THE INTRINSIC HYDROPHOBICITY OF ALIPHATIC AND AROMATIC HYDROCARBONS

by

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Approved G. L. Amidon

(Prof. G.L.Amidon)

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TABLE OF CONTENTS

I. INTRODUCTION

A. Hydrophobicity: General considerations. The behaviour of hydrocarbons and hydrocarbon side chains of complex molecules in water has been of considerable interest in the past due to their importance in such areas as protein and nucleic acid conformation, association phenomena, enzyme-substrate binding, lipid bilayers, membrane structures and drug-receptor interactions. The tendency of these hydrocarbon moieties to leave water or to form associated structures in water has been referred to as hydrophobic bonding or the hydrophobic effect (1,2).

A molecular interpretation of the factors involved in hydrophobic bonding was put forth by Frank and Evans(3) on the basis of their study on the thermodynamics of solution of small gas molecules and hydrocarbons in water. They ascribed this behaviour to the anomalous structuring of water upon the introduction of a hydrocarbon group into water, resulting in a large negative entropy change which dominated the positive free energy change for the transfer of a hydrocarbon from its pure liquid to aqueous solution. This explanation has continued to serve as the basis for interpreting the properties of hydrocarbon groups in water.

Since the pioneering studies of Frank and Evans, numerous studies(4,5,6,7,8) have been conducted on: the solubility of hydrocarbons in water, variation of solubility with chain length and the structural implications of longer chain length compounds, temperature dependence of

solubilities and enthalpies and entropies of micellisation. These studies confirmed the large contribution of the entropy to the free energy change and further supported the concept of hydrophobic bonding as being the result of the particular structural properties of water.

The importance of the hydrophobic effect in protein conformational equilibria has been discussed by Kauzmann(9). He noted that the association of the hydrophobic side chains of the constituent amino acids of a protein in aqueous solution could play an important role in the stabilisation of the native structure of the protein and that a major portion of the favorable free energy of association could result from hydrophobic bonding. Tanford (10,11) calculated the contribution of the side chains to the free energy of association by determining the transfer free energies of the side chains from non-polar solvents (alcohol,dioxane) to water. The non-polar solvents served as a model for the hydrophobic interior of the protein molecule. From these values, he proposed a hydrophobicity scale for the side chains. Side chains with aromatic residues were found to be more hydrophobic than aliphatic side chains, however on a per carbon atom basis the aromatic residues were less hydrophobic.

B. Surface area approach to estimation of free energy changes. An alternative approach to understanding the energetics of hydrophobic bonding is based on the cavity

model. Langmuir(12) was the first to suggest the use of molecular surgace area in the estimation of solution free energies and it has subsequently been employed in a variety of contexts. Sinanoglu(13) has used the cavity model approach to estimate free energies for the helix-coil transformation of DNA molecules in different solvents. His calculations indicated that the energy required to form a cavity in the solvent constitutes a dominant part of the free energy change of the transformation. The relative free energy changes for the different solvents were in good agreement with their denaturing abilities.

Recently surface area computational schemes have been developed and used to assess i) the solvent contribution to protein conformational equilibria and ii) smaller molecule hydrophobicity. Richards(14) using crystal structure data calculated the accessible surface area of proteins to the solvent. The accessible surface area of an atomic group is defined as the area on the surface of a sphere of radius R on each point of which the centre of a solvent molecule can be placed in contact with the atom without penetrating any other atoms of the molecule. The radius R is comprised of the Van der Waals radius of the group and the solvent radius. One of the advantages of the surface area approach used by Richards is that it allows the breakdown of the total surface area to individual group surface areas. Using this method he computed the changes in accessibility of surface area in terms of polar and non-polar contributions

for the transformations of a protein from its extended state to its native structure. He found that in the native protein an average side chain polar atom is nearly 3.5 times as accessible as an average non-polar side chain atom. This factor is about 2 in the extended chain conformation. Hence the change in accessibility in going from the extended chain to the folded conformation for polar and non-polar atoms differ by less than a factor of 2. This factor is not very large and Richards has suggested that this implies a much closer balance of polar and non-polar interactions to the solvent contribution term.

Studies on the hydrophobicity of smaller molecules using the surface area approach have been mainly directed to the solubility process. Hermann(15) found good correlation between the surface area and aqueous solubility of hydrocarbons. Amidon et al (16) extended this further to include various alkyl substituted monofunctional compounds and calculated individual functional group contributions to the solubility process. Results of these studies give a value of about 25cal/$\mathring{A}^2$ for the free energy change for hydrocarbons in the solution process. The results indicate that surface area is a very good parameter for use in estimating free energy changes in processes involving hydrophobic bonding. The surface area method has the additional advantage of handling aromatic compounds where it is not feasible to represent data on a carbon number basis.

C. Intrinsic Hydrophobicity. The thermodynamic analysis of hydrophobic bonding has been based mainly on data for small chain length hydrocarbons and alkyl substituted compounds for the following process:

pure liquid ——→ aqueous solution

This process however can be considered to consist of two steps,

1) pure liquid ——→ gas

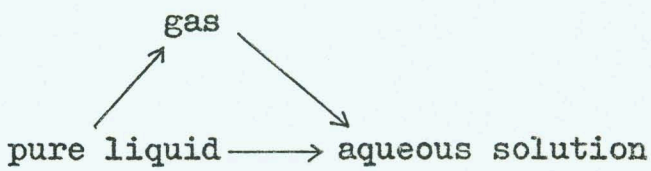
2) gas ——→ aqueous solution

This analysis separates out the interactions and structuring in the pure liquid and has been pointed out by several authors(16,17,18,19,20). The results for the individual steps show that about 80% of the total free energy of transfer from pure liquid to water is due to removal of the molecule from the pure liquid. The 2nd step may be taken to indicate the solvent contribution or intrinsic hydrophobicity (analogous to Hine's intrinsic hydrophilicity(21)) and represents the interaction of solute with solvent, free of solute-solute interactions. This suggests that the solvent contribution to the hydrophobic effect has been overemphasised and the use of energy factors based on the pure liquid ——→ aqueous solution process for estimating changes in free energy for protein-protein and protein-ligand interactions needs re-evaluation. In addition the term hydrophobicity has been used rather loosely particularly with aromatic residues. From literature

data on the solubility and vapor pressure of benzene and naphthalene, it is observed that naphthalene is more soluble than benzene at equivalent partial pressures and this implies that naphthalene may be intrinsically less hydrophobic than benzene. This however may be due to using the solubility-vapor pressure data to calculate the Henry's law constant which could be altered if significant association of solutes occurred.

The purpose of this study was twofold:

1) An analysis of existing thermodynamic data on aqueous solutions of straight chain hydrocarbons and alcohols in terms of the cycle:



and to compare the energetics of the aliphatic and aromatic residues with regard to their intrinsic hydrophobicity.

2) To determine Henry's law constant for benzene and naphthalene from the limiting slopes of the vapor pressure concentration curves of their aqueous solutions.

II. EXPERIMENTAL

A. Materials. Benzene and naphthalene were purified as reported by Riddick and Bunger(22).

Benzene(A.R.,Mallinckrodt) was initially distilled collecting the fraction at 80°C. This was then recrystallised from a methanol-water mixture cooled by an ice-salt bath. The slurry of crystals was filtered through a sintered glass funnel(The filtering system was also cooled by an ice-salt mixture). The crystals collected in the funnel were transferred to a separating funnel, allowed to liquefy and washed several times with water to remove any methanol. The benzene was then dried over silica gel and finally filtered through a silica gel bed (in a funnel). It was distilled once more and the distillate stored over sodium. The benzene so purified had a freezing point of 5.4°C.

Naphthalene(scintillation grade, 99+%,Aldrich Chemical Co.) was extracted with 10% sodium hydroxide, 50% sodium hydroxide and 10% sulfuric acid solutions, all extractions being done at 85°C(fused state). 50 grams were then treated with 0.4 grams of aluminium chloride. It was further extracted with 20% sulfuric acid and 15% sodium carbonate solutions. The naphthalene was then recrystallised from methanol. The recrystallised product gave a sharp melting point between 80-81°C(reported 80.21°C).

Anthracene (Aldrich Chemical Co.,99.9+%) was used as

supplied.

Ethanol 95%(Commercial Solvents Inc.) and methanol (A.R.,Mallinckrodt) were distilled before use.

Aqueous solutions were prepared using water distilled from alkaline permanganate in an all glass still.

B. Apparatus and Method. The vapor pressures of both pure and aqueous solutions of the aromatic hydrocarbons were measured by the gas saturation or transpiration method. The method has been used by many workers with good accuracy and precision. The principle involves the saturation of a known volume of gas with the vapors of the pure compound or solution. The quantity of the compound in the vapor phase is then determined by a suitable analytical technique.

The apparatus was set up similar to that used by Balson,et al(23) and is shown in figure 1. The 5 gallon container was filled with nitrogen. The gas was displaced by addition of a known amount of water from a reservoir through a burette. The displaced gas registered any back pressure(usually negligible) on a water manometer and was then passed through a couple of drying tubes packed with calcium sulfate. After these it entered the saturator column in the case of pure substances or first into the presaturators(two litre bottles with 1 litre of solution) and then the column in the case of aqueous solutions. For pure compounds the saturator column consisted of a 6 feet, 14mm O.D. glass column bent either in the shape of a coil

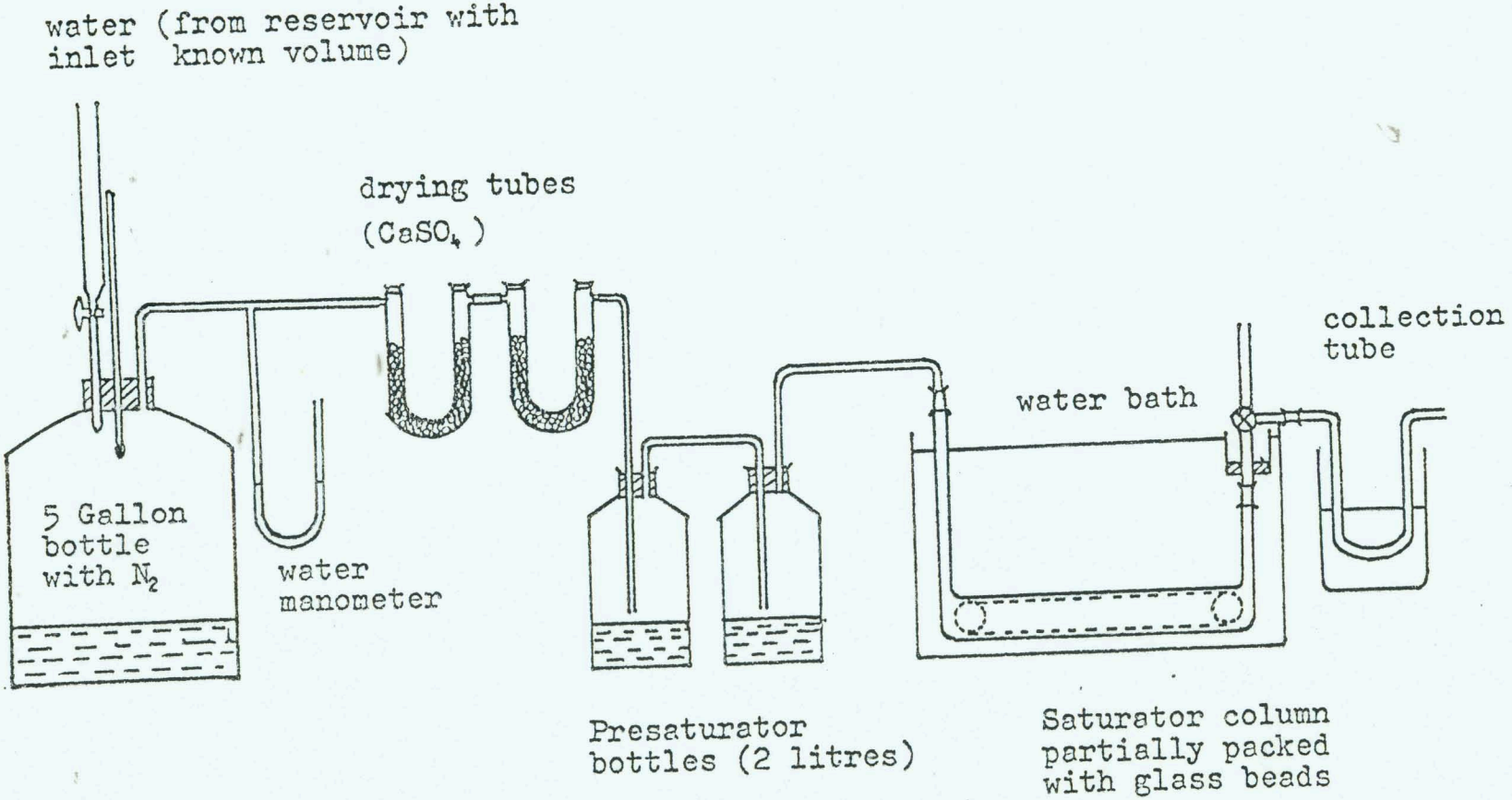


Figure 1. Vapor Pressure Apparatus

or a series of U's, with ground glass joints at the ends. The columns were packed with glass beads 4mm in diameter. The saturator column was placed in a water bath maintained at 25°C(± 0.05 C). For naphthalene, the column packed with a mixture of beads and naphthalene was placed in an oven so that the beads were coated with fused naphthalene. Anthracene was sublimed onto the beads and the column then packed with the beads and solid anthracene.

For aqueous solutions, the column was made up of a 4 cm. diameter glass tubing, 100cm. long and bent in the form of a square, with 14mm. O.D. glass tubing for outlets with ground glass joints at the ends. The reason for having a large column was that a large quantity of solution(600-700 ml.) could be introduced into the column. The column hence was only partially packed with glass beads(near the beginning and end).

To the outlet of the column was attached a 3-way stop cock with ground glass joints at the arms(Fig.2). The stop cock controlled the start and termination of the runs. One arm of the stopcock was connected to the U-shaped collecting tube which was packed with glass beads moistened 95% ethanol. The collecting tube was placed in a beaker containing dry ice- acetone mixture. After a run, the substance collected was washed into a volumetric flask with 95% ethanol and made to volume. The concentration was then determined(after appropriate dilutions if necessary) either on a spectrophotometer(Cary 118) or fluorimeter

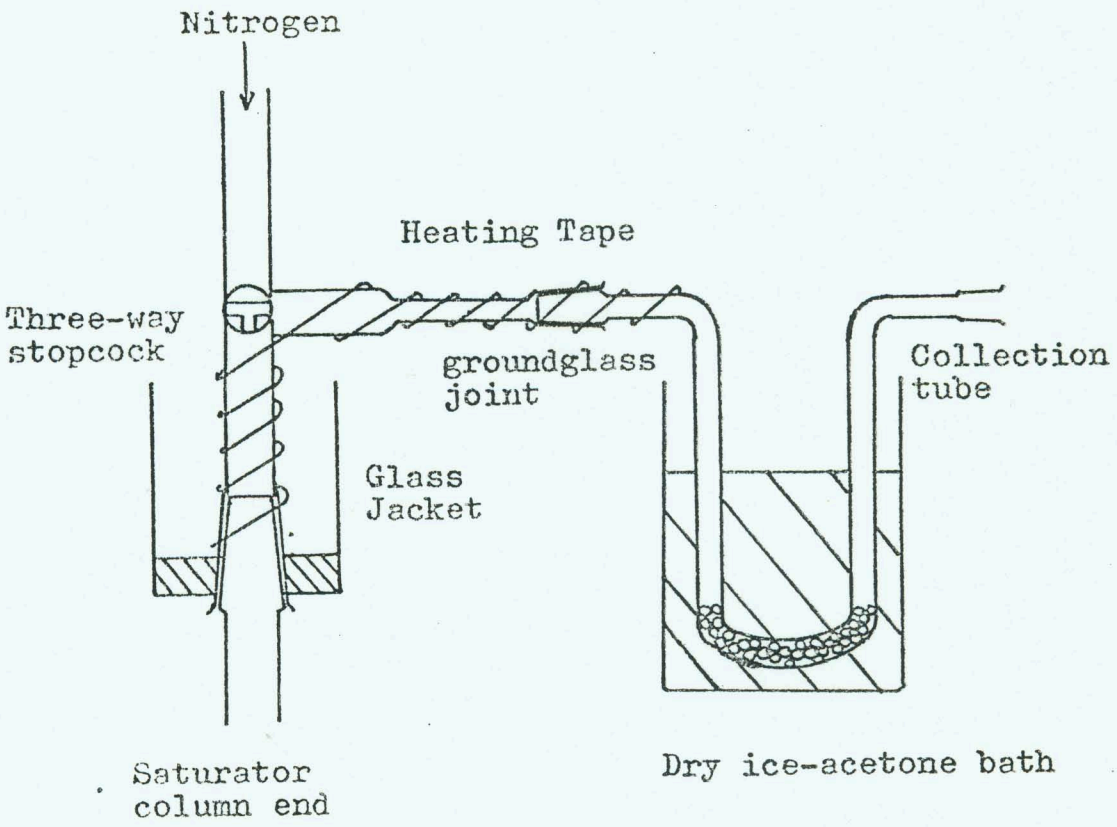


Figure 2. Magnified view of collection end

(Perkin Elmer MPF-4). One collecting tube was sufficient, no condensate being found in the second. The outlet of the column was heated by a heating tape to preventany condensation and the stopcock outlet was flushed with nitrogen after each run. To confirm the saturation of the gas with vapor, the runs were carried out at different flow rates ranging from 4 to 12 ml/min. No dependence on flow rates was observed.

For aqueous solutions, the solution in the saturator column was assayed both before and after the run. For benzene a change in concentration of 5% or less normally occurred whereas for naphthalene the upper limit was 2%. Due to the high volatility of benzene, losses during sampling can be high. To minimise these, a fine plastic tube was introduced into the column before and after each run and an exact quantity(5ml) of solution was withdrawn by a syringe and added to 5 ml. of 95% ethanol, the volume being made up with 47½% ethanol. This minimised loss of benzene. For naphthalene, the solution was pipetted out and assayed directly without dilution. The mean of the concentration before and after the run was taken as the concentration corresponding to the vapor pressure measured.

Since it is not possible to make solutions of known concentration of benzene and naphthalene in the column, repeat data points of vapor pressure for any particular concentration cannot be done. However data points can be

obtained for concentrations close to each other(that is continuous runs without changing column solution) and a vapor pressure concentration curve is generated. The presaturators prevent excessive change in concentration.

The volume of gas passed depends on the vapor pressure of the substance and varied from 250 ml. for benzene to 4000 ml. for anthracene. The reproducibility was very good for pure substances(0.5% for benzene, 2% for naphthalene and 5% for anthracene) but of the order of 5 - 7% for aqueous solutions of benzene(lower for naphthalene) particularly at higher concentrations. Between three to five runs were made for the pure substances depending on the reproducibility.

C. Calculations. The vapor pressure is calculated using the assumption that the substance follows the ideal gas law. It is given by the expression

$$Ps = \frac{g \times R \times (Pa-P'w) \times Tr}{MW \times Vg \times (Pa-Pw) + g \times R \times Tr}$$

where

Ps = vapor pressure of compound

g = amount of substance collected in grams

R = gas constant

Pa = atmospheric pressure

Tr = temperature of gas in container(room temperature)

MW = molecular weight of compound

Vg = volume of wet gas displaced from the container

P'w = water vapor pressure in column at 25°C.

Pw = water vapor pressure at room temperature

A detailed derivation of the above expression is given by Thomson and Douslin(24).

D. Spectral Measurements. Benzene and naphthalene were assayed spectrophotometrically on a Cary 118 whereas anthracene was assayed by fluorimetry.

The standard curve of benzene in 95% ethanol was linear throughout the concentration range required (absorbance =1). The molar absorptivity (max = 254.5nm) calculated from the slope was 2.206×10^2 which lies within the range of reported values. A standard curve of benzene in 47½% ethanol was also linear with a molar absorptivity (max = 254.5nm) of 1.99×10^2.

The standard curves for naphthalene in 95% ethanol and water were linear with molar absorptivities(max = 276nm) of 5.497×10^3 and 4.955×10^3 respectively, the latter in good agreement with reported values(25). The standard solutions in the latter case were prepared by dilutions from solutions made in 95% ethanol, none of the final solutions containing more than 0.5% ethanol.

Anthracene was assayed by fluorimetry on a Perkin Elmer fluorescence spectrophotometer model MPF-4 using methanol as solvent. The excitation wavelength was 250nm and the fluorescence intensity was measured at 378nm, the temperature being maintained at 25°C. A linear plot of fluorescence peak height versus concentration was obtained.

A concentration of 0.0040 µg/ml gave 27% of full scale deflection at 378nm with coarse sensitivity setting of 3 and both slits at 10mm openings.

III. RESULTS

A. Benzene. Figure 3 shows the vapor pressure-concentration curve for aqueous solutions of benzene. The initial vapor pressure points(upto 1.16×10^{-4} mole fraction) together with the value for the pure lipuid were fitted to a straight line by regression analysis giving a slopw of 23.7×10^{4} mm Hg, which is the Henry's law constant for benzene in water. This line also closely approximates that which would be obtained by joining the origin to the point for the vapor pressure of the pure lipuid(23.8×10^{4}mm Hg) and justifies the previous practise of estimating Henry's law constant from vapor pressure and solubility data.

At higher concentrations, the vapor pressure falls off increasingly until near saturation when the vapor pressure of the pure compound is reached. The lowering of vapor pressure at higher concentration probably indicates some association of benzene molecules. The curvature of the absorbance-concentration plot of aqueous solutions of benzene at higher concentrations(26) may be considered to support this observation. Reproducibility with the saturated solution was poor. This may be due to problems in equilibriation between the benzene and water. Since the solubility of water in benzene is low(3×10^{-3} mole fraction at 25°C), the vapor pressure of the saturated solution may be assumed to be equal to that of the pure liquid.

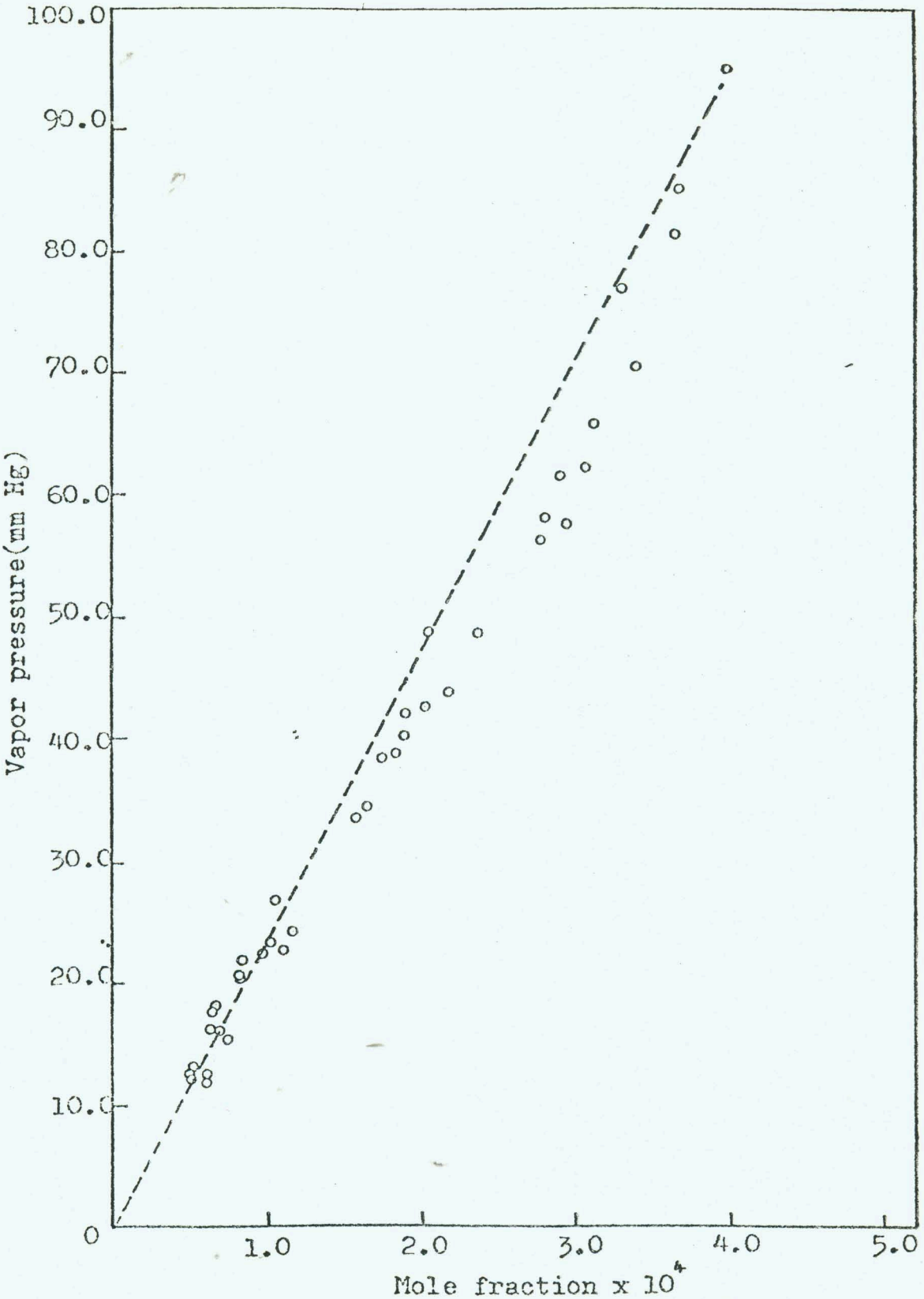


Figure 3. Vapor pressure-concentration results at 25°C for aqueous solutions of benzene.

B. Naphthalene. Figure 4 shows the vapor pressure-concentration curve for aqueous solutions of naphthalene. The Henry's law constant is 1.76×10^4 mm Hg. This is also in good agreement with that obtained by dividing the vapor pressure of the pure compound by the solubility.(At 25°C, Henry's law constant is 1.81×10^4 mm Hg.) The graph is linear throughout the concentration range although at higher concentration a few measurements fall below the line but do not significantly alter the slope from that obtained when considering only the initial points at lower concentrations. The linearity of the graph indicates that n naphthalene does not associate in aqueous solutions. Since in the case of benzene, deviations were observed at higher concentrations and could be ascribed to self association, one might expect naphthalene to show greater association. However if we compare the concentrations of the solutions, the naphthalene solutions are 100 times more dilute (on a mole fraction basis) and hence solute-solute interactions are apparently negligible. This is also supported by the linearity of the absorbance-concentration graph for aqueous solutions of naphthalene.

C. Anthracene. The vapor pressure of pure solid anthracene was measured to be 3.7×10^{-6} mm Hg. From the reported solubility(5.39×10^{-9} mole fraction), the Henry's law constant is 6.86×10^2 mm Hg. The vapor pressure-concentration curve for aqueous solutions of anthracene was not

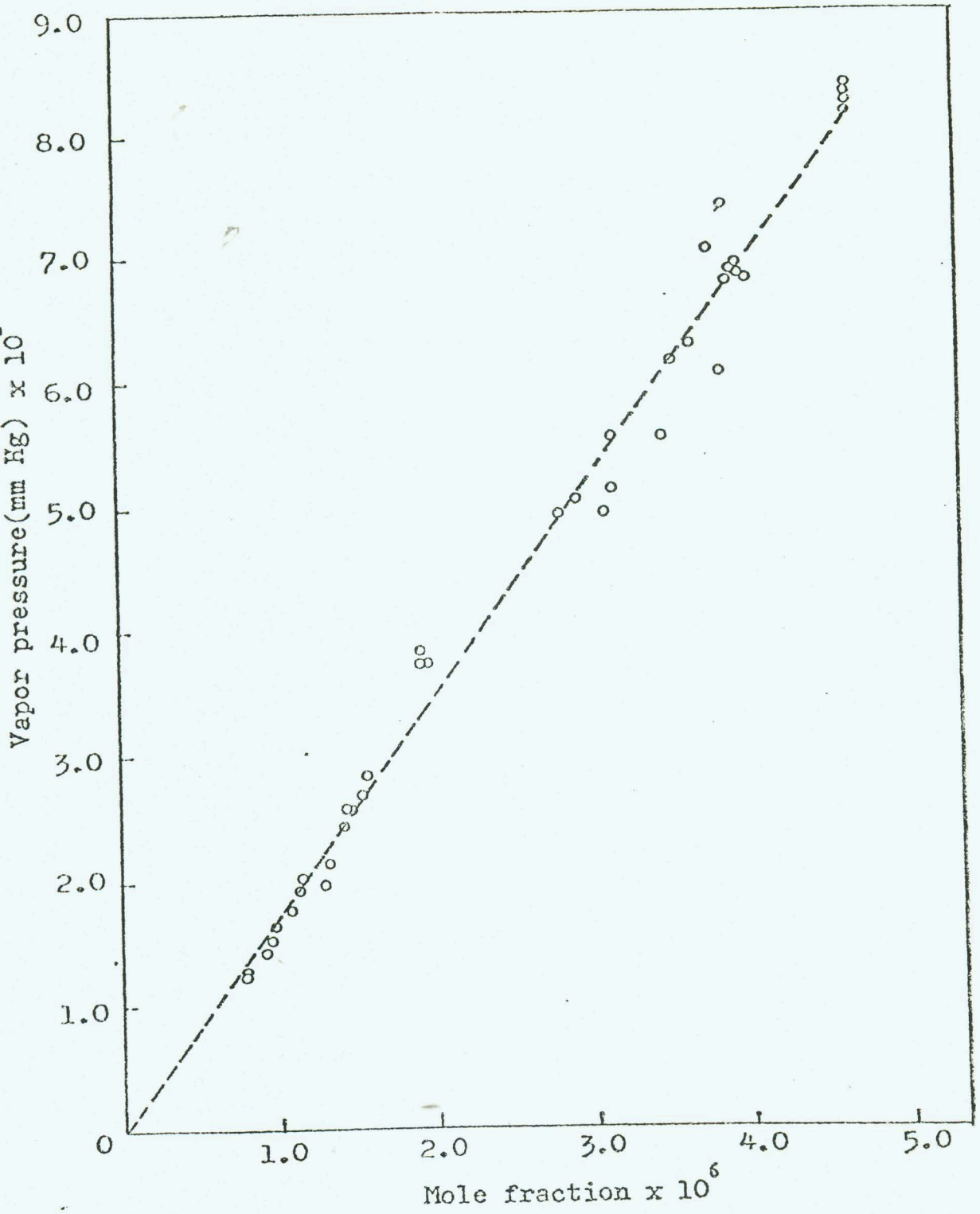


Figure 4. Vapor pressure-concentrarion results at 25°C for aqueous solutions of naphthalene.

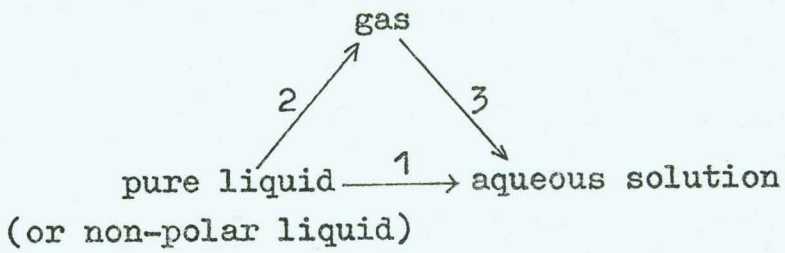
determined for two reasons: 1) The very low aqueous solubility of anthracene, and 2) from the results obtained for benzene and naphthalene, it is expected that the Henry's law constant for anthracene obtained from the solubility and vapor pressure values would be a reasonable estimate.

IV. DISCUSSION

A. Analysis of existing data and results. The usual analysis of the thermodynamics of the hydrophobic effect is based on the process:

pure liquid (or non-polar liquid) → aqueous solution

However, this process can be divided into two processes in the form of the cycle below:



SCHEME I

This separates the free energy, enthalpy and entropy changes of process 1 into two terms. Assuming no intermolecular interactions in the gas phase, process 2 gives the contribution of the inter and intra molecular interactions in the pure liquid whereas process 3 gives the contribution of the interaction of solute with solvent ie. the solvent contribution. The solvent contribution computed in this manner is a net difference between the solute-solvent interactions in solution and the solvent-solvent interactions gained when the solute is transferred into the gas phase. It is proposed that the free energy change associated with process 3 be termed the 'Intrinsic Hydrophobicity of the molecule.

The free energies for the various steps are given by:

Step 1: $\Delta G^{\circ}(pl \rightarrow soln) = RT\ln (1/Xs)$

Step 2: $\Delta G^{\circ}(pl \rightarrow g) = RT\ln (1/Vp)$

Step 3: $\Delta G^{\circ}(g \rightarrow soln) = RT\ln (Vp/Xs)$

where Xs is the aqueous mole fraction solubility and Vp is the vapor pressure in atmospheres for liquid or solid. Using the above equations, the free energies for the cycle steps for the aliphatic hydrocarbons and alcohols and for the aromatic hydrocarbons were calculated and are presented in table 1. Figures 5,6,7 show graphs of these values vs the molecular surface area. The values obtained for the free energy changes per $\AA^2$ (or per CH_2 group) determined from the slopes are given below.

Aliphatic hydrocarbons

$\delta\Delta G^{\circ}$ (pl→water) = 27.3cal/$\AA^2$ = 874 cal/CH_2

$\delta\Delta G^{\circ}$ (gas→water) = 5.0 cal/$\AA^2$ = 160 cal/CH_2

$\delta\Delta G^{\circ}$ (pl→gas) = 22.7 cal/$\AA^2$ = 726 cal/CH_2

Alcohols

$\delta\Delta G^{\circ}$ (pl→water) = 25.5 cal/$\AA^2$ = 816 cal/CH_2

$\delta\Delta G^{\circ}$ (gas→water) = 5.0 cal/$\AA^2$ = 160 cal/CH_2

$\delta\Delta G^{\circ}$ (pl→gas) = 20.3 cal/$\AA^2$ = 650 cal/CH_2

Aromatic hydrocarbons

$\delta\Delta G^{\circ}$ (pl/solid→water) = 29.6 cal/$\AA^2$

$\delta\Delta G^{\circ}$ (gas→water) = -26 cal/$\AA^2$

$\delta\Delta G^{\circ}$ (pl/solid→gas) = 55.8 cal/$\AA^2$

TABLE 1: Free energy data for the cycle steps

Compound	TSA(Å²)[a]	ΔG (Kcal/mole) pl→water[b]	pl→gas[c]	gas→water[d]
Butane	255	5.97	-0.5	6.47
Pentane	287	6.84	0.26	6.58
Hexane	319	7.77	0.98	6.79
Heptane	351	8.56	1.67	6.89
Octane	383	9.52	2.37	7.15
Ethanol	209	0.76	1.50	-0.74
Propanol	240	1.58	2.11	-0.53
Butanol	272	2.37	2.80	-0.43
Pentanol	304	3.17	3.40	-0.23
Hexanol	336	4.03	4.00	0.03
Heptanol	368	4.85	5.10	-0.25
Octanol	399	5.58	5.50	0.08
Nonanol	431	6.46	6.00	0.47
Decanol	463	7.42	6.70	0.72
Benzene	255	4.60	1.23	3.37
Naphthalene[e]	321	7.25	5.39	1.86
Anthracene[e]	382	11.10	11.05	0.05

[a] Computed with 1.5Å solvent radius.

[b] For hydrocarbons, Ref.13
For alcohols, Ref.7
For aromatics, Ref.14

[c] For hydrocarbons, Ref.15
For alcohols, Ref.16
For aromatics, Ref.15

[d] Obtained by difference from columns 3 and 4.

[e] Calculations refer to pure solid rather than pure liquid in these cases.

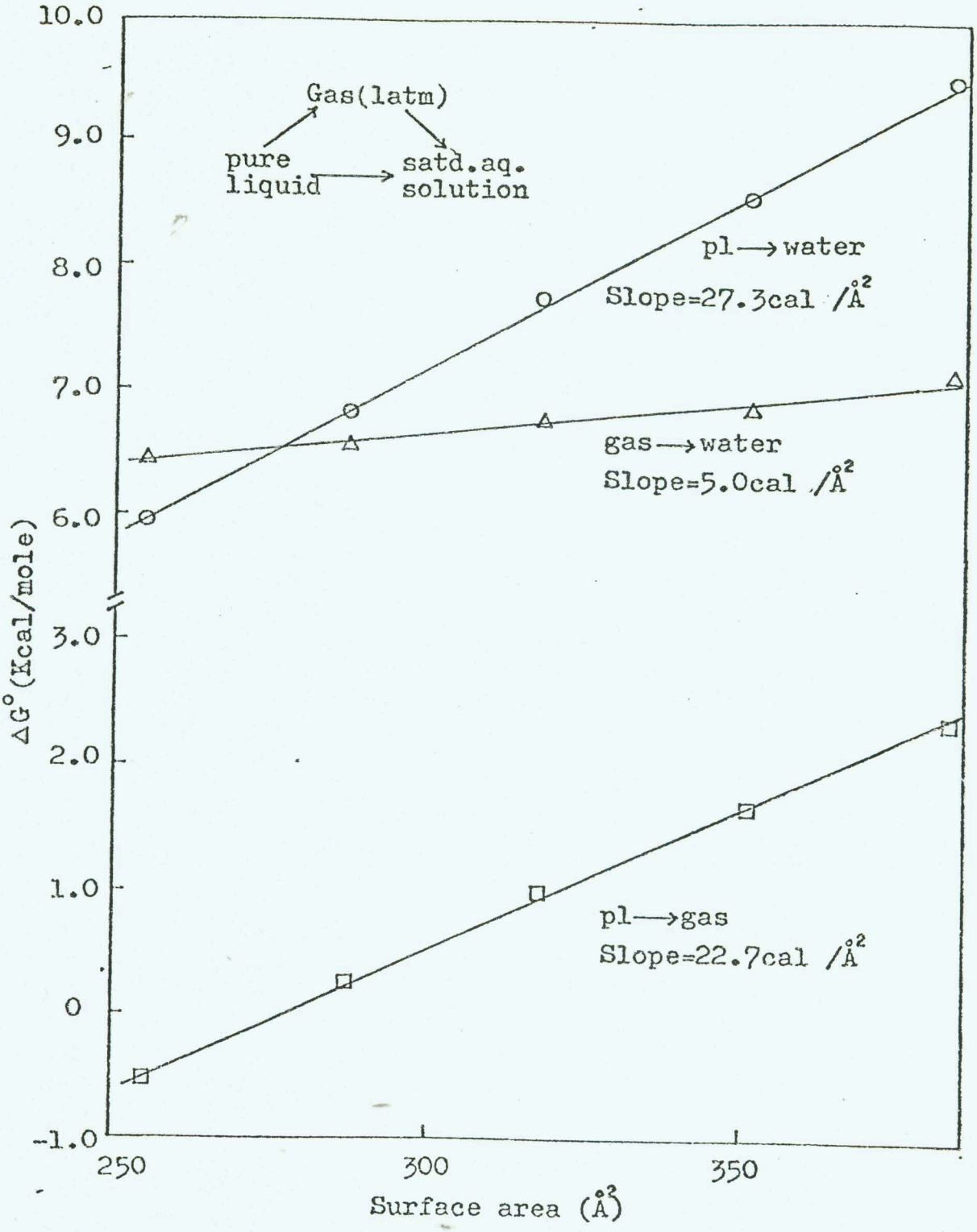


Figure 5. Free energy vs surface area for the cycle steps (Scheme I): Normal aliphatic hydrocarbons.

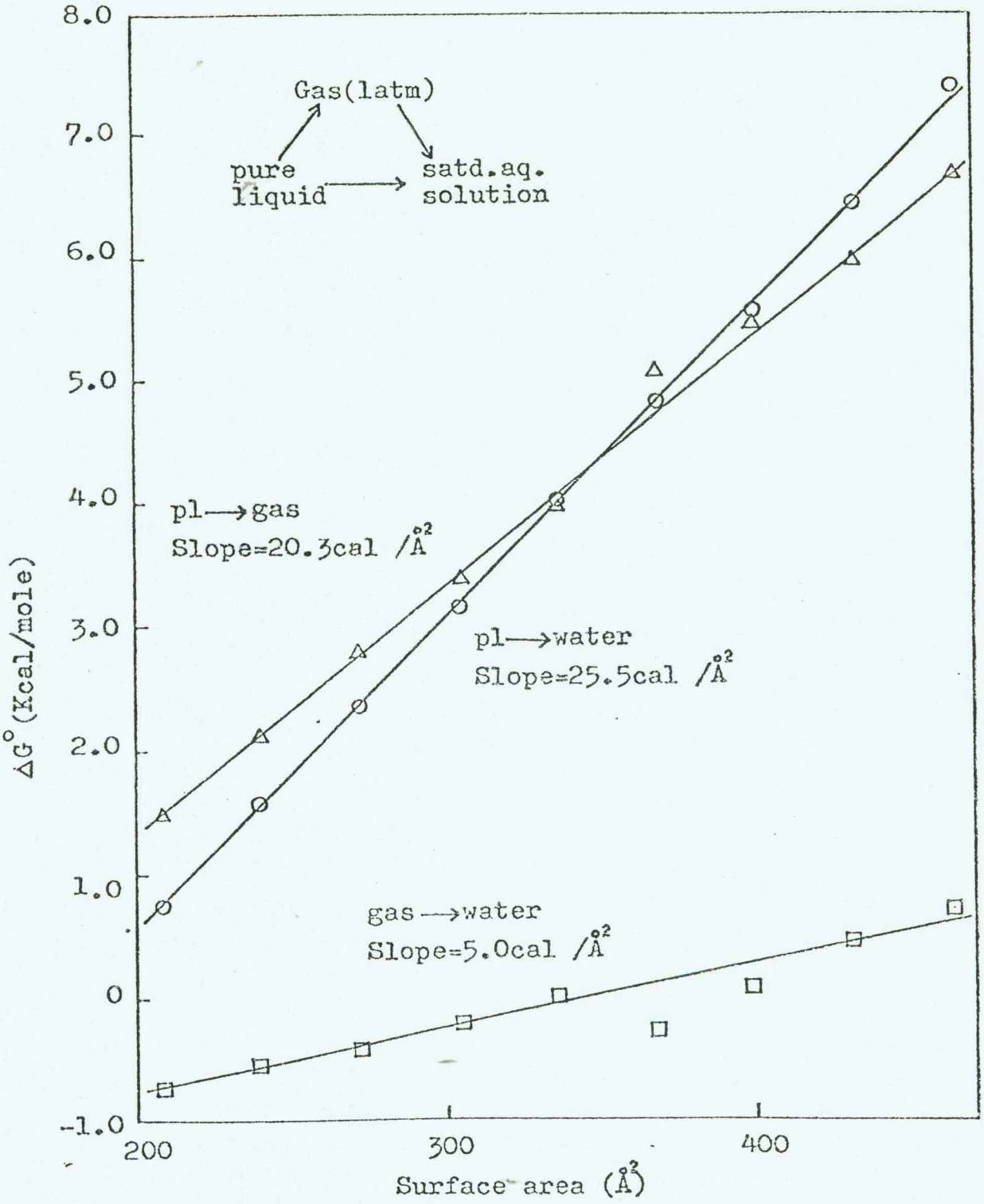


Figure 6. Free energy vs surface area for the cycle steps (Scheme I): Normal aliphatic alcohols.

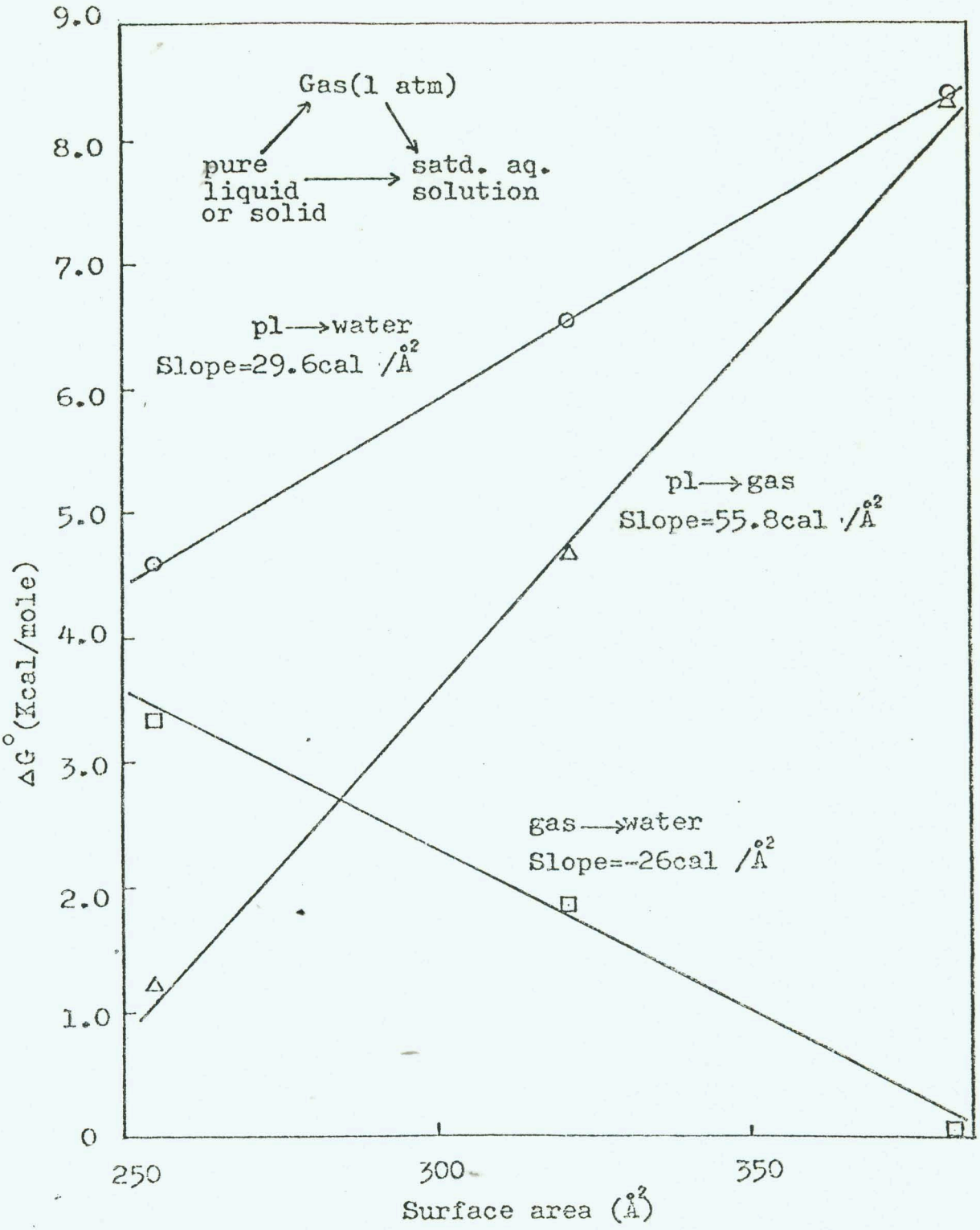


Figure 7. Free energy vs surface area for the cycle steps (Scheme I): Aromatic hydrocarbons.

The values for the aliphatic hydrocarbons and alcohols clearly indicate that approximately 80% of the free energy /mole/CH_2 group for process 1 is due to the interactions in the pure liquid itself(process 2). Hence only the remaining 20% is a result of the solvent contribution.

If we now examine the results for the aromatic hydrocarbons we find that the slope for step 3 is -26 cal/ $Å^2$. This suggests the rather surprising result that as we proceed from benzene to naphthalene, the interaction with water (on a per unit surface area basis) increases. Hence the aromatic systems rather than being hydrophobic, have net attractive interactions with water since $\delta\Delta G^\circ$ for step 3 is negative. The values obtained for the free energy changes for aromatic hydrocarbons for step 3 were calculated from vapor pressure-solubility data on the assumption that they gave a reasonable estimate of the Henry's law constant for the aqueous solutions. That this assumption is valid is evident from the vapor pressure-concentration curves (figures3,4) for aqueous solutions of benzene and naphthalene.

The above analysis is based on free energy changes only. It is also instructive to consider the enthalpy and entropy changes for the cycle steps. Availability of data restricted the analysis to C_2 to C_5 chain length alcohols. Table II lists the enthalpy and entropy changes for the cycle steps for alcohols. It is seen that the enthalpy term dominates the process: pure liquid$\longrightarrow$gas whereas the

Table II. Free energy, enthalpy and entropy increments for the cycle steps in Scheme I for normal alcohols $C_2 - C_5$.

	cal/mole[a]		
Process	$\delta\Delta G/CH_2$[b]	$\delta\Delta H/CH_2$[c]	$\delta(T\Delta S)/CH_2$
gas phase water	180	-957	-1147
gas phase pure liquid	-625	-1158	-541
pure liquid water	813	+187	-636

[a]The values in the table were determined individually from the experimental data and add to within experimental error.

[b]Free energy data from Table I.

[c]Enthalpy data for step 1 in Scheme I from ref.31 and for step 2, ref. 30.

enthalpy and entropy terms balance out for the process: gas phase aqueous solution. However if we consider the enthalpy and entropy contribytions of processes 2 and 3 separately, the enthalpy terms balance out whereas the large negative entropy term for process 3 is dominant and is indeed the basis of the entropic origin of the hydrophobic effect, although its magnitude in terms of the free energy provided by the solvent is small.

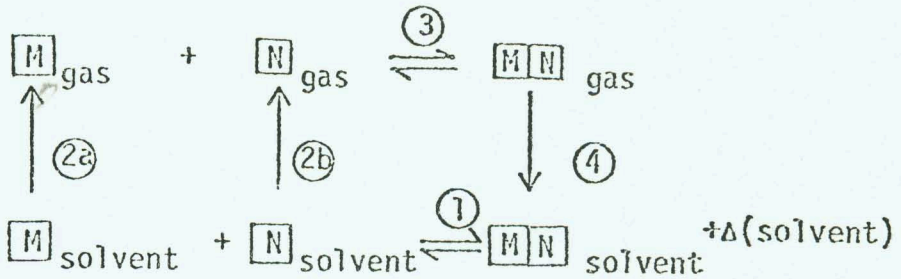
It appears from the literature that distinguishing the enthalpic and entropic contributions to the free energy of process 1, while failing to distinguish the intrinsic solute and solvent contributions has led to misleading statements regarding the solvent contribution. The term 'Intrinsic Hydrophobicity' emphasises the importance of the free energy change for the gas water process as a measure of the solvent contribution.

B. Implications on the calculation of solvent contribution to protein-ligand interaction energies.

In recent years(32,33) there have been attempts to calculate the free energy changes for protein-ligand or enzyme-substrate interactions by calculating the excluded hydrophobic surface area resulting form binding. It is necessary to reconsider these calculations in light of process 3(Scheme 1).

Consider the association of two non-polar molecules (M,N) in aqueous solution. The solvent and the M,N inter-

action energy can be partitioned by considering the following scheme.



SCHEME II

where ΔG°_{1} is the sum of the steps 2,3 and 4 and Δ(solvent) is the solvent released when the complex MN forms. The free energy change in step 2 is the intrinsic hydrophobicity contribution and for an aliphatic hydrocarbon is of the order of 5cal/Å^2(refer figure 5). This also includes the free energy of the solvent-solvent interaction released on removing the molecule from water. The free energy change for step 4 is calculated from the excluded surface area given by:

$$SA = (SA_M + SA_N)_{free} - (SA_{MN})_{bound}$$

The change in free energy then is given by $\Delta SA.(5cal/Å^2)$ where it is assumed that the excluded surface area is equivalent to hydrocarbon surface area. This calculation also includes the solvent term as in analogy with step 2 (scheme II). Previous calculations of solvent contributions (32,33) based on free energies for process 1(scheme I) used a factor of 25cal/Å^2 resulting in an overestimation of the solvent contribution by a factor of 5. The value of 5 cal/Å^2 is an upper limit since it is based on aliphatic

hydrocarbon surface area. For more polar residues the solvent contribution to association is expected to be less favorable.

In the case of association of aromatic residues, the solvent contribution is unfavorable to the process(-26cal/$Å^2$) implying that the aromatic residue is not hydrophobic at all but has net attractive interactions with water. Hence the importance attached to the solvent contribution in the base stacking interaction in DNA molecules or to the folding of proteins may need to be revised. The intrinsic hydrophobicity of aromatic residues suggests that in aqueous solution, association of aromatic residues is not solvent driven but is a result of stronger solute-solute interaction energy as compared to solute-solvent interaction energy.

V. SUMMARY.

The free energy change for the process pure liquid aqueous solution for aliphatic and aromatic hydrocarbons and aliphatic alcohols has been analysed in terms of two contributing processes, pure liquid⟶gas and gas→aqueous solution to gain further insight into the hydrophobic effect. The gas phase⟶aqueous solution process gives the interaction of the solute with the solvent free of solute-solute interactions and it is suggested that the term 'Intrinsic hydrophobicity' be used to characterise this process.

Analysis of aliphatic hydrocarbons on the above basis indicates that 80% of the free energy change of solution is due to solute-solute interactions in the pure liquid whereas the solvent contribution is only of the order of 5 cal/$\mathring{A}^2$. For aromatic compounds the trend is reversed in that the solvent contribution is unfavorable to association of aromatic residues in aqueous solution. This implies that aromatic residues are not intrinsically hydrophobic since an increase in interaction energy(-26cal/$\mathring{A}^2$) with water per unit aromatic surface area is observed. These observations are based on estimates of Henry's law constant from vapor pressure solubility data. The validity of these calculations for aromatic compounds was experimentally confirmed by determining the Henry's law constants for benzene and naphthalene from the vapor pressure-concen-

tration curves of their aqueous solutions. The vapor pressure of anthracene was determined and the Henry's law constant calculated on the basis of the results obtained for benzene and naphthalene.

A model is developed for calculating the solvent contribution to protein-ligand binding based on the accessible surface area of the free and bound species. From the preceding results, this model indicates that the solvent free energy contribution to the association process is considerably less than previous estimates suggested. In the case of the aromatic residues the results indicate that the actual solvent contribution is unfavorable to the binding process. Thus a larger role must be ascribed to the nonbonded interactions than here-to-fore has generally been the case.

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