{app.(M^+)}$ vs. $[S^-]_w$ or $P.C._{app.(S^-)}$ vs. $[M^+]_w$ at a constant $[R]_o$ as is done in Figures 12-16. The basic aqueous phase consisted of sodium citrate to make 1.6×10^{-3} molar sodium ion, the sulfonate isomer, sodium salt was 5.68×10^{-3} molar, and to this the cation sulfate or chloride was added to make the desired cation concentration. The pH was adjusted to pH 6.2 with dilute sulfuric acid and the solution brought to volume. The effect of the sodium ion at 7.28×10^{-3} molar present in the lithium and cesium ion studies was considered to be insignificant at the concentration these cations were used. Figure 12 shows that the extraction constant is independent of the sulfonate concentration, but that it is a function of the isomer used and the different cations may be seen throughout Figures 12-16. Table I lists the values of the extraction constants found in this series of experiments.

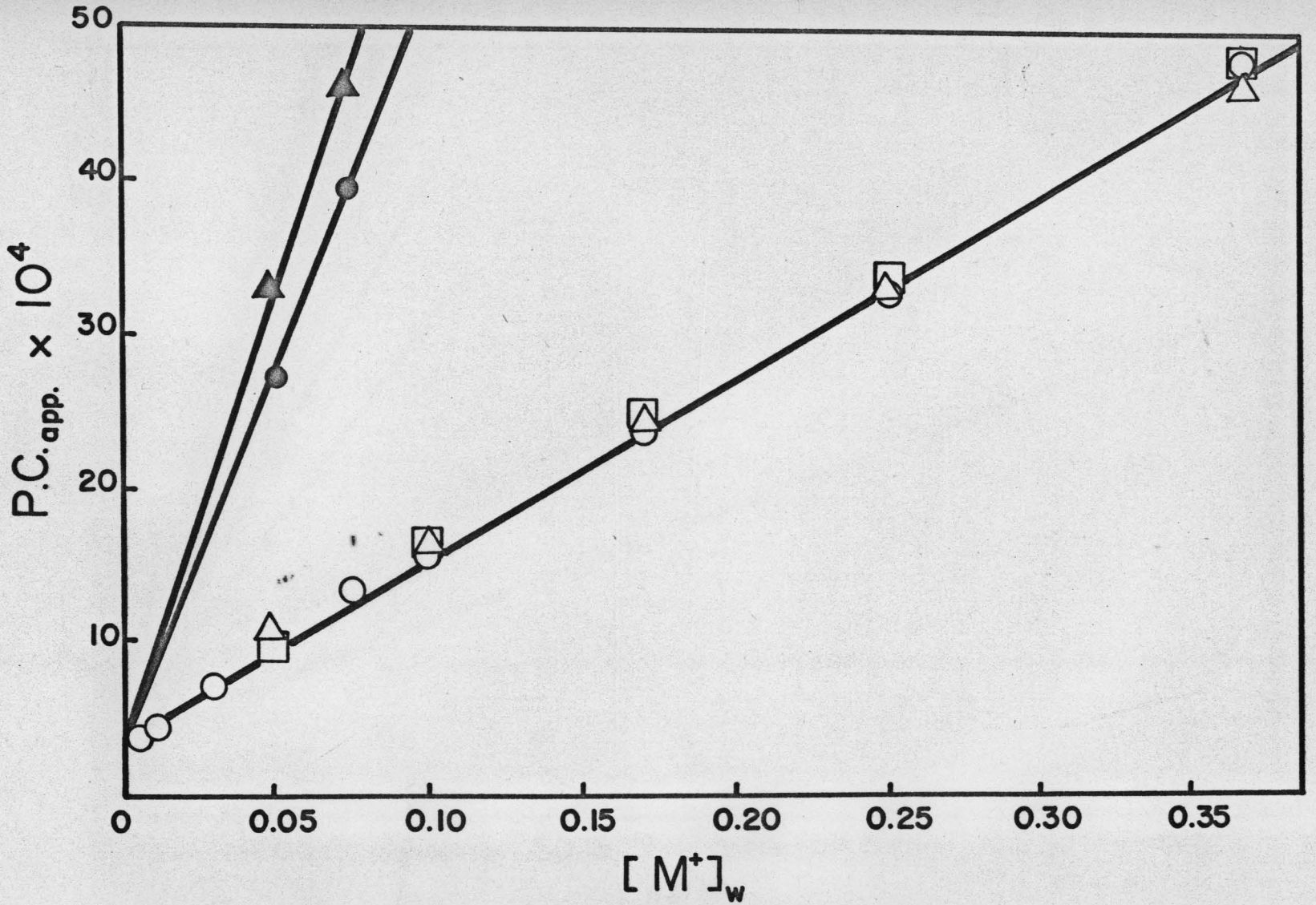


Fig. 12 Partitioning dependency of 1-naphthol-2-sulfonic acid, sodium salt. Sodium ion added: □ with sulfonate at 8.52×10^{-3} molar; ○ with sulfonate at 5.68×10^{-3} molar; △ with sulfonate at 2.84×10^{-3} molar. Lithium ion added: ● Cesium ion added: ▲

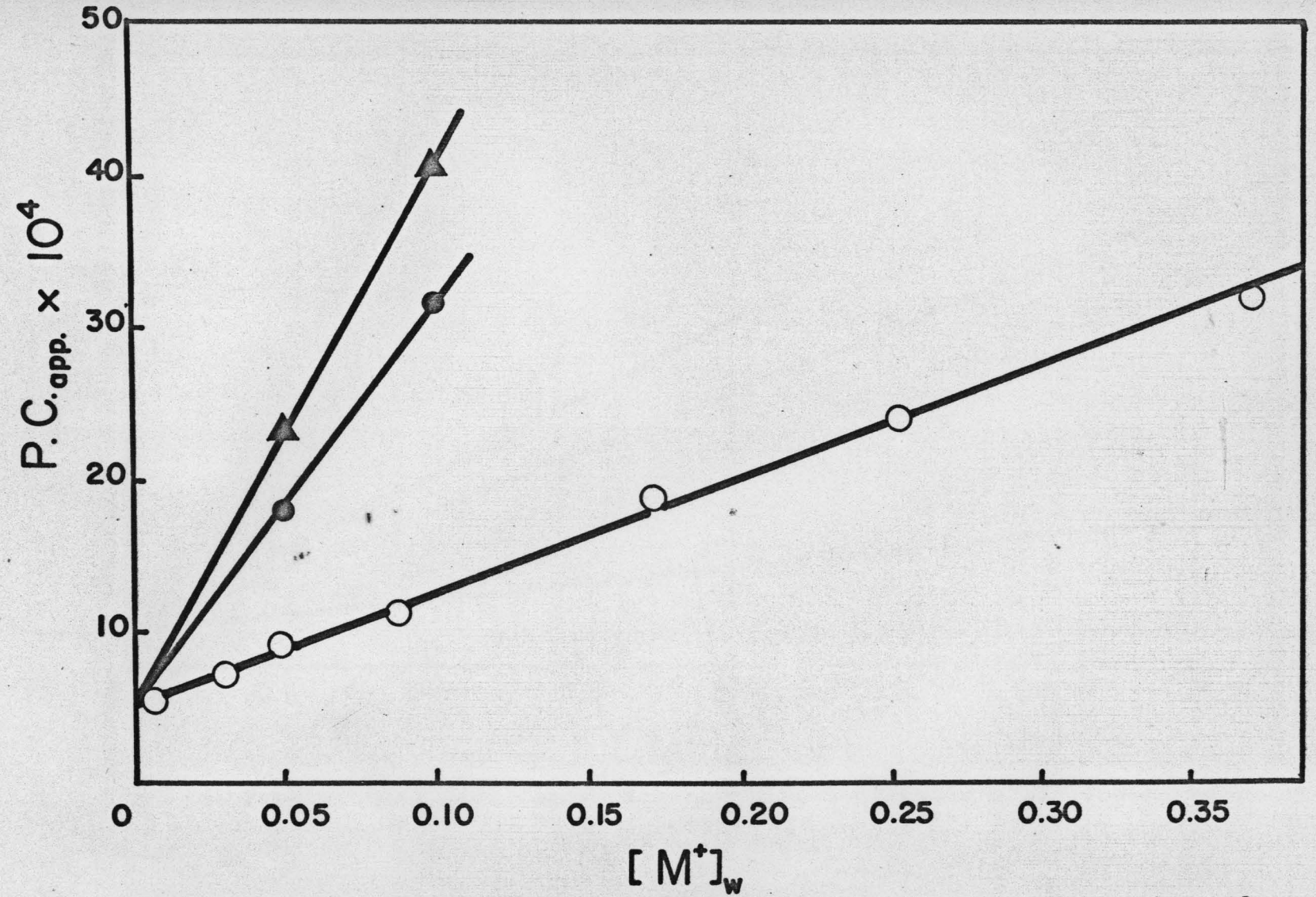


Fig. 13 Partitioning dependency of 2-naphthol-1-sulfonic acid, sodium salt. ○ Sodium ion added to the aqueous phase. ● Lithium ion added to the aqueous phase. ▲ Cesium ion added to the aqueous phase.

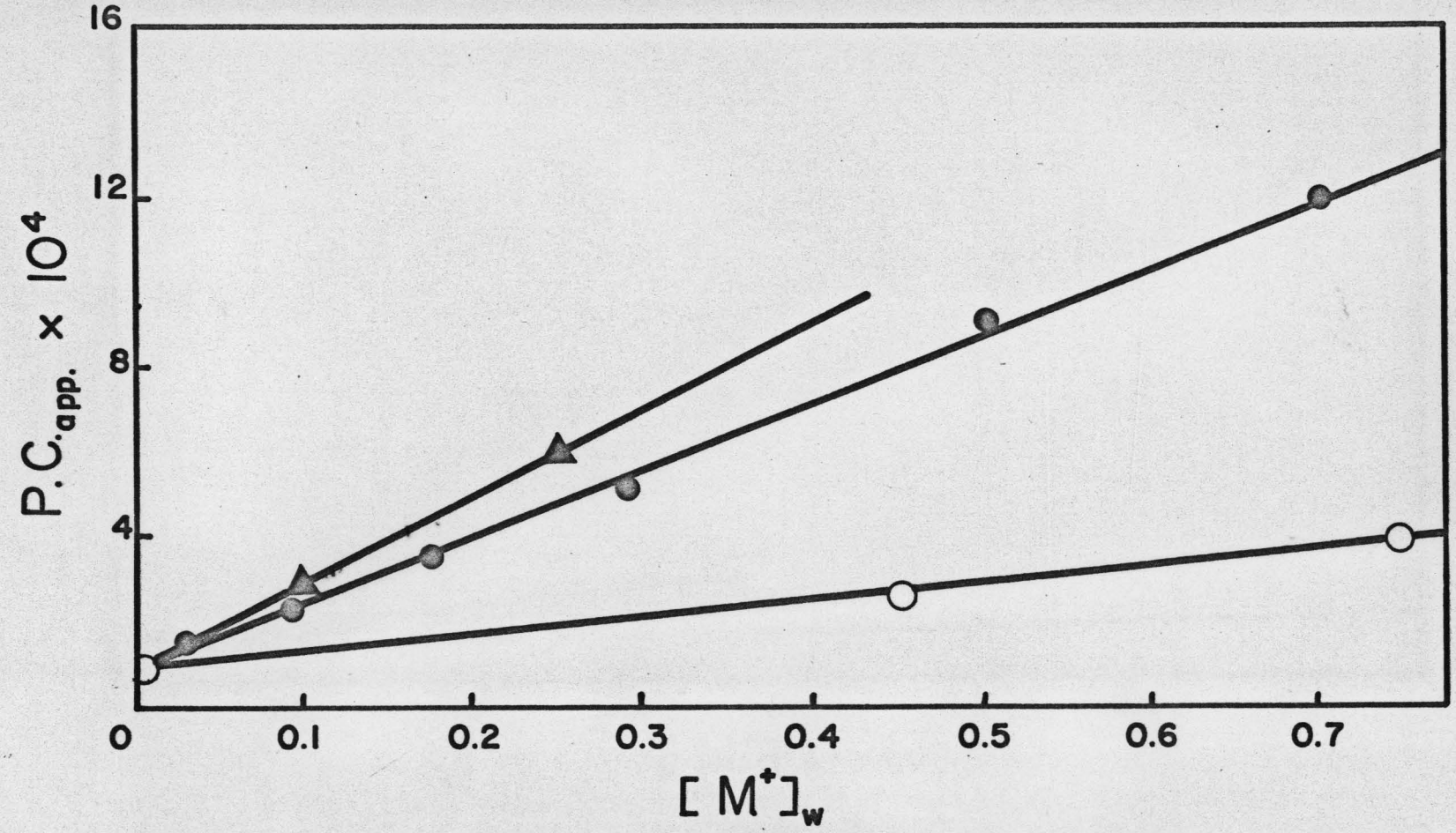


Fig. 14 Partitioning dependency of 1-naphthol-3-sulfonic acid, sodium salt. ○ Sodium ion added to the aqueous phase. ● Lithium ion added to the aqueous phase. ▲ Cesium ion added to the aqueous phase.

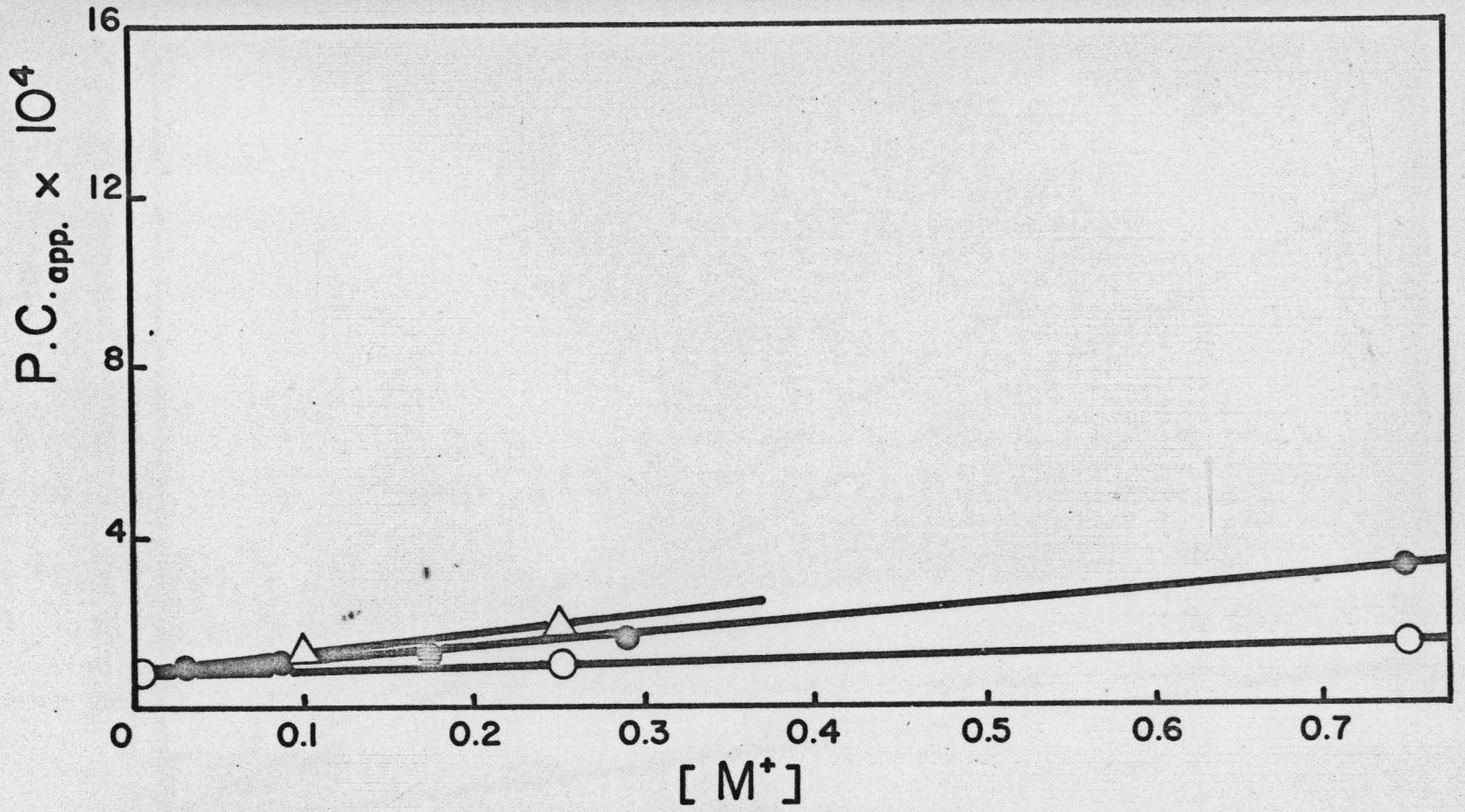


Fig. 15 Partitioning dependency of 1-naphthol-4-sulfonic acid, sodium salt. ○ Sodium ion added to the aqueous phase. ● Lithium ion added to the aqueous phase. ▲ Cesium ion added to the aqueous phase.

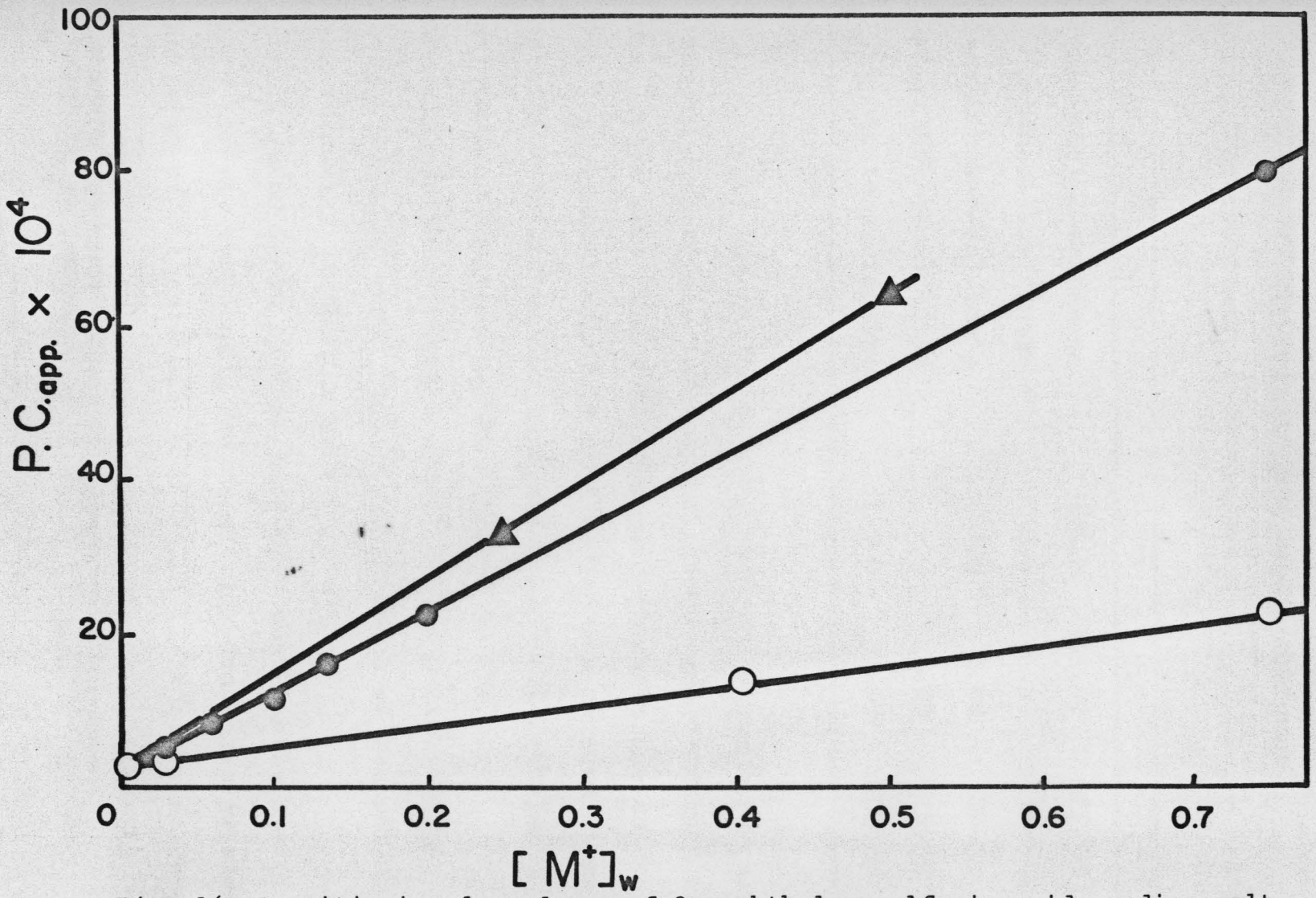


Fig. 16 Partitioning dependency of 2-naphthalenesulfonic acid, sodium salt. ○ Sodium ion added to the aqueous phase. ● Lithium ion added to the aqueous phase. ▲ Cesium ion added to the aqueous phase.

Table I

Extraction constants of sulfonates x 10^4 at $[R]_o$ = 1.842

Compound	Li^+	Na^+	Cs^+
1-naphthol-2-sulfonate	536.0	133.00	662.00
2-naphthol-1-sulfonate	338.0	96.50	411.00
1-naphthol-3-sulfonate	20.0	8.46	24.30
1-naphthol-4-sulfonate	6.0	3.88	7.75
2-naphthalenesulfonate	113.0	36.60	132.00

Clearly the sulfonate extracted was highest for the isomers with adjacent functional groups and decreased as the groups were separated from each other in the molecule. The 2-naphthol-1-sulfonic acid isomer was somewhat less sensitive to sodium ion than the 1-naphthol-2-sulfonic acid isomer and this may be ascribed to the steric crowding in this compound caused by the peri-hydrogen here (31) which would hinder the approach of the cation and formation of the ion pair.

Figures 12-16 show an intercept on the ordinate axis for all compounds, and this value decreases as the polarity of the sulfonate with which it appears increases. Conceivably the intercept could be due to an impurity though these compounds were all chromatographically purified as described in the appendix. This value was found to be independent of the hydrogen concentration

over a range of 1.5 pH units in the plateau region; it was unchanged among different lots of sulfonate supplied and among samples varying in the method of purification of the sulfonate. This point has not been resolved.

DISCUSSION

From the preceding observations on the tendency of the metal sulfonate ion pair to partition, the relation is: $Cs^+ > Li^+ > K^+ > Na^+$. Mg^{++} and Ca^{++} have very little, if any, effect. Clearly this does not fall into a pattern of ionic size, hydrated radii, charge density or location in the periodic table except that the divalent cations do not form extractable ion pairs. The supposed nature of the hydrated cation as indicated in the well documented tables of Stoke's radii or the derived hydration numbers does not fall in the above sequence either. Actually Stoke's radii values are a poor indication of the state of ions in aqueous solutions, fundamentally because Stoke's equation is not a universal law. It applies only to molecules falling through a continuum in which the molecules are much smaller than the falling particle; it does apply to spheres but only where there is no interaction between the particles or between particles with the medium. It assumes the viscosity to be constant, which is not correct because of the structure making and structure breaking effect of ions on the structure of water (32). Darmois (33) attempted to apply this law to the nature of the hydration of ions and concluded that it is particularly difficult to apply Stoke's law to the "non-hydrated" ions.

Accepting the general concept of the "non-intimate" type of ion-pair, it may be formed in the extractive process while the aqueous solution is being shaken with chloroform containing an agent, e.g., an alcohol, that can participate in solvating the ions. If there is any "positive" hydration of these cations in the aqueous phase, the dynamic nature of their hydration shell allows alcohol molecules to participate in the primary solvation which facilitates their formation and solubilization in the organic phase. Also, the presence of the alcohol helps to solubilize the hydrated sphere by hydrogen bonding with the water molecules remaining in the shell and thereby presenting a lipophilic appearance to the organic solution. Stoke's radii appear to be useful for hydrated ions. The hydrated radius of the lithium ion is 3.82 Å, that of the sodium ion is 3.58 Å, which presents a larger surface for alcohol solubilization in the lithium ion and enables it to be more solubilized by the alcohol. This is very important because the resulting effective size is quite large and somewhat lipophilic.

The "negatively" hydrated cations don't have to overcome the penalty of hydration to become more tolerated by the organic phase; in fact, because of their steric disturbance in the water structure, and the tendency of water to want to go back to this structure,

the negatively hydrated cation sulfonates have some assistance, relatively speaking, in their transfer to the organic phase. This effect is greater the larger the ion, yet alcohol is still required for the large cesium ion because the alcohol dependency was found to be 1.04 with cesium and 1.11 with the sodium ion. Evidently the nature of the hydration of these ions as indicated in the theoretical discussion is in agreement with the behavior found in these studies.

Schill (4) noted in working with bromthymol blue that blanks in his system increased in the order of $NH_4^+ > K^+ > Na^+$ with the ammonium ion causing a tenfold and potassium a fourfold increase over the effect of sodium ion added. This is in agreement with the data presented in the present study. Kavanau (32) places the ammonium ion in the negatively hydrated class of cations.

The divalent cations of calcium and magnesium are very highly hydrated in aqueous solution; the magnesium ion is the most hydrated by the very large factor of about sixfold and the calcium ion state of hydration appears between that of the lithium and sodium ions. If an extractable ion pair were formed from these cations and the sulfonates there should be a demonstrable effect and there should be a detectable difference between the two, but this is not apparent. It must be that the divalent cations do not form ion pairs detectable by this

procedure. This is in agreement with observations made by several other investigators in this laboratory (15,16) and Schill (9) while investigating a potential unsymmetrical ion pair. The slight apparent dependency with these ions in Figure 9 is probably due to the content of alkali metal ion in the magnesium acetate and calcium acetate reagents used to adjust the cation ion concentration there.

Alcohols possess the basic hydroxyl functional group associated with many of the chemical and physical characteristics of water. Furthermore, this group is in a favorable steric position to exhibit both its donor and acceptor character and as such solvates both cationic and anionic species, as does water. Solvation of the sulfonate anion is highly dependent on the freedom of the sulfonate hydroxyl to interact with water as noted in the previous pages.

As for the position of the functional groups on the naphthalene ring, the partitioning is in line with the ability of these groups to interaet with water and decrease the partitioning. In adjacent positions intramolecular hydrogen bonding not only decreases this interaction but presents a partially masked sulfonate group to the organic phase, making it more acceptable to the organic solution. The R_f values in the paper sheet chromatography done as noted in the appendix correlate with the partitioning of these isomers, the R_f values being the highest for the

isomers with functional groups adjacent and decreasing progressively as they are separated.

Conceivably, the sulfonate isomers with adjacent functional groups could act as a bidentate ligand to form a chelate with the metal ion which would explain the relatively high metal dependency of these isomers. For chelates of the primarily ionic type, such as those of the alkaline earth and alkali metals, the strength of the chelation should increase with increasing ionic charge and decreasing ionic radius of the metal ion (34). This would imply $Mg^{++} > Ca^{++} > Li^{+} > Na^{+} > K^{+} > Cs^{+}$ (35). It may be worthwhile to note here that coordination of a ligand with metals is a competitive process between ligand and water molecules for the metal ion with the metal coordination being somewhat stronger to overcome the existing coordination with water for complexing in general to occur. Both increase with the charge/radius ratio. Stability constants for chelates of Li^{+} and Na^{+} have been found for only a few strong ligands and there is only "evidence" (34) of complexing with the far less "hydrated" cations K^{+}, Rb^{+} and Cs^{+} which is in agreement with the state of water association involved with the latter group of cations.

The extraction of chelates in general is not markedly dependent on the particular solvent used whereas the extraction of ion pairs which pass into the organic

solvent by virtue of their ability to coordinate with the organic molecules to form some type of solute solvent complex is characterized by a strong dependence on the properties of the solvent used (34). A chelate is an inner sphere type of association; here the relationship follows the solvation characteristics of the outer sphere variety, the ion pair.

The sequence in this study appears like that of a chelate in respect to the order found with Li^+ and Na^+ only. If chelation were the process involved, the partitioning dependency on K^+ and Cs^+ should be less than Na^+ and that with the divalent cations greater than Li^+; furthermore, the sequence would be far different in the 2-naphthalenesulfonate (cannot form a chelate) which remains consistent with the hydroxyl containing isomers. Ion pair formation appears to be the process occurring in this study, but it seems that chelation may exist in some instances.

From the point of view of the blank in the acid dye method, Mg^{++} or Ca^{++} salts and sulfonate compounds with the sulfonate group separated as far as possible from any -OH group have the advantage.

EXPERIMENTAL

A) Apparatus

Aminco-Bowman Spectrophotofluorometer, #4-8106, equipped with a 1P28 photomultiplier tube, a Hanovia, #901C-1 Xenon arc light source and the X-Y recorder built by Electro Instruments for Aminco-Bowman. The Photomultiplier Microphotometer circuit was left on constantly for the purpose of humidity control and stability of the circuit. The equipment was standardized and the standardization checked frequently with a standard solution of quinine sulfate 1 mg/liter tenth normal sulfuric acid.

Cary models 11, 14A and 15 recording spectrophotometers were used.

Beckman Expandomatic pH meter, #76-A equipped with a calomel reference electrode and an appropriate glass electrode, or the Beckman combination electrode, #39142.

Chromatographic -- described in the appendix.

Separatory flasks for the distribution studies were 60 ml, globe type flasks equipped with teflon stopcocks.

Erlenmeyer flasks to contain the sample from the distribution flasks were siliconized with dichlorodimethylsilane and heat treated.

Burrell Wrist-Action flask shaker.

B) Reagents

Water was distilled from acid permanganate.

Chloroform, Analytical Reagent, Mallinckrodt, was shaken with phosphorous pentoxide and distilled over phosphorous pentoxide collecting the fraction with an atmospheric boiling point of 60-61°C.

Iso-amyl alcohol, "Baker Analyzed" Reagent.

Magnesium hydroxide prepared from magnesium sulfate and sodium hydroxide was washed with distilled water until free of sodium and sulfate ion.

Naphthalene sulfonates were isolated, purified and characterized as indicated in the appendix.

All other reagents were Reagent or ACS grade chemicals.

C) Distribution Procedures

The organic phases were saturated with water, and the water used to prepare the aqueous phase was saturated with the organic phases before use. Aqueous phases were prepared by the addition of the components studied and the pH adjusted with tenth normal sulfuric acid. Aqueous phase, 5.0 ml, and organic phase, 20.0 ml, were added to the distribution flask, temperature equilibrated for at least 1 hour and shaken in a temperature controlled room at 25±0.3°C for 15 minutes. The organic phase was drained into a siliconized flask, which picked up any

excess aqueous phase remaining; a sample of 10.0 ml was obtained with a syringe, added to a second siliconized flask containing 0.5 ml methyl alcohol for sample stability, and the fluorescence or absorbance determined.

PART II

DISTRIBUTION STUDIES WITH ORGANIC CATIONS

INTRODUCTION

Aromatic sulfonates, in the text of the present work, are employed as indicators in the ion pair extraction of compounds with basic amino functional groups. Many applications to this purpose have been reported (1), but theoretical investigations of these uses have only recently begun to appear.

Biles, et al. (13), reported that the distribution of amine tropaeolin ion pairs increased with the molecular weight of the alkyl amines and decreased with the branching of the alkyl amine; the latter observation was expected from the water solubility of branched versus straight chain alkyl compounds. Schill (6) concentrated a similar study on pharmaceutical amines and showed that increasing molecular weights usually increases extraction constants, this increase attributable to the increasing hydrophobic properties of the cation. Qualifications to a general rule result from functional groups in the pharmaceutical amine. Higuchi and Kato (15) established the extraction profile of chlorpheniramine and showed that the dibasic antihistamine and the monobasic dextromethorphan may be separated by extraction on the basis of pH control.

In the use of aromatic sulfonate anions, protonated amines have to compete with other cations present in the

formation of extractable ion pairs. The knowledge of the degree of their success is a great aid in predicting the type of sulfonate or other acid dye that would give the optimum extraction with a class of amines and ideally with no interference from the other amine classes. To this end the extraction constants of a series of amines of pharmaceutical interest were determined in the manner used to determine this constant in the previous section for the aromatic sulfonates. The dependency of the extraction on the alcohol content in the organic phase was also determined.

From the general overall equation,

$$(S^-)_w + (A^+)_w \overset{+nR}{\rightleftharpoons} (S,A\,R_n)_o$$

where $(S^-)_w$ represents the sulfonate concentration in the aqueous phase, $(M^+)_w$ the aqueous amine cation concentration, $(S,A\,R_n)_o$ the solvated ion pair in the chloroform solvated by n molecules of alcohol, R, the overall equilibrium constant may be defined as:

$$K_o = \frac{(S,A\,R_n)_o}{(S^-)_w(A^+)_w(R)_o^n}$$

Taking the logarithm of this equation and rearranging:

$$\log K_o + \log (S^-)_w + \log P.C._{app.} = n \log (R)_o.$$

At constant $(S^-)_w$ the first two terms are constant so a plot of log $P.C._{app.}$ vs. log $(R)_o$ gives n, the effective molecularity of the solvating agent in the solvated ion pair. The extraction constant in the case of the protonated amine at a single alcohol concentration is defined as:

$$K_e = \frac{(S,A)_o}{(A^+)_w(S^-)_w} \quad .$$

EXPERIMENTAL

A) Apparatus

That of the previous section.

B) Reagents

d-Amphetamine, Aldrich Chemical Co.

d-Desoxyephedrine, Aldrich Chemical Co.

N,N-dimethylamphetamine hydrochloride, Smith, Kline and French Research Labs.

Tetramethylammonium chloride, Eastman Kodak Co.

Silver oxide powder, Gen. Chem. Div., of Allied Chemical.

All other reagents were the same as in the previous section.

C) Procedure

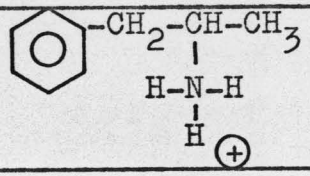
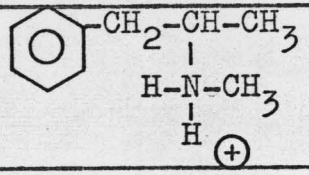
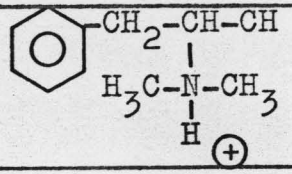
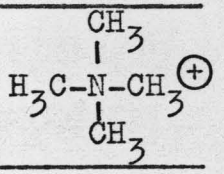
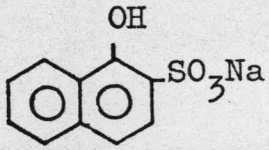
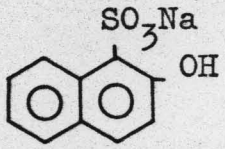
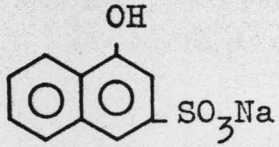
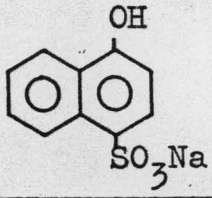
The amines were distilled under vacuum, dissolved in alcohol, their alcohol solutions neutralized with a calculated amount of standard sulfuric acid, recrystallized two times where possible from hydro-alcoholic or alcohol-ethyl ether solutions and dried to constant weight. The distilled N,N-dimethylamphetamine was titrated potentiometrically with dilute sulfuric acid to pH 5.5, diluted and used for a stock solution of the amphetamine sulfate. This salt is hygroscopic and

recrystallization was not successful. The quaternary ammonium sulfate was prepared from the chloride by reacting with silver oxide, filtering off the excess silver oxide through a fine sintered glass filter, and washing with ether to remove any amines; the residual ether was removed by vacuum and the quaternary ammonium was titrated with standardized sulfuric acid. Tests for halide were negative.

The aqueous phase was prepared to contain the amine in the concentration as listed in Table II, the sulfonate ranging from about 0.7×10^{-4} to 5.6×10^{-3} molar sulfonate in a citrate buffer, 5.33×10^{-4} molar in sodium citrate and the pH was adjusted to pH 5.40 with tenth normal sulfuric acid. The distribution and sampling procedures were identical to those in the preceding section. Blank samples were determined and the blank subtracted from the samples containing the amine. Equilibrium values of the amine and sulfonate concentrations were used in the calculations.

Table II

Molar concentration of the amine in the prepared aqueous phase

Isomer \ Cation				
	5×10^{-5}	3×10^{-5}	4×10^{-5}	7.5×10^{-4}
	5×10^{-5}	3×10^{-5}	4×10^{-5}	7.5×10^{-4}
	20×10^{-5}	12×10^{-5}	25×10^{-5}	7.5×10^{-2}
	40×10^{-5}	24×10^{-5}	50×10^{-5}	7.5×10^{-2}

RESULTS AND OBSERVATIONS

A typical $P.C._{app.}$ vs. anion plot to determine the extraction constant of amphetamine sulfate with 2-naphthol-1-sulfonic acid, sodium salt is shown in Figure 17, where a straight line fitted to the experimental points indicates a K_e of 600. The iso-amyl alcohol concentration was 20% v/v. An example of the alcohol dependency data is represented by Figure 18 for 2-naphthol-1-sulfonic acid, sodium salt at 9.64×10^{-4} molar sulfonate and 5.0×10^{-5} molar amphetamine sulfate. The value of n from the slope of this line is 1.70 which indicates the average number of alcohol molecules associated with each ion pair is 1.70.

The overall data obtained in this study with amines to represent a series of increasing methylation are listed in Tables III and IV. Both tables include the data with the inorganic sodium ion.

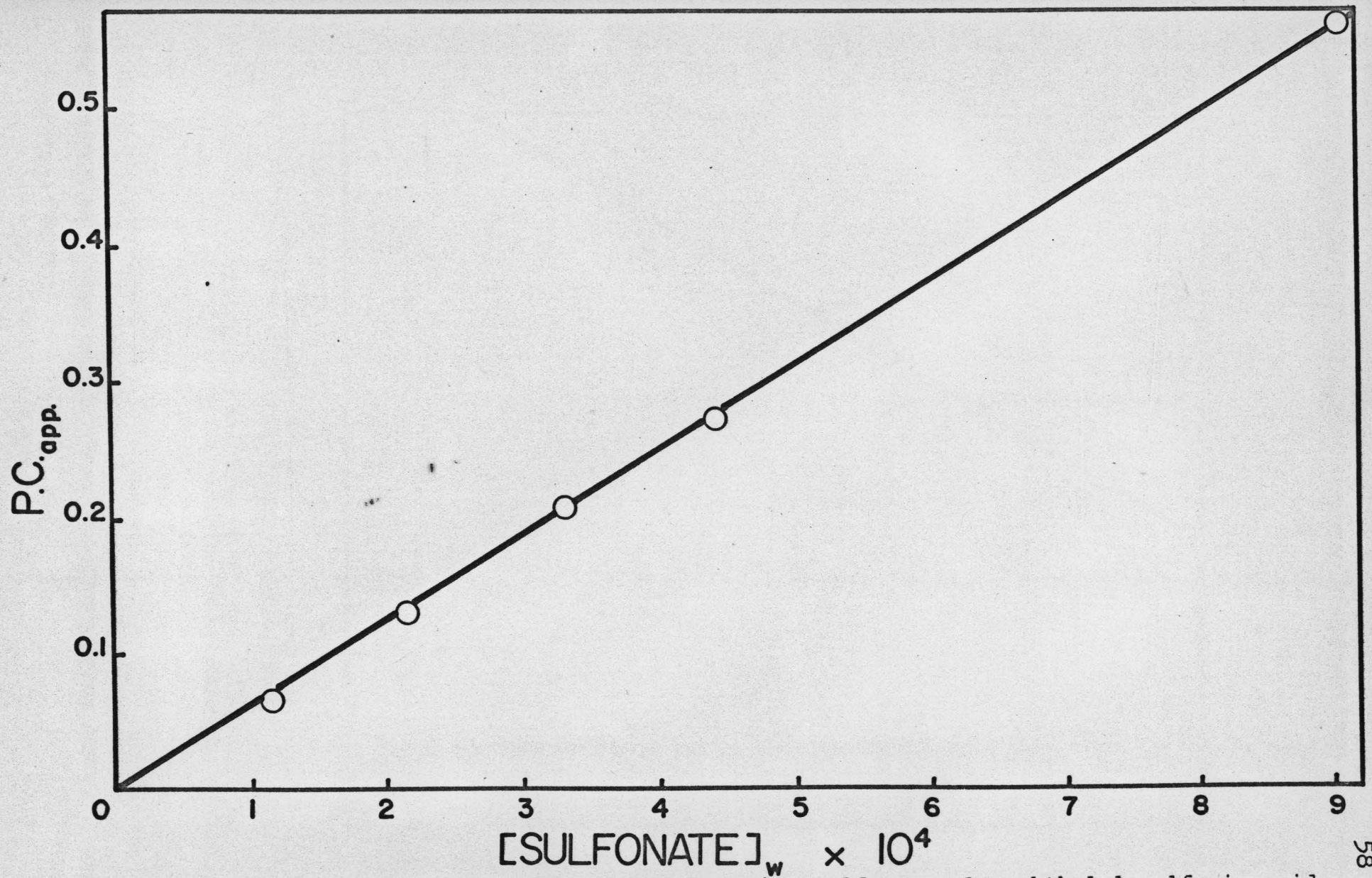


Fig. 17 The partitioning dependency of amphetamine sulfate on 2-naphthol-1-sulfonic acid, sodium salt, in the aqueous phase.

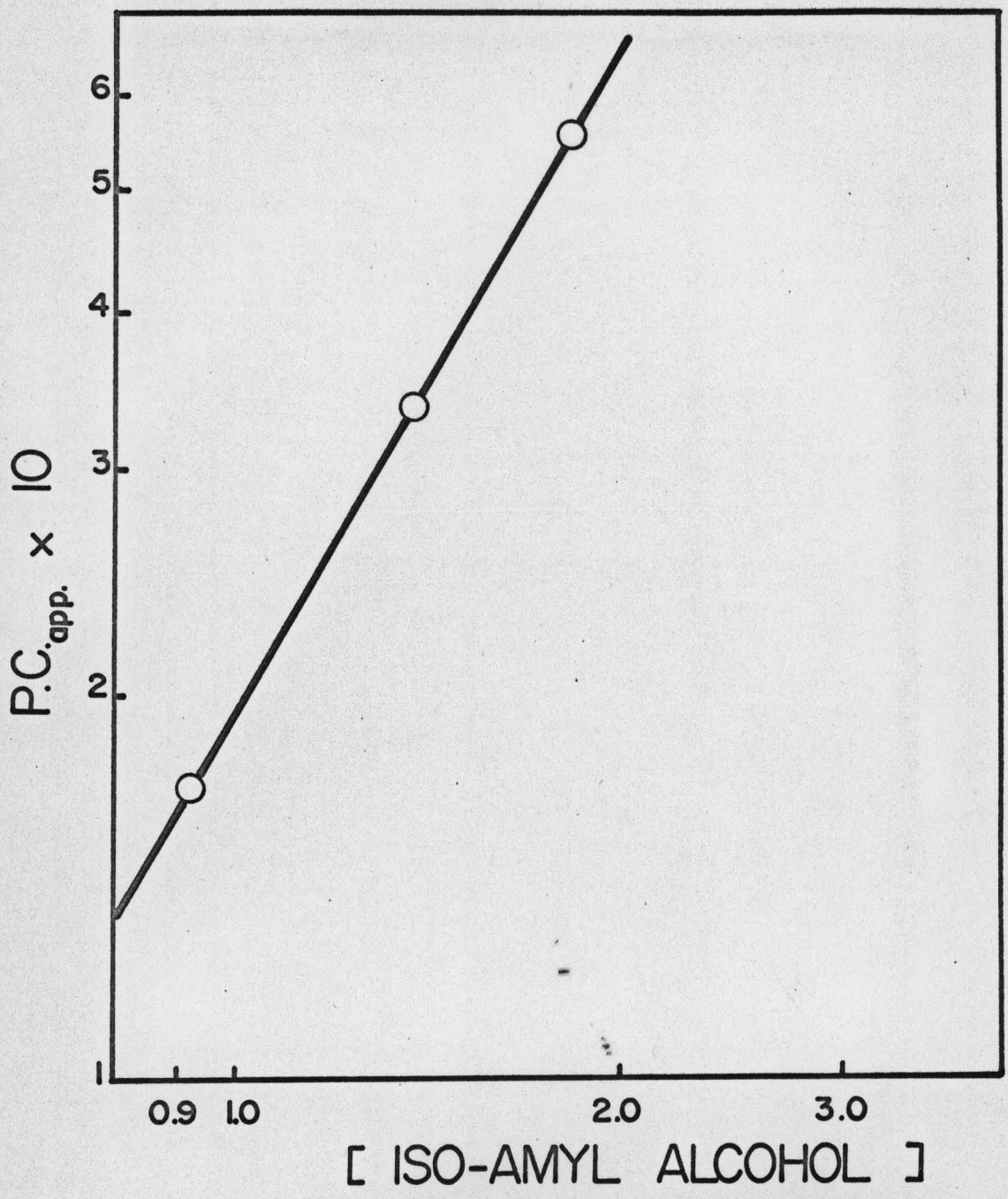


Fig. 18 Partitioning dependency of the amphetamine 2-naphthol-1-sulfonate ion pair on the iso-amyl alcohol concentration in chloroform.

Table III

The average number of iso-amyl alcohol molecules associated with each ion pair

Isomer \ Cation	Sodium ion	Amphetamine cation	N-methyl amphetamine cation	N,N-dimethyl-amphetamine cation	Tetramethyl-ammonium cation
1-Naphthol-2-sulfonate	2.25	1.50	1.20	0.43	1.43
2-Naphthol-1-sulfonate	0.71	1.70	0.93	0.49	1.00
1-Naphthol-3-sulfonate	1.11	2.34	1.82	1.47	3.21
1-Naphthol-4-sulfonate	1.17	3.09	2.46	1.82	3.14

Table IV

Extraction constants of sulfonates at $[R]_o$ of 1.842

Isomer \ Cation	Sodium ion	Amphetamine cation	N-methyl amphetamine cation	N,N-dimethyl-amphetamine cation	Tetramethyl-ammonium cation
1-Naphthol-2-sulfonate	1.33×10^{-2}	780	1750	1090	1.93
2-Naphthol-1-sulfonate	9.65×10^{-3}	600	1270	1190	1.97
1-Naphthol-3-sulfonate	8.46×10^{-4}	23	59	43	0.08
1-Naphthol-4-sulfonate	3.88×10^{-4}	7	18	14	0.02

Table V

$\frac{\text{Organic cation}}{\text{Sodium cation}}$ extraction constant ratio x 10^{-4}

Isomer \ Cation	Amphetamine cation	N-methylamphetamine	N,N-dimethyl-amphetamine
1-Naphthol-2-sulfonate	5.8	13.2	8.2
2-Naphthol-1-sulfonate	6.2	13.2	12.2
1-Naphthol-3-sulfonate	2.7	7.0	5.1
1-Naphthol-4-sulfonate	1.8	4.6	3.6

DISCUSSION

An ion pair consisting of the large sulfonate anion and the small sodium cation has the negative charge distributed throughout the organic part of the molecule by resonance, but the positive charge is localized in the electrical field composing the inorganic cation. The result is a dipolar ion pair with an exposed positive charge representing the case II type of ion pair as proposed by Higuchi and co-workers (17) in a paper on the role of solvating agents in ion-pair extraction.

In the present work the nucleophilicity of the alcohol masks the exposed positive center of the ion pair while the electrophilicity of this solvating agent has a similar effect on the remaining negative part of the dipolar ion pair. The alcohol, therefore, by its masking effect acts as a solvating agent and increases the solubility of the dipolar ion pair in the organic phases. In this series of anions the hydroxyl group contributes to the solvation by its intramolecular electrophilic character, the effect of which decreases as the hydroxyl is removed from the sulfonate group. This effect results in the increasing dipolar character of the ion pair or alcohol dependency observed in Table III while proceeding down the columns. The alcohol dependency for the 1-naphthol-2-sulfonic acid, sodium salt appears to be

anomalous since this value is far higher and out of sequence with the rest of the table. It may be noted that it is about twice as much as that for the isomers with the functional groups separated from each other. This value has been obtained with several lots of sulfonate supplied and is reproducible.

The effect of the methyl substituted amines on the alcohol dependency is in line with the hydrophobic nature of the cation; it decreases as this nature of the cation increases. The tetramethylammonium quaternary compound was used because of the surface activity of higher analogues. The quaternary compound requires more alcohol molecules associated with the ion pair for its solubilization in the organic solvent, which is in agreement with the general solubility behavior of the quaternary ammonium compounds; they are much more soluble in both acidic and basic solutions than the simple amine salts (36).

From Table IV and V it is clear that substituting the pharmaceutical organic cations for the sodium ion increases the extraction constant by 4 to 5 orders of magnitude with the most favorable organic cation K_e to sodium cation K_e ratio appearing with the sulfonate isomers containing the functional groups adjacent to each other. The ratio is much less favorable for the quaternary ammonium compound; however, this is not a good representative because of the great difference in

molecular weight between this and the other cations. The quaternary ammonium compound was of most value in the solvation study, but it may be noted here that the K_e value with the quaternary compound decreases in the same order and at about the same rate as the tertiary cation indicating that the steric hindrance due to replacing a methyl group for the hydrogen is not significant. The general order of K_e values among the $\overset{\circ}{1}$, $\overset{\circ}{2}$, and $\overset{\circ}{3}$ amines is $\overset{\circ}{2} > \overset{\circ}{3} > \overset{\circ}{1}$. This is also the order of their pK_a' values which are 9.87, 9.77, and 9.40, respectively (37).

Table IV suggests that the model compounds used in this study could be used for the analysis of pharmaceutical amines related to the types used here. The sulfonates with the adjacent functional groups have the advantage over those with these groups separated. Separation of one class of amines from another, however, would probably not have very good prospects, except possibly in the case of separation of amines from a quaternary compound via column partition chromatography.

APPENDIX I

SOME FLUORESCENCE BEHAVIOR

Chloroform-isoamyl alcohol solutions of hydroxy naphthalenesulfonates show characteristic activation and fluorescence curves that are unchanged on varying the concentration of the alcohol from 5% v/v to at least 20% v/v and remain the same if the sulfonate is added to the organic solution by partitioning from an aqueous phase or on adding the dry sulfonate to the organic solution. Examples of the spectral curves of 1-naphthol-2-sulfonate are given in Figure 19. The activation curve shows maximum activation at 303 mμ while the fluorescence maximum is at 350 mμ. The largest peak on the activation curve is due to resonance radiation which is really the reflection of incident radiation and cannot be used for quantitative purposes. It is large because the solution was very dilute and response greatly magnified. In this study, these maxima for a specific isomer did not shift with the different inorganic cations, the different organic cations, or the different masking agents and their concentrations used here. In general, with fluorescing compounds, the wavelength of fluorescence is greater than that of activation. With this type of information and while using the wavelengths of maximum activation and maximum fluorescence, the working curves were obtained in the usual way. Care must be used to be

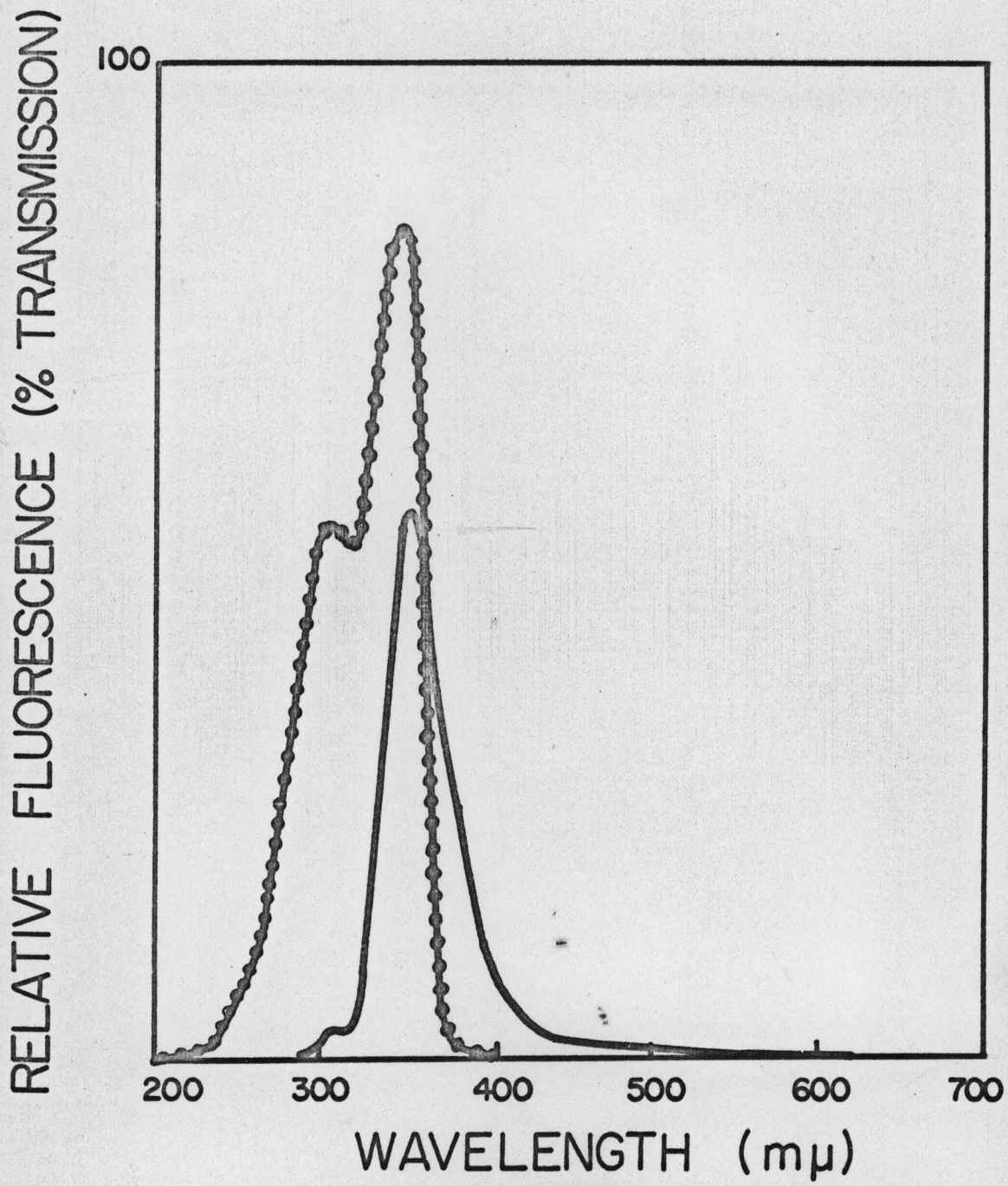


Fig. 19 The activation spectrum scan of 1-naphthol-2-sulfonate, at the fluorescence wavelength of 350 mμ ●●●●●●; and the fluorescence scan at the activation wavelength of 303 mμ ——. The solvent is iso-amyl alcohol 20% v/v in purified chloroform.

certain that the concentration of sulfonate in the solution being determined is not in the concentration quenching or non-linear range. An example of this phenomena is evident in Figure 20, where the linear region is the safest concentration range to work in. Higher concentrations may be diluted, the fluorescence determined, and the initial concentration calculated from this data.

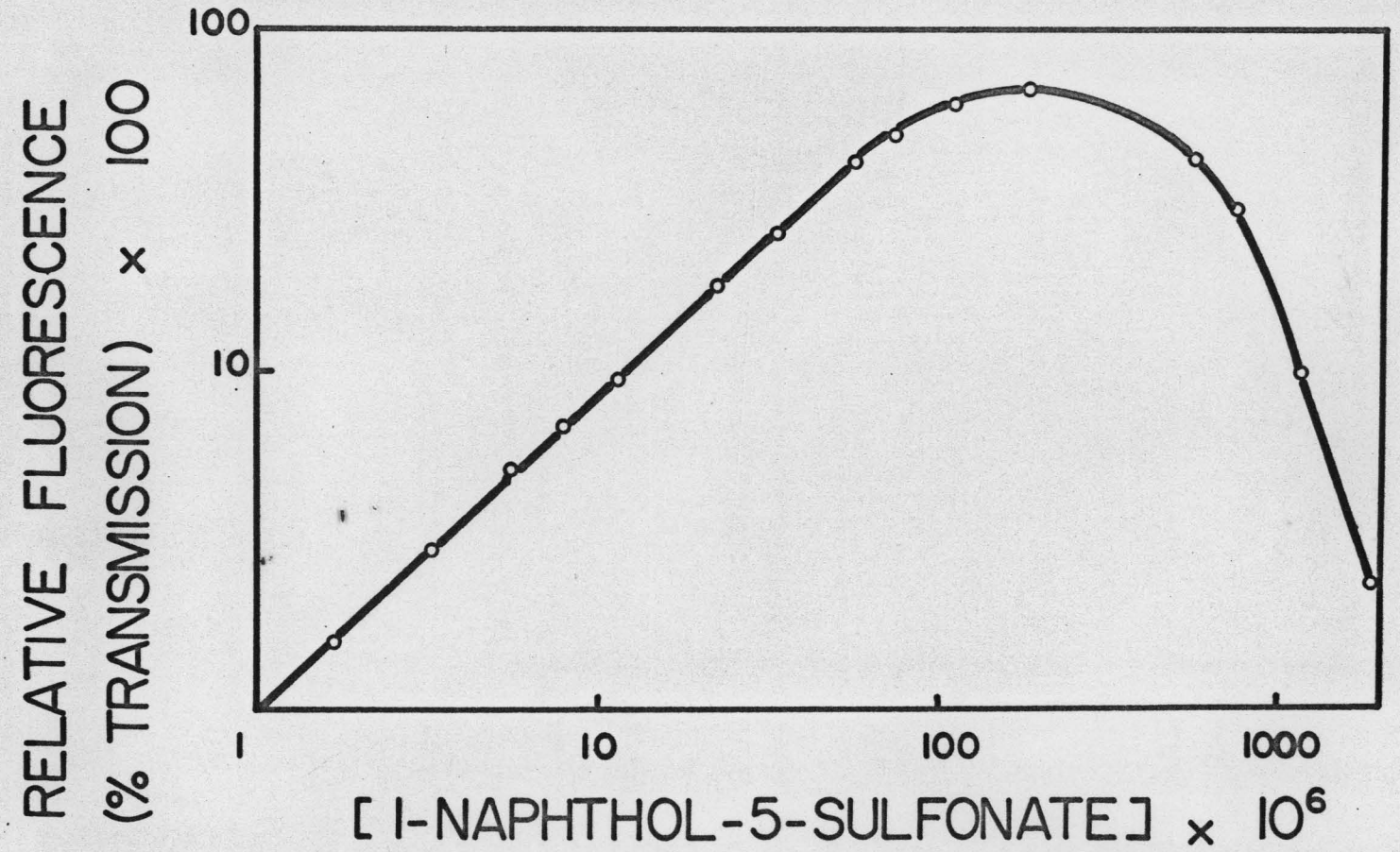


Fig. 20 A fluorescence working curve of 1-naphthol-5-sulfonate, in water.

APPENDIX II

THE PREPARATIVE PAPER CHROMATOGRAPHIC PURIFICATION AND CHARACTERIZATION OF SOME AROMATIC SULFONIC ACID SALTS

Powdered cellulose column partition chromatography was used for decolorizing, desalting, and isomer separation of naphthalene sulfonates and hydroxy naphthalene sulfonates. These compounds are common intermediates in the dye industry and are obtained from the reaction mixture by a salting out process. As supplied by the chemical sources they are a highly colored product containing oxidized material, tars, isomers, inorganic salts, and probably starting materials.

Latinak (38) and Kolsek (39) have reported on the paper chromatography of some aromatic sulfonates and qualitatively showed the common presence of several compounds in the sample. Terashima and Fujita (40) reported a quantitative electrophoresis study on some commercial sulfonic acids.

Kolsek and Perpar (41) used a developing solvent of tertiary butanol, normal butanol and water in a ratio of 4:3:3 for compounds similar to those used in this work. To investigate this system further chromatographic paper was charged with a mixture of the isomers of the sulfonates as supplied and with a deliberately oxidized sample of 1-naphthol-2-sulfonic acid, potassium salt and

the chromatogram developed. Except in the case of the similar compounds, 1-naphthol-2-sulfonic acid, sodium salt and 2-naphthol-1-sulfonic acid, sodium salt, the isomers were well separated during the 18 hours required for the solvent front to move about 40 cm, with R_f values of about 0.4 to 0.7. The oxidized sample was resolved into five spots though the unoxidized sample behaves as one compound. Since the separations were quite good, Kolsek's solvent system was used for routine checks on the fractions from the cellulose columns. For preparative purposes the eluting solvent ratio of the above system was changed as needed for each isomer sample to get better separation from a cellulose column. By this method a white crystalline product was obtained.

Purification of Sodium
1-Naphthol-2-sulfonate

This compound is available as the potassium salt which was converted to the free acid by passing through a column of Dowex 50X4 cation exchange resin. Freedom from potassium ion was followed by testing the eluant with tetraphenyl boron. The collected eluant was then neutralized with sodium hydroxide and the aqueous solution of the sodium sulfonate was evaporated to dryness under vacuum. A charge of 20 grams of this material was dissolved in about 50 ml of the eluting solvent of the butanols-water ratio of 4:3:0.5 and applied to a column of

about 2.5 liters in volume. Considerable colored material came off the column first followed by a large uncolored fluorescent band characterized to be the 1-naphthol-2-sulfonic acid isomer. After work up the yield was 85% and the product is free of chloride and sulfate ions which are present initially, probably from the salt used to salt these isomers out in their production.

Purification of Sodium
1-Naphthol-3-Sulfonate

The supplied sulfonate could not be decolorized with charcoal and was highly contaminated with inorganic salt as evidenced by a considerable amount of the sample being insoluble in the eluting solvent used to apply the charge to the column. The solvent was the same as that used for the above isomer. After work up the yield was 70% and the product was free of chloride and sulfate ions.

Purification of Sodium
1-Naphthol-4-Sulfonate

A very dirty looking supply of this isomer was chromatographed with the eluting solvent ratio of 4:3:1. Two strong bands visible with ultraviolet light appeared on the column with 65% of the charge appearing as the 1-naphthol-4-sulfonic acid, sodium salt isomer; the compound in the other strong band did not have the R_f value in paper chromatography of the 1-naphthol-2-sulfonic

acid, sodium salt isomer, a possibility if alkali salts of the 1-naphthol-4-sulfonic acid, sodium salt are heated (42). The product is also chloride and sulfate free.

Purification of 2-naphthol-1-sulfonic Acid, Sodium Salt

A supply of this isomer that had been extensively treated previously but still retained a light beige color was chromatographed with the eluant in the ratio of 4:3:0.5 giving a yield of 70% of a white chloride- and sulfate-free product.

Purification of 2-Naphthalenesulfonic Acid, Sodium Salt

A supply of this compound was eluted very slowly with the solvent ratio of 4:3:0.5, faster with 4:3:1.5 and much faster with the solvent ratio of 4:3:3 which gave a 70% yield that was free of chloride and sulfate.

EXPERIMENTAL PROCEDURES

The paper chromatography was carried out using Whatman #1 chromatographic paper in a deep square chromatographic glass tank with a lubricated ground glass jointed cover to insure a saturated atmosphere. The descending method was used to develop the chromatogram at room temperature. After the chromatogram was developed, it was air dried and viewed under both a 3660 Å and a "short" wavelength ultraviolet light. The paper was then sprayed with a 0.05% aqueous solution of Pinakryptol Yellow (K. and K. Labs, Inc.), air dried, and viewed under ultraviolet light again. The location of an arylsulfonic acid or sulfonate is readily noted by a characteristic fluorescent color (43) on a background of yellow fluorescence.

Tertiary butyl alcohol, technical grade, was distilled, the fraction boiling at 81-81.5°C was used, the n-butanol was Analytical Reagent, Mallinckrodt, and water used was distilled water.

Preparation of the cellulose column was done by making a thin slurry of about 800 grams of Whatman Cellulose Powder CF 11 (W. & R. Balston Ltd.) in the eluting solvent and pouring into a glass chromatographic column of 2.75 inches in internal diameter to make a final column of cellulose of about 30 inches. No packing was

done aside from the settling of the cellulose during the washing of the column with the eluent. Constant attention must be given to the column during this settling period because entrapped air bubbles will tend to move upward leaving channels in the settled column. This may be dealt with by slowly rocking the column of the thin slurry from an upright to an inverted position giving time for the air bubbles to rise while going back to the upright position. Channels that do appear after some settling of the cellulose may be filled by rapid twisting of the column which requires that the column not be rigidly secured. An elution rate of 3-5 ml per minute results from this procedure of column preparation.

The work up of the material from the cellulose column was done by collecting the fractions shown to contain one isomer by paper chromatography and evaporating off the eluting solvent under vacuum while warming. The dried product contains some material apparently from the cellulose column and may be slightly colored from the mild heat used earlier. This is solved by dissolving the dry product with distilled water, extracting with amyl alcohol, followed by washing the aqueous solution with ethyl ether to remove any alcohol dissolved in the water. The resulting aqueous solution of the sulfonate was freeze dried to prevent any oxidation of the phenolic group.

Characterization of sodium 2-naphthalenesulfonate was accomplished by making the S-benzylthiouronium derivative. Characterization of the hydroxyl-containing compounds was not successful by the usual methods to characterize sulfonic acids; the benzylthiouronium salt was never obtained and the arylamine salt melting points disagreed with the literature. Elsevier's Encyclopedia gives melting points of the sulfonyl chlorides when the hydroxyl group is first changed to the ethoxycarbonyloxy group with ethyl chloroformate in a heterogeneous reaction mixture (44). The sulfonyl chloride was prepared by refluxing this derivative with dry phosphorous pentachloride as per Shriner and Fuson (45) and recrystallizing the residual oil with an appropriate solvent. Carbon disulfide was used with good results here. The melting points agreed with the published melting points (44,46,47). In the characterization of 2-naphthol-1-sulfonic acid, sodium salt, no sulfonyl chloride was obtained. This may be due to desulfonation in the refluxing step since this is a labile position of the sulfonate group (48). Knuzli (47) suggests that the phosphorous pentachloride reaction be cooled. The sulfonate group is present in this isomer as indicated with the pinacryptol spray reagent and hydrolysis gives beta naphthol. N.M.R. curves show that this is not any of the above isomers and the color in ammonia vapor agrees with that reported by Latinak (38).

DISCUSSION

In both of these systems the more polar compounds move the slowest so inorganic salt remains near the starting point and the relatively nonpolar tars move most rapidly. Sulfonated naphthols also move according to their polarity; the monosulfonated naphthols having adjacent functional groups move fastest here, which is probably due to the intramolecular hydrogen bonding that exists in these isomers. As the functional groups are separated by one or two carbon atoms the R_f values decrease respectively. The polysulfonated compounds that could be present move still slower with the trisulfonated compounds having an R_f value less than the disulfonated compounds (28,38,49). The most polar compounds, the inorganic salts in these supplies, and the inert junk move very little if they move at all.

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SUMMARY

Aromatic sulfonates, in the "acid dye" method (1), are employed as indicators in this procedure for the analysis of compounds containing a basic amino functional group. Formation of the ion pair and its extraction is considered to occur on shaking aqueous solutions of the sulfonate and cations, inorganic and organic, with an immiscible organic solvent where the cation-sulfonate concentration is then determined photometrically.

Factors such as dye or anion to be used, pH of the aqueous phase, effect of inorganic salts commonly present in the unknown sample, extracting solvent, etc., have been considered on a rather empirical basis in the past. Only recently has some investigations begun to appear in the literature that enable these parameters to be chosen with some degree of certainty.

The present research has been concerned with experimental studies on some model sulfonate compounds that allow the use of the very sensitive fluorescence assay method. These investigations included:

a) The influence of isomeric structural changes on the selective extractability of a limited range of ion pairs.

b) The partition coefficient - pH profile of the model compounds.

c) Effect of inorganic and organic cations as measured by their extraction constants.

Among the four hydroxynaphthalene sulfonate isomers used and the 2-naphthalenesulfonate the partition coefficient-pH profile shows that lower aqueous phase pH values may be used with the 1-naphthol-3-sulfonate and 1-naphthol-4-sulfonate isomers before extraction of the protonated form of the sulfonate begins to contribute to the organic sulfonate level. These isomers may be used at a pH as low as pH 3.5 whereas protonated forms of the 1-naphthol-2-sulfonate and 2-naphthol-1-sulfonate isomers begin to appear at about pH 4.8. The hydroxynaphthalene sulfonates with the functional groups separated by at least one carbon atom in the naphthalene ring are also less sensitive to inorganic cations. Isomers having the functional groups located adjacent to each other are ten to thirty times more sensitive to excess sodium ion than those with the functional groups separated from each other. Sensitivity to inorganic cation was found to be in the following order: $Cs^+ > Li^+ > K^+ > Na^+$ with excess magnesium and calcium salts having very little influence on the extraction of these sulfonate ion pairs. This observation suggests that blanks in the acid dye method would be minimized if extraneous salts in the unknown sample and in the aqueous buffer were limited to, for example, calcium or magnesium salts; sodium salts

would be the next choice. Extraction constants for the above systems are tabulated. The effect of the inorganic cations is in agreement with their state of hydration or degree of "negative hydration" as discussed by Samoilov (2).

As in the case of inorganic cations, the hydroxy-naphthalene sulfonates with the functional groups adjacent to each other are also most sensitive to amphetamine and its N-methylated analogs. Extraction constants with these organic cations were four to five orders of magnitude greater than with the sodium cation.

Tabulated values of the alcohol dependencies of the extraction of the organic and inorganic sulfonate ion pairs indicates that the sulfonate isomers with the functional group separated from each other in the naphthalene ring are more dependent on the solvating agent.

A method for the preparative paper chromatographic isolation and purification of naphthalene sulfonates using powdered cellulose column chromatography is described.

SUMMARY REFERENCES

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