THE DESIGN AND EVALUATION OF AN AUTOMATED DRUG PARTITIONING SYSTEM FOR THERMODYNAMIC STUDIES

by

Graham Noel Elson

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The measurement of partition coefficient data is now a routine part of drug research. Octanol-water partition values are used by Medicinal Chemists in studies on structure activity relations (Hansch Analysis). And in preformulation investigations partition coefficient values are determined at different pH to give insight into the absorption properties of a drug. Partition data also play an important role in the development of a group contribution approach to solution thermodynamics and the prediction of solution properties.

The usual method of measuring partition coefficient values is the laborious shake-flask technique. The values determined from such batch-wise determinations can be inaccurate and measurements at temperature considerably different to room temperature are often difficult. The derived data cannot normally be used to calculate the thermodynamic quantities; enthalpy and entropy. Consequently the utility of a rapid, continuous method for obtaining accurate partition data has been investigated.

A commercially available Swedish apparatus (AKUFVE) has been used in recent years for studying the important factors in solvent extraction. This system has been evaluated using pharmaceutical systems. Briefly it consists of a mixer which provides rapid efficient contact between two immiscible solvents, a unique centrifugal separator that ensures rapid "absolute" separation of the two solvents and connections permitting sampling or on-stream measurement of the concentrations of solute in both solvent phases. Equilibrium is normally attained in a matter of minutes. The experiment conditions can be changed at will with the result that a temperature or pH profile can be determined in a few hours rather than over a period of days. The performance of the AKUFVE has been assessed using a range of compounds (alkyl phenols, weak acids, weak bases) at different solute concentration, temperature, pH, ionic strength, phase ratio. Three different solvents (octanol; cyclohexane; chloroform) and their mixtures have been examined. The derived partition values were compared with experimentally determined or literature values obtained in the conventional way. Ion-pair extraction systems and drugs undergoing simultaneous phase transfer and hydrolysis have also been investigated.

The AKUFVE apparatus has been found to provide a rapid and accurate method for determining the partition coefficient values of drug substances in instances where a range of experimental conditions are investigated. For example solute concentration, pH, temperature. It can also be used to measure the partition coefficients of unstable substances. However, it is not suitable for simple routine one-off partition coefficient determination.

Key Words: Partition coefficients; Structure-activity relationships; Solution thermodynamics; AKUFVE

ABSTRACT

The object of this work was to develop an experimental procedure which would provide an accurate means of acquiring thermodynamic data on partitioning for a wide range of solute and solvent systems.

The importance of partition coefficient data in pharmacy is discussed and the relevant theoretical aspects are described. Previous attempts at developing automated partitioning techniques are considered, particular attention being focussed on the "AKUFVE" method developed in Sweden by Rydberg and Reinhardt (1969). The AKUFVE technique has been examined in detail under a wide range of experimental conditions.

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CHAPTER 1. INTRODUCTION

1.1. PARTITION COEFFICIENTS IN PHARMACY

Partition coefficients are of fundamental significance to a wide range of processes which find applications in pharmacy. A few of the most relevant of these are reviewed in the following pages.

1.1.1. STRUCTURE-ACTIVITY RELATIONSHIPS

Medicinal chemists have striven for years to produce a reliable method for the correlation of drug activity with chemical structure in an attempt to save considerable amounts of time and money in the search for useful pharmacological agents.

The biological activity of a drug is thought to depend on two fundamental processes that occur at any given receptor site:-

(i) structurally specific.

(ii) structurally non-specific.

The former depends upon the functional groups present in the molecule as well as its general stereochemistry.

The second process is more non-specific in character, depending mainly upon physical properties e.g. thermodynamic activity of the drug molecule.

When a drug reaches the receptor site in a sufficient concentration then a biological response is observed. This phenomenon depends to a large extent on the distributive tendencies of the drug molecule between aqueous phases and biological phases with varying degrees of lipophilicity i.e. biological activity is related to the partition coefficient of the drug.

Meyer (1899) and Overton (1901) were amongst the first workers to observe structure activity relationships when they related narcotic activity to partition coefficients. Ferguson (1939) introduced a more rigorous concept when he suggested that an equilibrium exists between the extracellular phase and the phase at the receptor site such that substances at about equal thermodynamic activity produce equal degrees of narcosis. This thermodynamic approach was further developed by Crisp and Marr (1957)

who concluded that the mechanism of narcotic activity is only consistent with an equilibrium condition between the narcotic in the biophase and that in the external medium, an approach which has more recently been adopted by Higuchi and Davis (1970).

Hansch and co-workers (Hansch et. al. 1963; Fujita et. al. 1964; Hansch and Fujita, 1964; Iwasa et. al. 1965; Hansch, 1971; Leo et. al. 1971), introduced a linear free energy approach to structure activity correlation which has since found wide acceptance. Originally a four parameter empirical approach was suggested:-

$$- \log C = k\pi^2 + k'\pi + \rho\sigma + k''$$

where C is the concentration producing a given biological response.

k, k', k" and ρ are constants.

σ is the Hammett electronic substituent constant. (Hammett; 1937).

π is the group contribution parameter.

$$\pi_x = \log K_{D(RX)} - \log K_{D(RH)}$$

Where $K_{D(RH)}$ is the partition coefficient of the unsubstituted parent compound and $K_{D(RX)}$ is the partition coefficient of the substituted compound.

This group contribution approach for partition coefficients will be discussed further in a later paragraph.

Hansch also expressed biological response in the free energy terms as being governed by one rate limiting process for which K_{BR} is the equilibrium constant:-

$$\Delta G_{BR} = \Delta G_{hydrophobic} + \Delta G_{electronic} + \Delta G_{steric}$$

Proportional to $\log K_{BR}$.

i.e. $\Delta G_{BR} = -RT \ln K_{BR}$

Because of the possibility of associative effects e.g. dimerisation, and phase saturation the structure-activity relationship can become concentration dependent.

Also in the case of acidic or basic drugs, the form and degree of ionisation of the molecular species are pH dependent with consequent effects on the biological activity.

1.1.2. THERMODYNAMICS OF DRUGS IN SOLUTION

It has been thought for many years that a knowledge of the solution thermodynamics of drug molecules could lead to a predictive method for the physical and biological properties of drug systems. Some degree of success has been achieved by adopting a semi-empirical group approach the more rigorous attempts being confined to non-polar systems.

The basic assumption that is made in this method (Hansch; 1971) is that the free energy of the solution process is additively composed of independent contributions from the constituent functional groups. By calculating group contribution values it should be possible to use them in 'a priori' fashion to predict solution behaviour for a wide range of drug molecules since the activity coefficient, excess free energy and partition coefficient could be found by summing contributions for the different groups comprising the molecule. In multi-functional molecules some perturbation of the determined values may arise as a result of interactive processes, but account should be taken of this in the form of correction factors.

Furthermore it is to be hoped that the enthalpy and entropy parts of the free energy will also be additive, so the prediction of all three terms would be possible. Such data would help clarify the physical processes occurring upon solution and the causes of non-ideal behaviour.

The first extensive study of group contributions to partition coefficients was made by Collander (1951) who partitioned alkanoic acids, alkanols and alkylamines between water and solvents such as diethyl ether, isobutanol, octanol and oleyl alcohol. He concluded that there was a logarithmic relationship between the partition coefficients of a given homologous series of compounds partitioned between water and two different solvent systems (i, j):-

$$\log K_{D(i)} = a \log K_{D(j)} + b$$

where a and b are constants.

This basic approach of Collander's has been extensively studied and developed by Hansch and his colleagues who have determined the partition coefficients of hundreds of compounds for the octanol water system. The group contribution was given the symbol π referred to in the previous section on structure activity relationships. Studies have been carried out on the dependence of π on different molecular environments e.g. aromatic; aliphatic; branched chain; conjugated; stereochemical; hydrogen bonded etc. thereby arriving at accurate additive π-values for a wide range of functional groups in a variety of compounds.

Lough, Currie et al (Lough et al., 1968a, 1968b, Currie et al., 1966; Delaney et al., 1967) carried out similar studies using cyclohexane, instead of octanol, as the organic phase in an attempt to facilitate assessment of the effects of very polar groupings. They assumed that the algebraic sum of the π-values for the component groups within the molecule was equal to the logarithm of the partition coefficient i.e.:-

$$\log K_D^m = a\Sigma\pi + b$$

a and b are constants.

Currie claims that the electronic environment of a substituent is more relevant than had previously been thought and he assigned an interaction parameter in the case of gem or vicinal polar groups:-

$$\log K_D^m = a\Sigma\pi + \Sigma\pi_{interaction} + b.$$

More recently Higuchi and Davis (1970) have characterised structural changes within a drug molecule by a group constant "F"which is defined as the ratio of the oil-water partition coefficients of a substituted and parent compound in the absence of dimerisation and ionization. In free energy terms the F constant can be written as:-

$$\Delta (\Delta G)_X = -RT\ln F_X$$

and the Hansch π value as equal to log F.

Davis et al. (1972) suggested that if F constant values for all common substituent groupings became available then it would permit a much greater insight into the potential distribution behaviour of drugs and also allow one to make a priori estimations of solution behaviour.

The free energy of solute transfer can be further analysed in terms of the constituent quantities enthalpy and entropy. These are determined by studying the change in partition coefficient with temperature and then applying the normal thermodynamic arguments to the data obtained (c.f. theory section). A knowledge of both enthalpy and/or entropy of solute transfer can lead to an understanding of the solute-solvent interactions existing in both phases. Such interactions can undergo considerable variation depending upon the solvents chosen for the two phases e.g. a non-polar solvent of low dielectric constant tends to exhibit virtually complete immiscibility with aqueous phases thus tending towards a thermodynamically ideal state, whereas more polar solvents can undergo solvent-solvent interaction between the two phases and thus complicate the interpretation of results. Rytting et al (1972) have in fact produced a potent case for using a non-polar solvent as a reference system for further partition studies.

In the applications outlined above it is essential that the data used must all be absolutely reliable.

1.1.3. SOLVENT EXTRACTION.

The determination of partition coefficient values, as well as the kinetics leading to equilibration, form an essential part of the design of solvent extraction procedures. The efficiency of a solvent extraction process can assume major economic proportions when applied to large scale production units found throughout the pharmaceutical industry. These are used mainly for the concentration of antibiotic products from large volumes of fermentation liquors, prior to a second solvent extraction process which purifies the product and renders it suitable for the final freeze drying stage.

The manufacture of penicillin typifies this type of process, the crude antibiotic being extracted from the fermentation liquors into methyl isobutyl ketone by means of continuous solvent extraction using a centrifugal, counter-current extractor (Podbielniak). Fast equilibration coupled with a high partition coefficient obviously leads to the most efficient process.

The optimised conditions for such a process are arrived at by means of a thorough study of many parameters which often undergo a complex series of interactions. However, the most important parameter is usually the value of the partition coefficient.

The above implies that solvents are often required to extract selectively one component of a solution in preference to another. The ideal solvent would dissolve a maximum of one component and a minimum of the other(s). A numerical system of the degree of selectivity of a solvent has evolved being designated under the term β. Thus the selectivity β of solvent B for component C in a system including a third component A is given by the relationship:-

$$\beta = K_D \cdot \frac{X_{AA}}{X_{AB}}$$

where X_{AA} is the concentration of A in the A-rich phase.

X_{AB} is the concentration of A in the B-rich phase.

K_D is the distribution (partition) coefficient. $\left(\frac{[A]}{[B]} \text{ convention}\right)$

For systems with K_D less than one, β will always exceed unity, and thus the process will be selective and successful. In a practical sense the more that β exceeds unity the better since selectivities close to unity will result in large plant equipment, greater numbers of extraction stages and in general costly investment and operation. If β equals one, separation is impossible and thus a knowledge of the partition coefficient can give a good indication as to the viability of a projected process.

However, distribution coefficients can frequently be altered by changing the pH and deliberate attention to this factor can lead to a more suitable value of partition coefficient when the extracted solute is to be an ionic species.

Another important factor is the rate of mass transfer from one solvent (R) to another immiscible solvent (E) which is given by the equation:-

$$N_A = K_E \ (C_E^* - C_E)$$

$$= K_E \ \Delta C_{OE}$$

where K_E is the mass transfer coefficient for solute entering the E phase.

C_E^* is the concentration of solute at the liquid-liquid interface.

C_E is the concentration of solute in the bulk of phase E.

For a rectilinear equilibrium distribution it can be shown that:-

$$K_R = K_D \cdot K_E$$

where K_R is the mass transfer coefficient for solute entering the R phase.

Thus

$$K_D = \frac{K_R}{K_E}$$

and can thus determine the rate of mass transfer in such a system.

In industrial processes the separation of the components of a solution by extraction are often undergone by a stage-wise contact, i.e. solvent and mixture to be separated are intimately contacted, allowed to approach equilibrium and then separated. This operation can then be repeated in a variety of sequences. A stage is defined as a mechanical device, or series of devices, wherein the solution to be separated and an immiscible solvent are intimately mixed, allowed to approach equilibrium and then settled or separated into two immiscible liquid phases, which are then withdrawn. The solvent rich phase leaving the stage is termed the extract, the solvent lean phase the raffinate. A theoretical or ideal stage is one where contact between phases is sufficiently intimate and maintained for a sufficient period of time to establish distribution equilibrium such that raffinate and extract are equilibrium solutions.

It can be shown that the extraction factor ε (which is the measure of the efficiency of a series of stages) is given by the relation:-

$$\varepsilon = \frac{K_D B}{A} = \frac{\text{quantity of solute in extract}}{\text{quantity of solute in raffinate}}$$

Where A and B are the phase volumes.

and thus a knowledge of K_D is required to determine the minimum number of stages required for a given extraction process.

Finally for stagewise contact of multicomponent systems where the fraction of B leaving in the A phase equals that of C leaving in the D phase (but not necessarily the concentrations).

$$\frac{A}{D} = \sqrt{(K_D^B \cdot K_D^C)^{-1}} \qquad \text{eqn. (i)}$$

or $$\varepsilon_B = \sqrt{\beta_{B,C}}$$

and $$\varepsilon_C = \sqrt{\beta_{B,C}^{-1}}$$

i.e. the degree of separation is a maximum when the solvent ratio is given by eqn.(i).

1.1.4. PREFORMULATION. (c.f. Dearden 1977).

Since the process of drug absorption can be regarded as the passage of a solute from an aqueous (hydrophilic) environment across a membrane into a hydrophobic (lipophilic) area then it follows from the discussion on structure-activity relationships above that a knowledge of the partition coefficient for that species (in a suitable bi-phasic solvent system) could lead to a prediction of the absorption characteristics of that drug.

In many systems, for solutes of similar structure, the activation energies for both the forward and reverse phase-transfer mechanisms are often approximately equal; hence the transfer rate constants become proportional to the equilibrium constants or partition coefficients, i.e. the hydrophobicity of the drug species is related to its partition coefficient. Dissolution rates of drugs, administered in solid form, can also determine the degree of absorption if the dosage form passes the site for optimal absorption before total release has occured. Partition coefficients determined in the biphasic system of chloroform and water have been correlated with percentage of drug absorbed, and also with amount adsorbed onto carbon black surfaces, (Nogami, 1969).

However, general trends can be modified and even reversed, due to hydration and other effects, such that experimental evaluation using different solvent systems and even mixed solvents becomes particularly useful. Also since many drugs are either acidic or basic it is usual to study the system at two or more pH values.

Thus it is shown that a knowledge of partition coefficients can lead to the most effective formulation for the administration of a particular drug at a particular site. Implicit in this statement is the type of problem often encountered in drug delivery systems in that there usually exist conflicting requirements for the transport of the drug to the receptor site and its subsequent binding to the receptor

If a drug has a large number of membranes to cross before it reaches the receptor site then its lipophilicity (partition coefficient) should be minimal in order not to be lost on its way there. However, upon arrival at the receptor, for optimal binding, a high lipophilicity is usually desirable.

One technique which is adopted to solve this dichotomy is to use a hydrophilic derivative of a lipophilic drug which will revert to the original form (e.g. by enzymatic hydrolysis) on approaching the site of action; these derivatives are called pro-drugs. Thus it is required to"design" the drug to have an optimal partition coefficient for both transport and binding processes, leading to maximum bioavailability.

It has also been found that the dissolution rate of a drug in solid dosage form depends to some extent upon the value of its partition coefficient.

For a wide range of organic compounds it has been shown that non-specific protein binding can be directly related to partition coefficient. The dual process of protein binding and deposition during transport through fatty tissue is enhanced by increasing partition coefficient and results in a reduction of drug concentration at the receptor site and a prolongation of drug activity unless of course the attainment of a therapeutic level is prohibited by the transport processes.

Other examples of partition coefficient studies being useful in formulating techniques occur in the design of topical vehicles in which there exists a competing partitioning process between the vehicle and the skin which requires optimising of the partition coefficient for any particular application.

More recently, as an alternative to pro-drugs, liposome encapsulated forms of drugs are being administered. In such systems the drug has to partition out of its cell before it can interact with the receptor and thus the partition coefficient again plays an important role in this particular type of formulation.

Finally since solubilization is a partitioning between a relatively non-polar micelle and an aqueous matrix it would be expected that the more lipophilic members within a congeneric series would have lower absorption rates from the aqueous phase containing solubilizer, a trend which has been confirmed by partition coefficient studies on some systems.

1.1.5. PHARMACEUTICAL ANALYSIS.

Extraction processes are often used to purify compounds and separate them from their co-products prior to analysis. Biphasic systems have also been used as indicators in volumetric analysis e.g. the Andrew's titrimetric method in which iodine is extracted from a carbon tetrachloride phase until at the end point a faint violet colour persists in the organic layer.

It is however, in the chromatographically related analytical methods such as thin layer, paper, gas and liquid chromatography as well as ion exchange and ion-selective processes, that a knowledge of partition coefficients is of prime importance.

In ion-selective electrodes the partitioning of ions occurs between a non-polar solvent and water, the selectivity of the device being dependent on the relative partition coefficient for the various ions in the aqueous phase e.g. nitrobenzene has been used in an electrode to measure lithium ion concentration in the presence of rubidium ions. (Eisenman, 1969).

It has been shown (Hilmi et. al. 1970) that a relationship between retention volume (V_n) and partition coefficients exists in gas-liquid chromatography viz.

$$K_D = \frac{V_n}{V_l}$$

where V_l is the volume of the liquid phase.

By further assuming ideal gas law behaviour the activity coefficient at infinite dilution is given by:-

$$\gamma_1^{\infty} = \frac{RT}{Mp_1^{o}V_n}$$

(Littlewood, 1962)

where M is the molecular weight of the solvent.

where p_1^{o} is the vapour pressure of the pure solute.

Similarly in paper and thin layer chromatography Bate-Smith and Westall (1950) showed theoretically that:-

$$-\Delta G = RT\ln K_D = \ln k + \ln R_M$$

where k is a constant.

and R_M is a chromatographic parameter related to R_f (the distance moved by the solute relative to the distance moved by the solvent front) by:-

$$R_M = \log\left[\left(\frac{1}{R_f}\right) - 1\right]$$

Hence it can be observed that the choice of the optimal phase system for separating a given mixture by chromatography is facilitated by a knowledge of the partition coefficients of a wide assortment of liquid-liquid systems. In many such systems a linear relationship can be assumed between retention parameters and partition coefficient, viz:-

$$t_{Ri} = t_{Ro} + t_{Ro} q K_i$$

where t_{Ri} = retention time measured from the moment of the sample injection until the indication of the peak maximum by the detector.

t_{Ro} = average residence time of the mobile phase.

$q = \frac{V_s}{V_m}$ = volume ratio of the stationary and the mobile phase.

K_i = partition coefficient of the i^{th} component.

The most important separation method based on liquid-liquid distribution in chemical analysis is liquid-liquid chromatography. To obtain the best conditions it is necessary to operate in the linear range of the distributive isotherm. The chromatographic resolution of two components is determined mainly by the ratio of their distribution coefficients in the stationary and the moving liquids (phases).

The choice of the optimal phase system for the separation of a given mixture by chromatography follows from consideration of the equation for the resolution of the successive components:-

$$K_i = \frac{C_i^{\beta}}{C_i^{\alpha}} = \frac{A_{io} - A_i}{A_i} \left(\frac{V_{\alpha}}{V_{\beta}}\right)$$

where K_i = partition coefficient of component i in the phase β

C_i^{α} = concentration in phase α

C_i^{β} = concentration in phase β

A_i = quantity proportional to the concentration in phase α

A_{io} = initial value of A_i.

V_{α} = volume of phase α

V_{β} = volume of phase β

Compounds separated by liquid-liquid chromatography can be characterised by their partition coefficients. These values can be used for the identification of the compounds. The efficiency of such identification techniques depends upon the precision of the data obtained by methods which should not only be rapid but also be amenable to further development by the combination of static and dynamic measurements by applying statistical methods to the above relationships.

By further development of the preceding arguments it has been shown that a linear relationship exists between partition coefficients and chromatographic parameters (McCall. 1975) i.e.:-

$$\log K_D = \log k + R_M$$

k is a constant.

It has been shown that the retention time in liquid chromatography can be expressed by a term (k') which is defined as:-

$$k' = \frac{(t_R - t_O)}{t_O}$$

where t_R is the elution time of a retained peak

t_O is the elution time of a non-retained peak.

The quantities of k' and $(\frac{1}{R_f} - 1)$ are analogous, hence for partition between a stationary and mobile phase:-

$$\log K_D = \log k + \log k'.$$

Thus for liquid chromatography with a stationary lipid-like phase and an aqueous mobile phase, k' should be linearly related to a measured liquid-liquid partition coefficient.

Techniques have been evolved from this theory enabling liquid-liquid chromatography to be used in the determination of structure-activity relationships and these will be discussed later.

1.1.6. COMPLEXATION

Partition coefficients can be used to study complexation phenomena. A good example is provided by the study of hydrogen bonding interactions in biphasic systems containing hydrogen donors and acceptors. (Higuchi et al. 1969). The partitioning of a hydrogen donating species in the aqueous phase is influenced directly by the extent of complex formation in the organic (non-polar) phase when a suitable hydrogen acceptor is present in the system. The resultant complex is normally extracted into the organic phase after equilibrium has been attained. Measurement of the concentrations of the two interacting species in the two phase allows calculation of the appropriate stability constant.

In their studies of the thermodynamics of group contribution effects to partitioning Higuchi et al. (Mamtora 1969; Weber 1969), utilised the property of ion-pair formation to overcome poor water solubility problems with certain organic solutes. The compounds were rendered soluble by formation of a charged species i.e. acids were converted to salts; alkanols were sulphated; amines protonated etc. and then by choosing a suitable ion of opposite charge the resultant ion pair was extracted into the organic phase.

The interaction of drug molecules with proteins (including enzymes) is referred to as protein binding and this is an important example of the fundamental property of non-specific hydrophobic interaction which occurs in many biological and drug systems. Franke (1968, 1969, 1970a, 1970b) has made a detailed study of the interaction of hydrocarbons and other species with albumin and has attempted to correlate thermodynamic quantities with partition coefficient.

Hymes et. al. (1965) has shown that the free energy of interaction between a solute and solvent i.e. enzyme and enzyme inhibitor, is a linear function of the surface area of the solute, which was subsequently shown to be exactly analogous with the relationship between free energy of complex formation and partition of the inhibitor between water and non-polar solvent (Wildnauer and Canady 1966).

Modern concepts of drug action and drug specificity are all based on the assumption that the initial process in drug action is the formation of some type of reversible complex between the drug and a cell compartment that is known as the drug receptor. The occupation of the receptor by the drug induces a change in some property of the cell which persists for as long as the drug occupies the receptor. The various theories that have been presented regarding the drug receptor complex all lead to the conclusion that the reponse under equilibrium conditions is likely to be some function of the concentration of the drug receptor complex, a feature which is very dependent upon the partitioning process for that system.

1.1.7. REACTION RATES.

Finally, having considered all aspects of the thermodynamic parameters pertaining to the system at equilibrium reference must be made to the kinetics of equilibration which are also a highly relevant feature of partition coefficient studies.

In a two phase system overall transfer of solute from one phase to the other occurs until the chemical potential of that species is the same in both phases whereupon a dynamic equilibrium is established in which the forward mass transfer rate equals that of the reverse process.

The rate of attainment of this dynamic equilibrium varies over a wide range of time for different systems of stable species. The kinetics of such processes can be modified to a greater or lesser extent by the presence of other reacting species or even in their absence if the solute molecules are unstable in either phase.

If the extracting species is a non-polar adduct of more than one ionic species then the rate of formation of this adduct in the aqueous phase may be less than the partitioning rate and will therefore become the rate determining step.

In other systems instability of the solute in either phase will produce a time dependent concentration of derived species, which can subsequently alter the equilibrium of the distribution process in either direction.

Many drugs are susceptible to both types of time dependent processing and thus are amenable to kinetic investigation.

Other factors also govern the rates of interphase movement e.g. a reduction in the electrical charge of a water soluble molecule would cause a reduction in its aqueous solubility which in the presence of an oil phase would result in a net increase in the rate of transfer to the less polar (oil) phase. This phenomenon is observed in systems which undergo ion-pair formation the rate increase being proportional to the molar charge and concentration of such paired species.

The addition of specific solvating agents will also affect the kinetics of the partitioning process and studies of the effect of adding polar species to the organic phase have in fact been carried out.

Another factor which has been found to affect the rate of attainment of equilibrium in a biphasic system is temperature. This effect is minimised in the AKUFVE, presumably due to its very efficient mixing and rapid recycling process. However, one drug (secobarbitone) was found to need several hours for a true determination of its partition coefficient to be established after a rise in temperature of only $5^{\circ}C$. This probably explains why previous workers had obtained such erratic results for this particular system when using the conventional and much less efficient shake flask technique. (Doyle and Proctor, 1973).

In fact for such "slow" systems the AKUFVE is invaluable in enabling the kinetics of partitioning to be completely monitored up until true equilibrium, a process which is impossible to carry out by conventional techniques.

Conversely the efficiency of mixing processes etc. could also be studied with the AKUFVE.

1.2. THEORY OF THE PARTIONING PROCESS.

Davis et al. (1974).

A resume of the relevant thermodynamic arguments as applied to solutions will be given in this section.

It must be emphasised at the outset that normal thermodynamic concepts can only be applied to systems which are in a state of reversible, dynamic equilibrium.

1.2.1. NON IDEAL SOLUTIONS AND THE CONCEPT OF ACTIVITY COEFFICIENT AND STANDARD STATE

For an ideal solution the chemical potention (μ) of every component is related to its mole fraction (X) by:-

$$\mu_i = \mu_i^o + RT\ln X_i$$

where μ_i^o is the chemical potential of the "i^{th}"component when in its standard state and which is a function of the intensive factors (temperature and pressure) only. It can be seen from the equation that this condition exists when X_i is equal to one and is thermodynamically said to be at unit activity.

Activity is the product of mole fraction and the activity coefficient:

$$a_i = \gamma_i X_i$$

This concept has arisen because in real systems although many thermodynamic properties are concentration dependent, e.g. activity is proportional to mole fraction, it is virtually always necessary to introduce a fractional quantity to the right hand side of the equation in order to attain exact equivalence i.e.:-

$a_i \propto X_i$

and

$a_i = \gamma_i X_i$

since γ_i varies with concentration as well as with other thermodynamically relevant parameters e.g. temperature, pressure, ionic strength etc.; it is termed the activity coefficient. Hence:-

$$\mu_i = \mu_i^{\circ} + RT\ln\gamma_i X_i$$

for real systems.

The activity coefficient is defined by reference to an arbitrarily chosen standard state. In the case of the solvent the standard state is for pure solvent at given temperature and pressure (usually one atmosphere). However, for the solute a variety of conventions is adopted e.g. when the solute and solvent are completely miscible the pure solute at a given temperature and pressure is chosen as the standard state. For cases of limited solubility and generally for aqueous solutions, a hypothetical state in which the solute has properties of unit molality or mole fraction behaving as if it were at infinite dilution is usually chosen i.e.

Either (i) $\gamma_i \longrightarrow 1$ as $X_i \longrightarrow 1$

or (ii) $\gamma_i \longrightarrow 1$ as $X_i \longrightarrow 0$

1.2.2. RAOULT'S LAW AND HENRY'S LAW

In convention (i) the activity coefficient becomes unity when the mole fraction is unity and for a non-ideal liquid mixture in equilibrium with the vapour phase the partial pressure of the solute in solution (p_1) will be related to the vapour pressure of the pure component (p_i°) in its standard state, the mole fraction of the solute and the activity coefficient:-

$$p_i = p_i^{\circ} a_i = p_i^{\circ} \gamma_i X_i$$

where $\gamma_i = 1$

we get

$$p_i = p_i^{\circ} X_i$$

which is the formal definition of Raoult's law.

This states that the partial pressure of each volatile component in a solution is equal to the vapour pressure of the pure component multiplied by its mole fraction in solution. Raoult's law holds in the limit as one approaches the pure substance, $X_i \longrightarrow 1$. i.e. pure solvent.

When considering the non-volatile components in solution case (ii) usually applies at infinite dilution and the system is said to obey Henry's law, i.e. the partial pressure of the component is proportional to its mole fraction:-

$$p_i = k_H X_i$$

where k_H is Henry's constant.

For ideal solutions Henry's and Raoult's laws are identical. When applying Henry's law to non-ideal solutions we get:-

$$\gamma_i^* = \frac{p_i}{kX_i}$$

where γ^* is the Henry's Law activity coefficient (c.f. γ_i for the Raoult's law activity coefficient).

At infinite dilution $X_i \longrightarrow 0$, γ_i is often referred to γ_i^{∞} and $\gamma_i^* = 1$.

$$\therefore \gamma_i^{\infty} = \frac{k_H}{p_i^{\circ}}$$

In the limit as $X_i \longrightarrow 1$, $\gamma_i \longrightarrow 1$

$$\gamma_i^* = \frac{p^{\circ}}{k_H} = \frac{1}{\gamma_i^{\infty}}$$

Thus, theoretically data obtained using one standard state should be readily convertible to the other.

1.2.3. PARTIAL MOLAR PROPERTIES AND EXCESS FUNCTIONS.

The rate of increase in the content of a particular (extensive) thermodynamic quantity (M) of a system to which that particular component is being added at constant temperature and pressure is known as a partial molar quantity:-

$$\bar{M}_i = \left(\frac{\delta \bar{M}_i}{\delta n_i} \right)_{n_2, T, P}$$

For an ideal solution the partial molar free energy is:-

$$\bar{G}_i = \mu_i - \mu_i^o = RT\ln X_i$$

For a non-ideal solution:

$$\bar{G}_1 = RT\ln a_1 = RT\ln X_1 + RT\ln \gamma_1$$

$$\bar{G}_2 = RT\ln a_2 = RT\ln X_2 + RT\ln \gamma_2$$

the subscripts 1 and 2 refer to solute and solvent respectively. The free energy of mixing is given by:-

$$\Delta G^m = RT(X_1 \ln X_1 + X_2 \ln X_2) + RT(X_1 \ln \gamma_1 + X_2 \ln \gamma_2)$$

The first part of the right hand side of this equation is equivalent to the free energy of mixing of an ideal solution so that the remainder of the equation may be thought of as the excess free energy due to non-ideal behaviour:-

$$\Delta G^E = RT(X_1 \ln \gamma_1 + X_2 \ln \gamma_2)$$

Positive deviations from Raoult's law occur when the excess free energy is positive ($\gamma_1 > 1$) and negative deviations from Raoult's law occur when it is negative ($\gamma_1 < 1$)

1.2.4. THE GIBBS-DUHEM EQUATION.

This relates the variation of activity coefficient with composition and essentially takes the form:-

$$X_1 d\mu_1 + X_2 d\mu_2 = 0$$

$$\text{or } X_1 \left(\frac{\gamma \ln_1}{\delta X_1} \right)_{T,P,} + X_2 \left(\frac{\ln \gamma_2}{\delta X_2} \right)_{T,P,} + \ldots = 0$$

For binary systems $X_1 + X_2 = 1$

$$\therefore \quad X_1 \left(\frac{\delta \ln \gamma_1}{\delta X_1} \right)_{T,P,} = X_2 \left(\frac{\delta \ln \gamma_2}{\delta X_2} \right)_{T,P,}$$

The integrated forms of the Gibbs-Duhem equation can be used with success to predict activity coefficient values in the limit of infinite dilution.

1.2.5. THE EFFECT OF TEMPERATURE ON ACTIVITY COEFFICIENT & PARTITION COEFFICIENTS.

From the general Gibbs-Helmholtz relation for a system at equilibrium and constant temperature and pressure:-

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

where ΔG° is the Gibbs free energy change

ΔH° is the enthalpy change

ΔS° is the entropy change.

T is the absolute temperature in degrees Kelvin.

also $\Delta G^\circ = - RT\ln a = \Delta H^\circ - T\Delta S^\circ$

rearrangement gives:-

$$\frac{d\ln a}{d\left(\frac{1}{T}\right)} = \frac{-\Delta H^\circ}{R}$$

or for unit mole fraction

$$\frac{d\ln \gamma_i}{d\left(\frac{1}{T}\right)} = \frac{-\Delta H^\circ}{R}$$

Solutions that give positive deviations from Raoult's law will have a positive ΔH° and γ_i will decrease with increase in temperature. For mixtures that give negative deviations ΔH° is usually negative. In both cases of non-ideality the solutions usually approach ideal behaviour as the temperature increases.

All that has been said with regard to activity coefficients is also directly applicable to the partition coefficient (K_D) for a solute distributed between two immiscible liquid phases since:-

$$\Delta G^{\circ} = - RT\ln K_D$$

$$\therefore \frac{d\ln K_D}{d(\frac{1}{T})} = - \frac{\Delta H^{\circ}}{R}$$

and a plot of $\ln K_D$ vs. $(\frac{1}{T})$ will give a slope of $-\frac{\Delta H^{\circ}}{R}$ enabling one to obtain a value for the enthalpy of distribution.

Also it can be shown that:-

$$\frac{\delta \Delta G^{\circ}}{\delta T}_p = -\Delta S^{\circ}$$

Hence a plot of ΔG° (or $- RT\ln K_D$) vs. temperature gives a slope of $- \Delta S^{\circ}$. Thus we can also obtain values for the entropy of partitioning which could indicate changes in molecular environment in passing from one phase to the other.

1.2.6. IONIC STRENGTH.

In dilute solutions, particularly of ionised species, the activity coefficients, solubilities, rates of ionic reactions and other related properties become functions of the ionic strength, which is defined by:-

$$I = \frac{1}{2} \Sigma m_i z_i^2$$

where m_i is the molality of the "i^{th}" species

z_i is the ionic charge on the ion "i".

or in terms of concentration (i.e. molarity).

$$I = \frac{1}{\rho_o} \cdot \frac{1}{2} \Sigma\, c_i z_i^2$$

where ρ_o is the density of the solution

c_i is the molarity of the "i^{th}" species.

The variation of activity coefficient with ionic strength is given by the Debye-Huckel limiting law, which for point charges in aqueous solution at 25^oC is:-

$$\log \gamma_\pm = -0.509 |z_+ z_-| I^{\frac{1}{2}}$$

where $\gamma_\pm$ is the geometric mean activity coefficient.

z_+; z_- are the valencies of cations and anions respectively.

$\gamma_\pm$ is the only experimentally measurable property in terms of activity since for:-

$$C_{\nu+} A_{\nu-} \rightleftharpoons \nu_+ C^+ + \nu_- A^-$$

$$a = a_+^{\nu+} \cdot a_-^{\nu-} = a_\pm^{\nu}$$

also $a_+ = \gamma_+ m_+$

$a_- = \gamma_- m_-$

only $\gamma_\pm$ can be determined by experiment:-

now $\gamma_\pm = \gamma_+^{\nu+} \gamma_-^{\nu-}$

$$a = m_+^{\nu+} m_-^{\nu-} \gamma_+^{\nu+} \gamma_-^{\nu-}$$

$$a_\pm = a^{1/\nu} = (m_+^{\nu+} m_-^{\nu-} \gamma_+^{\nu+} \gamma_-^{\nu-})^{1/\nu}$$

$$\gamma_\pm = \frac{a_\pm}{(m_+^{\nu+} m_-^{\nu-})^{1/\nu}}$$

now $m_\pm = \nu_+ m$; $m_- = \nu_- m$

Then $$\gamma_\pm = \frac{a_\pm}{m(\nu_+^{\nu+} \nu_-^{\nu-})^{1/\nu}} = \frac{a_\pm}{m_\pm}$$

$a_{\pm}$ can be determined for dilute solutions by the usual physical methods and applied in typical partitioning processes

For ions of finite size, a more rigorous relationship is given by:-

$$\log \gamma_{\pm} = \frac{-0.509|z_+ z_-| I^{\frac{1}{2}}}{1 + \beta d\, I^{\frac{1}{2}}}$$

d - average effective diameter of the ions

$$\beta = \left(\frac{8\pi L^2 e^2 \rho_o}{1{,}000\ ERT} \right)^{\frac{1}{2}}$$

where L is the Avagadro constant

e is the electronic charge
E is the dielectric constant

βd usually tends to unity.

1.2.7. THERMODYNAMICS OF THE PARTITIONING PROCESS.

Assuming completely immiscible solvent phases, with minimal and uniform solute-solvent interactions we can apply the usual ideal thermodynamic relationships to a bi-phasic system at equilibrium, viz:-

By the Gibbs equation.

$$\mu_i (P,T,X) = \mu_i + RT \ln a$$

where μ_i is the chemical potential of the "i^{th}" component.

By Raoult's law

$$\mu_i^o = -RT \ln a$$

$$= -RT \ln \gamma_{\pm} X_i$$

$$= -RT \ln \gamma_{\pm}$$

since the pure solute is taken as standard state and therefore X_i (mole fraction is one.

$\gamma_{\pm}$ is the mean activity coefficient and is given by the relationship:-

$$\gamma_{\pm} = \frac{P_i}{P_i^{o} X_i}$$

$$\left(\text{since } a_i = \frac{f_i}{f_i^{o}} = \frac{P_i}{P_i^{o}} \right)$$

where P_i is the partial pressure of solute in solution at mole fraction X_i and P_i^{o} is the vapour pressure of the pure solute.

It has been observed by Davis, Higuchi and Rytting (1972) that for infinitely dilute drug systems it is preferable to utilise Henry's limiting law, rather than than of Raoult, hence:-

$$P_i \propto a_i = ka_i = k_H \gamma_{\pm}^{*} x_i$$

$$\text{or } k_H = \frac{P_i}{\gamma_{\pm}^{*} X_i}$$

where k_H is the Henry's law constant

At infinite dilution $x_i \longrightarrow 0$, and the activity coefficient tends to unity ($\gamma_{\pm}^{*} \longrightarrow 1$) which contrasts with Raoult's law in that

$$\gamma_{\pm} \longrightarrow 1 \text{ as } x_i \longrightarrow 1$$

$$\therefore k_H = \gamma_{\pm}^{\infty} P_i^{o}$$

The Nernst partition coefficient K_D^{x} is defined as:-

$$K_D^{x} = \frac{X_o}{X_w}$$

where X_o is the mole fraction of solute in the organic phase

X_w is the mole fraction of solute in the aqueous phase

The Gibbs free energy of transfer of one mole of solute from one phase to the other is:-

$$-\Delta G = RT \ln K_D$$

thus in the Henry's law limiting case:-

$$K_D = \frac{\gamma^{\infty}_w}{\gamma^{\infty}_o} = \frac{k^w_H}{k^o_H}$$

where γ^{∞}_w and γ^{∞}_o are Raoults law activity coefficients.

and k^w_H and k^o_H are the Henry's law constants for the two phases.

Since in practice it is normally the molar partition coefficient which is determined by experiment, it is necessary to convert such results to the Nernst thermodynamic equivalents i.e.:-

molar partition coefficient $K^m_D = \frac{C_o}{C_w}$

where C_o is the molar concentration of solute in the organic phase.

C_w is the molar concentration of solute in the aqueous phase.

'thermodynamic' partition coefficient

$$K^x_D = \frac{X_o}{X_w}$$

To convert one to the other we use the relationship:-

$$\frac{K^X_D}{K^m_D} = \frac{V_o}{V_w}$$

Where V_o is the molar volume of the organic phase

V_w is the molar volume of the aqueous phase.

It can be seen from the above that the value of partition coefficient quoted will be dependent upon the concentration scale used. Hence it is desirable to adopt a standard state concentration to facilitate general application of the recorded literature values. To this end Davis (1973) has proposed that the thermodynamic partition coefficient should always be quoted, particularly as in many instances this bears a direct linear relationship to the additive π values used in Hansch analyses.

One effect of using an organic phase with a relatively high dielectric constant is to reduce the electrostatic interaction between solute ions and therefore to encourage ion-pair formation (dimerisation) which is an example of association. This process becomes more prevalent as the solute concentration in the system is increased.

A related property to association is that of ionisation, which is another concentration dependent parameter, as is the limiting case of phase saturation. It is possible to eliminate the latter complication by careful experimental design and to rationalise association and ionisation by the following procedure:-

Symbols:-

C_1 = concentration of total solute in aqueous phase in mole/l

C_2 = concentration of total solute in organic phase in moles/l (monomer molarity).

X = concentration of ions in aqueous phase.

$N = C_1 - X_1$ = concentration of unionised molecules in water at the first concentration level.

$n = C^1 - X_1^1$ = concentration of un-ionised molecules in water at the second concentration level.

P = $\frac{\text{concentration of single molecules in organic phase.}}{\text{concentration of single molecules in aqueous phase.}}$

K'_D = dissociation constant of double into single molecules in the organic phase.

K_A = dissociation constant of single molecules into ions in the aqueous phase.

$$K_A = \frac{X^2}{(C_1 - X)}$$

$$\underset{(C_1-X)}{HA} \rightleftharpoons \underset{(X)}{H^+} + \underset{(X)}{A^-}$$

and

$$x = \frac{-K_A \pm \sqrt{K_A^2 + 4K_A C_1}}{2}$$

For organic phase equilibrium, assuming dimerisation:-

$$(HA)_2 \rightleftharpoons 2HA$$

$$K'_D = \frac{2\left[P\,(C_1 - X_1)\right]^2}{C_2 - P\,(C_1 - X_1)}$$

$$= \frac{2\,(PN)^2}{C_2 - PN}$$

$$= \frac{2\,(Pn)^2}{C_2^1 - Pn}$$

$$P = \frac{C_2 n^2 - C_2^1 N^2}{(n-N)\,nN}$$

From the above it can be shown that

$$\frac{C_2}{N^2} = P\left(\frac{1}{N}\right) + \text{constant}$$

Hence a plot of $\left(\frac{C_2}{N^2}\right)$ vs $\left(\frac{1}{N}\right)$ will produce

a slope of P and this straight line plot can be used to eliminate any spurious results, as well as checking the range of the rectilinear relationship.

Furthermore it has been claimed that from the relationship

$$\frac{C_2}{N} = P + \frac{2P^2}{K'_D}.N \qquad \text{(Davies. et al 1951).}$$

a plot of N vs. (1/N) yields the value of P, from the intercept, at zero concentration where dimerization can be ignored. This is theoretically incorrect since a rectangular hyperbola of intercept infinity will result.

Thus in a system where the measured partition coefficient varies with concentration,, then the above procedures have been applied in order to determine the degree of association, or ionisation, involved. When association constants of acids are studied as a function of temperature, by this method, then values of ΔH, ΔS and ΔG for the association reaction can be obtained, except in cases where hydrate formation is involved.

This produces another variation in the measured partition coefficient since aqueous phase is transferred along with the solute into the organic phase, thereby altering the physical properties of the non-aqueous phase as well as the volume of the aqueous phase.

Acids and Bases

If the solute is either an acid or a base then only the undissociated (non-ionic) form is extracted into the non-polar organic phase. Thus the true partition coefficient differs from the measured apparent partition coefficient according to the Nernst equation viz:-

$$pH = pK_a + \log \left| \frac{\text{dissociated}}{\text{undissociated}} \right|$$

It can be shown from this equation that for bases:-

$$\frac{P}{P^1} = 1 + \text{antilog} \ (pK_a - pH)$$

and for acids

$$\frac{P}{P^1} = 1 + \text{antilog} \ (pH - pK_a)$$

where P is the true partition coefficient.

P^1 is the measured (apparent) partition coefficient.

CHAPTER 2: REVIEW OF METHODS FOR DETERMINING PARTITION COEFFICIENTS.

2.1. METHODOLOGY

The normal method of determining partition data is the simple shake flask technique. Although inexpensive this method is extremely tedious when one wishes to vary the experimental conditions and has a tendency to lead to errors, as well as limiting the scope and speed of measurement.

For our studies we required a method which could produce, rapidly, large quantities of precise and accurate data, under a wide range of experimental conditions. The requirement was to study the variation of partition coefficient with respect to the following parameters:-

A. Solute.

(i) Concentration

(ii) Ionization.

(iii) Complexation.

(iv) Molecular structure.

B. Solvent.

(i) Chemical nature

(ii) Density

(iii) Mixtures

(iv) Phase ratio

C. Experimental.

(i) pH

(ii) Ionic strength

(iii) Temperature

A survey of the various methods currently used in the acquisition of partition coefficient data was then made with a view to finding a method which would meet all our requirements. The results of this survey are presented in the next section.

2.1.1. METHODS FOR THE RAPID DETERMINATION OF PARTITION COEFFICIENTS

The determination of the partition coefficient of a solute distributed between two immiscible solvents involves a process of mixing of the two solvent phases until equilibrium is attained, followed by a settling or separation period, prior to assay of one or both liquid phases.

The classical experimental technique of shaking flasks in a thermostatted bath is both slow and tedious and involves a relatively crude method of sampling and monitoring of system changes. The inherent limitations of such an arrangement prevent full attainment of the degree of speed and flexibility desirable for a complete thermodynamic study of any two phase system.

The purpose of this project was to establish a suitable automated method which ideally would meet the following requirements:-

1) Rapid equilibration between the two solvent phases.

2) Absolute and rapid separation of the mixed phases.

3) Accurate and precise temperature control throughout the complete cycle.

4) On-line assay of the separated phases, e.g. spectroscopic, radiochemical, or other techniques.

5) The facility to easily vary and monitor the thermodynamic environment in terms of ionic strength, pH, solvent polarity (mixed solvents) etc.

6) Allow the study of extreme partition coefficients by enabling large solvent volume ratios to be adopted if required.

7) Ultimately to combine several, or even all of these operations into a programmed cycle under the control of some master functions e.g. a computer.

A review of some of the many "continuous"methods utilised by various workers was carried out, details of which are given below. The final method is the one that has been adopted for studies in the pharmaceutical field.

2.1.2. (1) A PHASE-SEPARATING CENTRIFUGE FOR AUTOMATED RADIOANALYSIS

Sutton and Vallis (1969)

In this apparatus the mechanism of liquid-liquid phase separation depends on either density or surface tension differences in the two solvents.

Fig. 1 shows the basic form of the apparatus for separating liquids by virtue of their differing densities. Figs. 2 & 3 describe its operation.

The multichannel spray collector can be moved vertically to collect different phases from the centrifuge. This obviates the need for elaborate multiway stopcocks.

The tube guide directs plastic tubes, used to deliver samples and reagents, into the central rotating centrifuge vessel. This centrifuge is always operated at a sufficient speed to eject liquid horizontally across into the adjacent receptor unit of the spray collector.

The apparatus was designed primarily to enable the aqueous phase to be continuously extracted by the organic phase for purification procedures in radioanalysis which involve precipitation or solvent extraction.

An interesting variant on this design is to replace the solid P.T.F.E. barrier (B) with a porous barrier, which can be of either a hydrophilic or hydrophobic type of material e.g. sintered glass (hydrophilic); P.T.F.E. (hydrophobic).

If the barrier is hydrophilic, then at low rotational speeds (600 - 900 r.p.m.) the aqueous phase is ejected, the organic phase remaining in the centrifuge. This residual organic phase can be subsequently ejected by increasing the centrifuge speed (2,500 - 4000 r.p.m.).

Conversely with a hydrophobic barrier the organic phase is ejected at low rotational speeds, the residual aqueous phases being removed at the higher speed. Normally mixing of the two immiscible phases is carried out in the stationary centrifuge vessel prior to separation by centrifugation.

Sutton and Vallis' apparatus

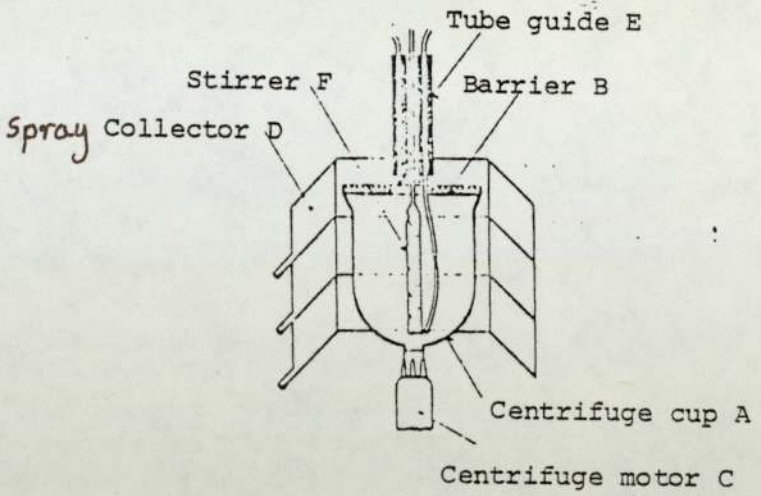


Fig. 1. Basic parts of the apparatus.

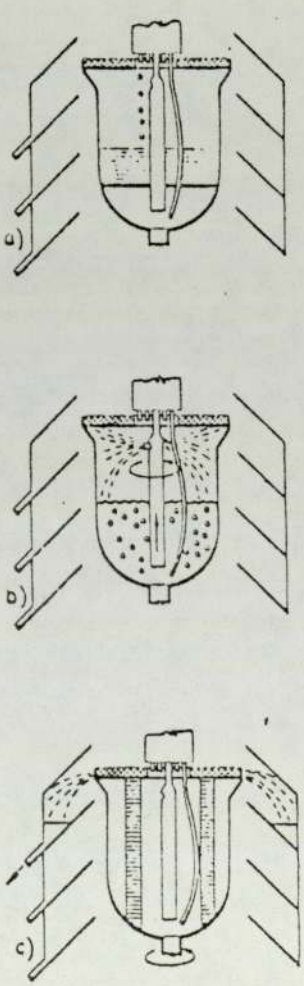


Fig.2. Solvent extraction with porous glass barrier. a) Aqueous and organic phases are introduced. - b) Aqueous and organic phases are mixed. - c) Aqueous and organic phases are separated. Aqueous phase ejecting.

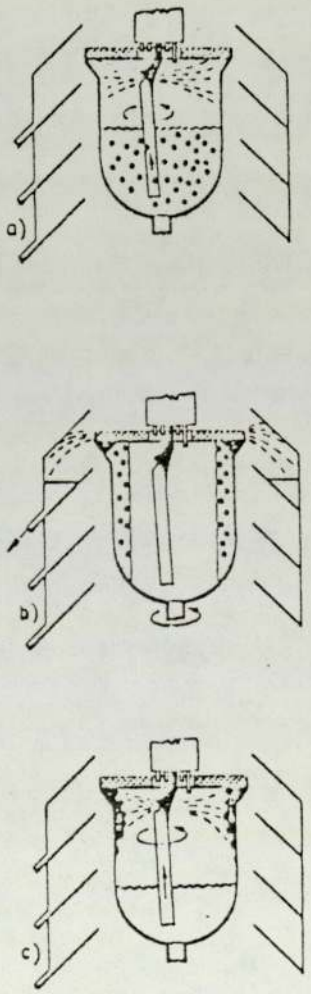


Fig. 3. Precipitation with a porous barrier. a) Reagents are added, solution is stirred, precipitate forms. - b) Suspension is being centrifuged, supernatant ejects into collector, precipitate remains behind porous barrier. - c) Precipitate is redissolved.

This technique precludes the use of this apparatus for our type of thermodynamic study, although if mixing of the two phases occurred in a separate vessel then by continually withdrawing and replenishing of the residual phase via the sampling tubes and recycling of this together with the ejected phase through the mixer a state of equilibrium could be obtained. The efficiency of the centrifuge would be the determining factor in such an apparatus and this aspect of the above design has not been fully investigated.

The equipment is commercially available in a programmable form.

2.1.3. A SIMPLE APPARATUS FOR ON SITE CONTINUOUS LIQUID-LIQUID EXTRACTION OF ORGANIC COMPOUNDS FROM NATURAL WATERS.

Ahnoff and Josefsson (1974).

This equipment was designed to continuously extract large volumes of aqueous phase, with relatively small volumes of organic solvent prior to assay. It is used in the analysis of large effluent volumes e.g. river water.

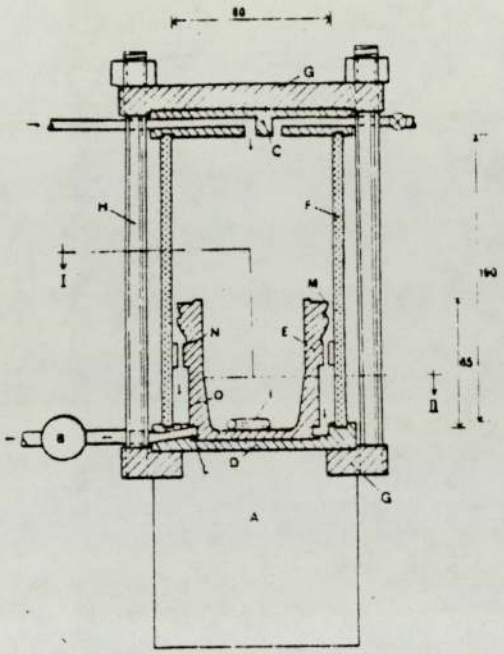
The mode of operation is outlined in fig. 4. With a pump rate of five litres per hour, extraction efficiencies of 75 - 85% are attained. This relatively low flow rate and related extraction efficiency, combined with the tendency towards emulsification in the separation phase makes the apparatus a doubtful proposition for our purpose.

2.1.4. CHROMATOGRAPHY

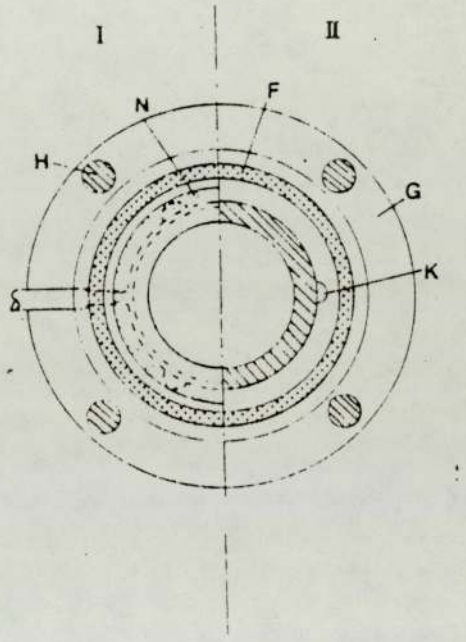
It has already been shown above that a relationship can be established between chromatographically determined parameters and partition coefficients. It is therefore possible to use the standard techniques of gas-liquid chromatography; thin-layer chromatography; liquid-liquid chromatography etc. in the determination of partition coefficients.

A recent elegant and highly versatile method which is finding increasing acceptance is that of high pressure liquid chromatography (H.P.L.C.).

Fig. 4. Ahnoff and Josefsson's apparatus for on-site continuous liquid-liquid extraction.



Schematic diagram of the mixed settler.



Cross section at I and II from the apparatus in the adjacent diagram.

2.1.5. HIGH PRESSURE LIQUID CHROMATOGRAPHY.

In this technique the organic layer forms a stationary phase on an inert support medium, whilst the aqueous phase is forced under high pressure down a column packed with this organic-support compound. The eluent is assayed by standard techniques such as ultra-violet/visible spectroscopy etc.

It can be seen that the process is analogous to that used in gas-liquid chromatography, the solute being progressively partitioned as it travels down the chromatography column.

Theoretically it has been deduced that a relationship exists between partition coefficient (K_D) and the R_f values obtained in liquid-liquid partition chromatography viz:-

$$t_{Ri} = t_{Ro} + t_{Ro} K_i$$

where t_{Ri} = retention time from injection to peak maximum.

t_{Ro} = average residence time of the mobile phase.

$q = \frac{V_s}{V_m}$ = volume ratio of the stationary and mobile phase.

K_i = liquid-liquid partition coefficient.

It can be shown that

$$qK_i = k^i = \left(\frac{1}{R_f} - 1\right)$$

where k^i is an expression for the retention of a compound in H.P.L.C.

Now

$$R_M = \log \left(\frac{1}{R_f} - 1\right),$$

and

$$\log K_D = \log k + R_M$$

$$= \log k + \log k^1$$

k is a constant for the system.

Thus the chromatographic retention time of a compound is characterised by its partition coefficient.

Attempts have been made to utilise this technique by Taylor, et al. (1975), in obtaining data relevant to a Hansch type analysis of structure activity relationships i.e. the partition coefficient is related to the chromatographic retention parameter as in the equation:-

$$\log K_D = \log k + R_M$$

It follows that $\log K_D$ is linearly related to R_M and hence ΔR_M is therefore related to π

In these experiments silanised Kieselguhr supports, impregnated with octanol as stationary phase were used. The solutes were then injected in octanol-saturated water at a concentration appropriate for u.v. detection; the same medium was used as eluent, pyridine N-oxide being included as an internal standard.

A high degree of reproducibility was achieved over a range of $\log K_D$ values of -0.5 to +2.5 and it is hoped to extend this to -1 to +3 by more recent refinements in the technique. Correlation with literature values was within ± 0.04 units of $\log K_D$.

Two limitations of the technique which were stressed are:-

(1) compounds of very low solubility gave anomalously low $\log K_D$ values, probably because the partitioning process has not achieved equilibration before complete elution occurs.

(2) it was found that in systems containing weakly dissociated electrolytes, both ionic and neutral species were extracted into the organic phase, thus precluding the use of the standard ion correction techniques.

In another typical study, (McCall, J.M. 1975) a stationary phase support such as CORASIL C18, which is hydrolytically stable, is impregnated with octadecyl chains, chemically bonded to a pellicular silica gel. Although such supports have a low percentage of active silanol sites these can interfere with the desired liquid-liquid partitioning.

These sites are blocked by the usual methods to produce an optimised column condition, i.e. until a good correlation between k^1 and K_D is obtained.

High pressure liquid chromatography experiments were then performed on a family of hypotensive triamino pyrimidine 3-oxides, using either 1% triethylamine (TEA) in water, or 15% CH_3CN in water as the mobile phase.

The difference between log K_D and log k for each compound with each eluent was determined and the term k_N defined by the relationship

$$k_N = \frac{(\log k)_{compound}}{(\log k)_{benzene}}$$

Ideally k_N should tend to unity, a condition which was achieved in these experiments only when a vigorously silylated octadecylsilane support was employed. This was predicted to be due to steric effects since the greatest deviations from unity occurred with solutes containing small basic (amino) groups. In such cases 1% TEA in water produced the superior correlation, possibly due to its affinity for any free "silanol" sites.

The technique was also found to be suitable for phenolic compounds and it is envisaged that similar experiments will be performed on carboxylic acids.

Finally it was established that compounds with widely variant lipophilicities could be measured by altering the components of the mobile phase. This was one area where it was accepted that classical techniques still had the advantage in that the ratio of the volumes of the two phases could be varied to accommodate divergent lipophilicities and thereby maintaining a constant solvent type.

Throughout these experiments it was found necessary to correlate particular eluent types with a given family of substrates which although capable of a high degree of precision could be viewed as an added complication.

The advantages claimed for this technique are:-

i) reproducible and fast

ii) because of i), compounds which are unstable in solution can be assayed.

iii) solvent lipophilicity can be adjusted and compounds whose partition coefficients vary by several orders of magnitude can be quickly measured.

iv) samples need not be pure since contaminants do not interfere with 'k' determinations.

v) because both refractive index and u.v./visible absorbance detectors are available, many compounds can be detected.

However, for our intended course of study we need to vary such parameters as pH; ionic strength; temperature etc., which would transcend the experimental versatility of existing HPLC devices. Also because of the need to use exotic solvents and derived functional groups on the columns another stage in the correlation between data and final calculated results is introduced. Additionally the system cannot be regarded to operate under strict thermodynamic equilibrium conditions, since the solute is partitioned at an ever decreasing concentration between its own phase and "pure" extracting phase, a phenomenon which is unpredictable in its effect over the range of thermodynamic environments to be studied.

In a recent review by Tomlinson (1975) it was observed that progress in the use of quantitative structure activity relationships (QSAR) has shown the importance of the hydrophobic or lipophilic nature of drugs. The hydrophobicity of a drug is usually characterised by the partition coefficient (K_D), obtained from distribution studies of the drug between an immiscible polar and "non-polar" solvent pair. The work of Martin and Synge (1941) and of Consden, et. al. (1944) in establishing relationships between the R_f values obtained from partition chromatography and the partition coefficient has led to the limited use of hydrophobic parameters obtained from chromatographic measurements in QSAR models.

Data collected by Green, et.al. (1963) of R_M values for n-alkyl dinitrobenzoates measured in a paper reversed-phase system of liquid paraffin - 50% aqueous ethanol, illustrated that log K_D values can be substituted by these R_M measurements to give a good correlation with the derived theoretical relationship:-

$$\log K_D = \frac{n\Delta\mu^\circ_H}{2.303RT} + \frac{\Delta\mu^\circ_L}{2.303RT} + \log \frac{\bar{V}^\circ_{(o)}}{\bar{V}^\circ_{(w)}}$$

where μ°_H and μ°_L are the chemical potentials of the hydrophi lic and lipophilic groups respectively.

R is the universal gas constant.

T is the absolute temperature ($^\circ K$)

$\bar{V}^\circ_{(o)}$ and $\bar{V}^\circ_{(w)}$ are the partial molar volumes for the organic phase and aqueous phase respectively.

n is the number of carbon atoms in the alkyl group.

From the slope of the graph 'R_M' vs. 'n' a value for the standard free energy of transfer per methylene group was obtained.

It has already been shown that for a pair of homologues distributed between identical solvent phases that:-

$$\pi_X = \log K_{D(RX)} - \log K_{D(RH)}$$

Clifford, et al. (1969) in correlating fungicidal activity with chemical constitution of some alkyl-dinitrophenols found that the analogy with R_M values also applied viz:-

$$\pi_X = R_{M(HX)} - R_{M(HD)} \propto \Delta R_{M(X)}$$

In fact they ignored the ΔR_M term and expressed π directly as:-

$$\pi \propto \log \frac{\left(\frac{1}{R_{f(RX)}} - 1\right)}{\left(\frac{1}{R_{f(RH)}} - 1\right)}$$

Remember that

$$R_M = \log \left(\frac{1}{R_f} - 1\right)$$

Further, for acids or bases, R_M can be related to R_f by the following expression, providing the degree of association in the organic phase can be ignored:-

$$R_M = \log \left(\frac{1}{R_f} - 1\right) + \log \frac{K_a + |H^+|}{|H^+|}$$

where K_a is the dissociation constant of the solute.

$|H^+|$ is the hydrogen ion concentration of the mobile phase

A study of the literature will show that R_M values are determined either in non-reversed (straight) or in reversed-phase systems and also by paper and thin layer methods.

Partition coefficients may be regarded as equilibrium constants and as such there should be extrathermodynamic relationships between partition coefficients measured in different solvent systems. It has been claimed that although R_M values are not obtained from true equilibrium parameters, they can be regarded as being derived from steady state functions and as such may be expected to show these same extrathermodynamic relationships.

Collander (1951) proposed that rectilinear relationships exist between partition coefficients found in one system (K_{D1}) and those found in a second (K_{D2}), providing the polar phase is water and the non-aqueous phase contain the same functional group:-

$$\log K_{D2} = a \log K_{D1} + b$$

'a' and 'b' are constants.

Leo and Hansch (1971) and Leo et. al. (1971) have extended the Collander expression to many partitioning solvent systems although they found that it fails when comparisons are attempted between hydrocarbons (e.g. cyclohexane) and hydrogen bonding solvents such as alkanols, esters, etc. It is then necessary in deriving theoretical relationships to generate two regression equations, one relating to "acidic" solutes and the other to "basic" solutes depending on their hydrogen acceptor or donor abilities. Similar arguments should hold for R_M determinations.

A small number of workers have shown this to be true in various systems eg. Lien, et al. (1971) using Bakerflex sheets precoated with silica gel IB and two solvent systems viz., dioxan and butanol-acetic acid/water, were able to give derived regression equation for the relationships between R_M values of some thiolactams measured in the two systems.

Dearden and Tomlinson (1972) in a study relating ΔR_M values to the biological activity of some para-substituted acetanilides showed an excellent correlation for the series between π (octanol-water) and the ΔR_M values obtained against two stationary phases.

A silica gel thin-layer reversed phase system impregnated using one of either two non-aqueous solvents, liquid paraffin or octanol was used. The mobile phase was acetone-water (20% v/v acetone for liquid paraffin, 10% v/v acetone for octanol.)

To summarise, the advantages claimed for chromatographic methods over direct partition methods for obtaining an index of hydrophobicity are:-

(1) simple to use, rapid and less tedious e.g. up to twenty-five different solutes can be developed simultaneously on a thin layer plate, so enabling a direct comparison of R_M values to be made.

(2) little material needs to be used. This may be extremely important when considering hydrophobicity of molecules of biological origin.

(3) chromatographic methods are able to accommodate drug molecules of very high or low K_D values.

(4) the material to be examined need not be ultra-pure, for impurities are normally separated during development.

(5) there is no need for a quantitative analysis of the solute.

(6) more reproducible results are usually found over those derived from direct partition coefficient techniques.

(7) reversed phase paper or thin-layer chromatography in a range of solvent mixtures can give R_M values for any of these mixtures provided that the linear relationship between solvent composition is established so enabling R_M values in a chosen standard system to be derived.

On the debit side, however, are the possibilities of the "streaking" of spots, especially in reversed phase systems, due to overloading of solute in an attempt to obtain visualization. This situation can lead to subjective errors when measuring the R_F values.

Also in reverse phase systems again, an even distribution of the non-aqueous phase upon impregnation of the support is not known for certain. This could affect the R_M value, though replication should overcome this.

2.1.6. MICROELECTROMETRIC TITRATION.

This technique has been used to obtain the pK_a's and partition coefficients of narcotics, as well as their pH and temperature dependence. (Kaufman, 1975).

In this method the substrate is titrated isothermally, under an inert atmosphere (Nitrogen), using pH change to monitor the course of the reaction. The experiment is carried out on a microscale using microcells, microburettes and concentrated solutions of titrant to avoid large volume changes.

pKa determination

First a curve of volume of titrant added is plotted against corresponding pH values, then a blank titration curve is similarly produced but in the absence of a sample. The difference of these curves represents the proton affinity of the substrate as a function of pH. The mid-point(s) of the curve inflection(s) represent the pK_a value(s) of the dissociation group(s). The theoretical curve is given by:-

$$pH = pK_a + \log \frac{(\alpha)}{(1 - \alpha)}$$

where α is fraction of sample in dissociated state for acids.

or fraction of sample in associated state for bases.

This equation is useful when the attainment of a full titration curve is precluded by practical difficulties, e.g. precipitation.

Determination of oil-water partition of a drug.

A solute distributed in a biphasic system undergoes dissociation in the aqueous phase according to its dissociation constant (K_a) which can be determined as above. The presence of the oil layer produces a shift in the apparent pK_a of the compound by an amount dependent on the oil volume as well as that of the aqueous phase. This apparent shift is related to the distribution coefficient (K_D) by the relation:-

$$K_D = V_w \frac{(\text{antilog } \Delta pK_a^1 - 1)}{V_o}$$

where V_w is the volume of the aqueous phase.

V_o is the volume of the organic phase.

ΔpK_a^1 is the shift of the pK_a^1 by the addition of the oil.

This method gives the distribution of the undissociated form of the compound between oil and water (true partition coefficient). The apparent partition coefficient is obtained by multiplying K_D by the fraction of drug in the undissociated form in the aqueous phase at that pH value.

The chief advantages of this method are the relatively simple technique and small quantities of materials required to give a direct determination of the true partition coefficient at different temperatures.

However, there has to be a measurable shift in pK_a when partitioned and the predominance of precipitation in many systems necessitates the adoption of a somewhat questionable technique with regard to the composition of the aqueous phase.

2.1.7. APPARATUS FOR THE RAPID DETERMINATION OF PARTITION COEFFICIENT DATA USING A CONTINUOUS SOLVENT EXTRACTION SYSTEM (AKUFVE).

The metals of interest to nuclear chemists and physicists occur in such low concentrations in their natural state that it is essential to utilise some form of intensive extraction in separating them from their native ores.

Solvent extraction soon established itself as the most flexible and economic method and it is now standard procedure to optimise the selectivity and efficiency of this technique when applied to a given metal extraction, or separation procedure.

The extraction of the transuranic elements poses particularly acute problems to the nuclear chemist and it is in this area that the most sophisticated techniques of study have been developed. An elegant instrumental technique for obtaining accurate partition data was developed by Rheinhardt and Rydberg (1969) and Rydberg (1969).

This method was evolved after an exhaustive evaluation of commercially available equipment (Rydberg, 1969) failed to produce a centrifuge capable of achieving this very rapid, and virtually absolute, phase separation essential for accurate, on-line, assay of the two phases during the partitioning process.

Early trials on the AKUFVE which is a Swedish abbreviation for "apparatus for continuous measurement of distribution factors in solvent extraction", at Leeds University, demonstrated that this equipment could potentially fulfill all our requirements with regard to a thermodynamic analysis of the partitioning process of drug type systems. This would be a novel technique of study within the pharmaceutical field, although as indicated above it has been developed and established in metal extraction studies.

The principle of the AKUFVE is illustrated in Fig. 5. By addition of suitable reagents the chemical compositions of the two liquid phases are adjusted in the mixing chamber. The bi-phasic mixture then flows down into the continuous flow centrifuge, in which "absolute" phase separation takes place i.e. there is no entrainment or droplet formation of one phase in the other when leaving the exit ports of the centrifuge. The two pure phases can then be passed through on-line measuring devices for the purpose of assay e.g. spectroscopic; radiometric etc. The liquids are then usually allowed to flow back into the mixing vessel, thus completing the cycle, or more rarely they are collected into separate vessels for a single pass system.

As can be seen the system is fairly straight-forward owing to the outstanding capabilities of the centrifuge in terms of the degree of phase separation achieved in a single pass. The centrifuge is a special "H-type" developed by Reinhardt and Rydberg (1969) after rigorous assessment of three commercially available centrifuges i.e.:-

(i) the Luwesta type LG205; bowl volume 0.66 litre

(ii) the de Laval type 1225; bowl volume 0.45 litre

(iii) the Sharples; bowl volume 0.016 litre

Tests on the ability of these centrifuges to achieve absolute phase separation of benzene-water mixtures were carried out in a closed cycle arrangement, the efficiency of the process being checked by the following two, independent methods:-

A) by use of absorption spectroscopy the presence of entrained impurities (air, or droplets of the other phase) can be detected. The organic phase (benzene) was passed through a cell placed in a spectrophotometer, with a reference cell containing benzene saturated with water. Both optical cells were maintained at a slightly higher temperature than that of the AKUFVE separator in order to counteract any temperature dependent solubility effects on the system.

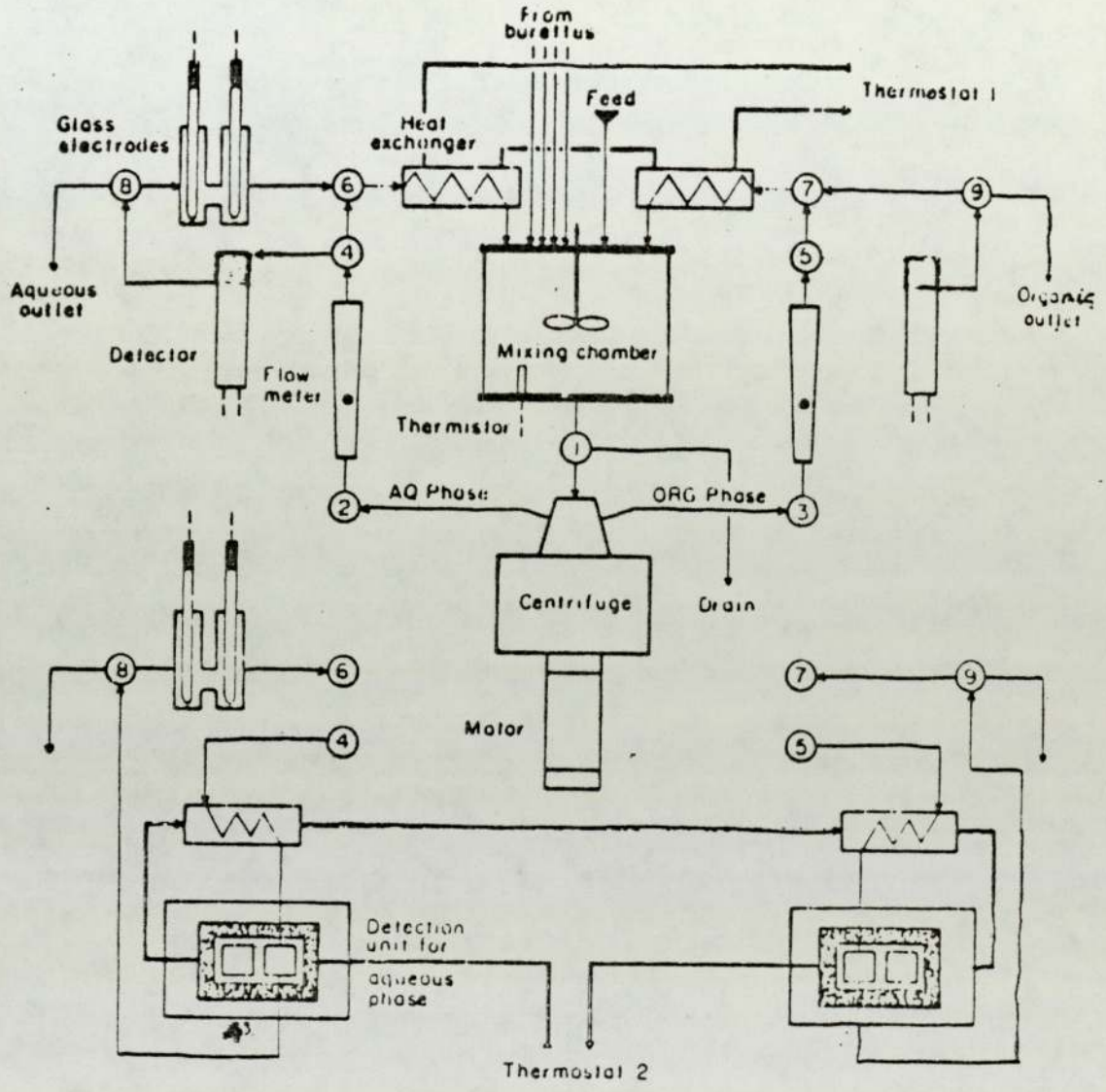


FIG.5. Diagram of the AKUFVE liquid-flow system for two different means of on-line measurements. The upper part indicates the connections for radiometric detection, which is most commonly used, while the lower part shows part of the corresponding arrangement for spectrophotometric detection.

This method is sensitive down to less than 0.01% which is an order of magnitude better than that detectable by the naked eye, (a just detectable haze occurs at approximately 0.1%).

(B) After a long circulation time, the concentration of each phase in the other was determined and the result checked against literature solubility data. Any excess values were assumed to be due to solvent entrainment.

Two starting conditions were employed in all the tests viz.

(i) The centrifuge was first filled with heavy phase prior to introduction of the mixed phases.

(ii) The centrifuge was allowed to attain maximum speed before feeding the solvent mixture into it.

Many hundreds of runs were made on these centrifuges, such as varying centrifuge condition (counter pressures, weir sizes and speed of rotation), physical conditions of the liquids (flow rate, aqueous-organic volume ratio, density differences, temperature) and chemical conditions (water with and without acid or salt, different organic solvents with and without dissolved reagents). All centrifuges could rather easily give one very pure phase which is satisfactory for most industrial purposes. Discharge over rotating weirs, used on the de Laval and Sharples type centrifuges, often gave air or gas bubbles in one or both of the outgoing phases and produced foam with some chemical system.

Several mechanical improvements to the centrifuges were introduced in order to obtain absolute phase separation and steady conditions, but none were entirely successful.

THE H-CENTRIFUGE

This was designed to overcome the deficiencies of the commercially available units. The mode of operation is illustrated in Fig. 6.

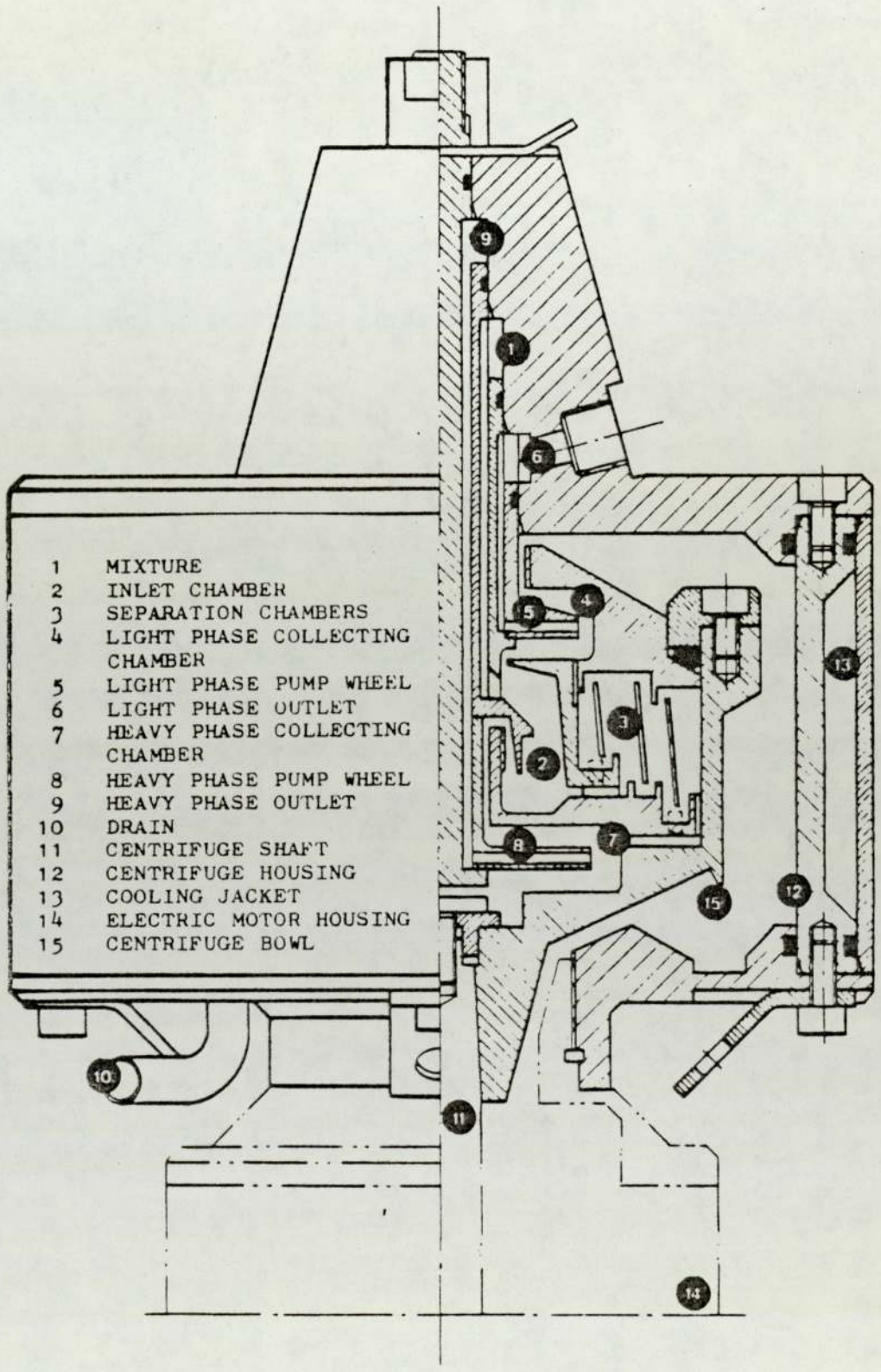


Fig. 6. H-Centrifuge Separator

The solvent mixture is fed to the centre of the centrifuge bowl where it is accelerated through the inlet chember (1) into the separation volume (2) This consists of eight sector shaped chambers totally isolated from one another. The liquid is subjected to a zig-zag motion by the presence of peripheral partition walls which are interspersed with baffle ridges. The lighter phase is diverted to an upper collecting chamber (3), the heavier phase going to the lower chamber (4). From these chambers the separated phases are taken out axially upwards with pump wheels of different types.

Heat exchanger coils are installed in the mixing chamber to maintain the system at a constant temperature, primarily to prevent heating due to frictional processes in the system, leading to droplet formation as a result of cooling within the external detection system.

The H-centrifuge is driven by a pneumatic motor of 0.6 H.P., or an 800W variable speed induction motor, which provides a maximum of 18,000 r.p.m. The bowl volume is 100 ml. and has a maximum flow capacity of 200 l/h. Materials in contact with the solutions are pure titanium and teflon (P.T.F.E.).

The particular merits of the AKUFVE system were found to be:-

(a) mixing and phase separation are instantaneous.

(b) the length of mixing and the time from mixing to separation are variable from zero upwards.

(c) absolute phase separation takes place.

(d) there is no time lag between separation and detection.

(e) a large variety of detection systems may be used, radiometric, spectrophotometric, refractive index, pH etc.

(f) all liquid volumes are small in order to permit work with dangerous or expensive substances.

(g) the temperature is kept constant at a selected value in the range 0 - 100°C.

These factors lead to extensive applications for the AKUFVE method in solvent extraction studies. Firstly data can be collected at least ten times as fast as with the shake flask technique and ultimately will use less materials if full use is made of the properties of the machine.

Secondly any changes are continuously and immediately monitored which can be used in conjunction with some form of feedback mechanism to precisely control the process under study. Also resulting from this is the ability to investigate the kinetics which lead to the overall equilibrium conditions, or the effect of any perturbations on such a system. This knowledge could lead to the development of more efficient separation processes, as well as giving an insight into non-equilibrating solvent extraction processes of interest to pharmacokineticists. The fast "once through" AKUFVE technique could also be used to study the solvent extraction of short lived species.

Although partition coefficients (K_D) can be measured at various constant temperatures both with the AKUFVE and classical methods, only the AKUFVE permits the continuous measurement of K_D as a function of temperature.

Certain limitations have been experienced with the AKUFVE, some of which can be overcome to a greater or lesser extent in the manner indicated. In some solvent-solute systems emulsions are easily produced e.g. surfactant solutes, and the AKUFVE is incapable of breaking these emulsions. However by careful control of flow rates and centrifuge speeds the initial formation of such emulsions can usually be avoided.

When using volatile solvents for the organic phase, excessive losses due to evaporation were experienced in the earlier experiments. These losses were considerably reduced by sealing off the system, using moderate centrifuge speeds and reducing experimental times to an absolute minimum. Other workers (Flett 1975) have reduced these solvent losses by

bubbling gas saturated with the solvent through the mixing chamber.

Owing to the relatively large volumes of solvent to be used and the time required for cleaning and assembly it was found to be impractical to use the AKUFVE for "one-off" determinations.

Memory effects have been reported when using on-line detection techniques due to flow phenomena and assay response times, although these problems are usually mastered ultimately.

2.1.8. REVIEW OF PREVIOUS APPLICATIONS OF THE AKUFVE METHOD

Rydberg, Reinhardt, and Liljenzin (1973)

A. Fundamental equilibrium studies

The temperature dependence of the extraction of copper, zinc, americium and neptunium acetylacetonates from an aqueous phase by an organic solvent such as benzene, chloroform, methylcyclohexane etc. (Liljenzin, Stary et.al. 1969) was greatly facilitated by the fast data aquisition achieved using automatic recording procedures. This method produced precise results leading to the determination of the thermodynamic functions, enthalpy and entropy of complex formation in the aqueous phase, which helped in the identification of such complexes, as well as giving values for their equilibrium constants and an insight into the solvation effects.

B. Extraction kinetics.

Results of the extraction kinetics of any system studied are a natural consequence of the AKUFVE method.

The extraction of the gallium^{III} - acetylacetone complex from aqueous perchlorate solution into benzene (Liljenzin et.al. 1972) was found to be far more rapid than the reverse process. This was established by plotting the gallium radio-activity in the organic phase as a function of time for several pH values thus indicating the first order kinetics pertaining to this system.

Equilibrium constants were subsequently calculated with a generalised least squares program

C. Extraction dynamics

The dynamic behaviour of commercial solvent extraction equipment can be studied by connecting the H-centrifuge directly to the mixing vessel of the system and monitoring the relevant concentrations in the two phases.

Excellent agreement was found between the mathematical treatment of the mixer - settler dynamics of an amine/copper extraction process (Aly, Jernquist et al. 1971) and the measured dynamics of the four stage mixer-settler battery used in the extraction plant. By using a radiometric detection technique, the response time of the measurement was reduced to five seconds.

In a similar case atomic absorption spectrometry was used to follow the separation of nickel and cobalt in various stages of a seven stage mixer-settler the response time here being thirty seconds, mainly as a consequence of a necessary on-line dilution prior to assay.

Preliminary investigations into the modification of this technique for use in process control of industrial solvent extraction equipment have also been made.

D. Fast separation

Because of the short hold-up time in the H-centrifuge of about three seconds, the AKUFVE can be used for the fast separation of short lived species.

In a typical experiment (Aronsson et. al. 1970) a continuously flowing solution of aqueous uranyl nitrate was irradiated by fast neutrons. By using two H-centrifuges the fission product ^{116}Pd, and its radioactive daughter, silver were isolated, and the half life of the parent compound found to be thirteen seconds.

More recently, three centrifuges were used in an on-line experiment to separate ^{234}Pa ($t_{\frac{1}{2}}$ = 1.18 min.) and $^{234}_{Pa}$($t_{\frac{1}{2}}$ = 6.7h) from ^{234}Th ($t_{\frac{1}{2}}$ = 24.1 days).

E. "Anomalous" extraction.

Whilst working with surface active compounds, it was found that the AKUFVE sometimes gave partition values different to those obtained by the more classical techniques. Closer study revealed that this was due to droplet formation and entrainment produced by the rapid mixing process used in the AKUFVE i.e. "anomalous" extraction is a characteristic of these chemical systems under conditions of vigorous mixing, the AKUFVE merely serving to enhance the detection of such emulsification processes, a feature which may enable the equipment to be used in more detailed investigations of such phenomena.

F. Evaluation of industrial solvent extraction processes

The AKUFVE has been used for evaluation of a number of potential or actual industrial solvent extraction processes.

In Norway (Gaudernack et. al. 1971) a process has been designed for the production of very pure yttrium and other rare earths, utilising extraction with di-2-ethylhexyl phosphoric acid from sulphate or nitrate solutions as one of the main steps.

At H.M. Government laboratories, Warren Spring, Flett., et. al. (1971) have used the AKUFVE to investigate the synergistic extraction of copper (II) and iron (III) by hydroxyoxime/carboxylic acids in kerosene from sulphate/nitrate solutions of different pH.

Other areas of study include copper (II) extraction from synthetic leach solutions by the reagents LIX-64N and Kelex-120 (Andersson, Spink, Okuhara 1971, 1973), the recovery of valuable metals from scrap alloys dissolved in hydrochloric acid solutions using Alamin 336, and Lorol in kerosene, after a rigorous study of the distribution curves of Mo, Zn, Fe, Sn, Cu, Co, Mn,

Ni and Cr (III) had been made using the AKUFVE.

Finally, the P.V.C. version of the AKUFVE was used in the development of a solvent extraction process for the recovery of Ni, Cr, and Mo from 3,000 tons per annum of stainless steel pickling waste solution (Rydberg et al. 1973).

CHAPTER 3: EXPERIMENTAL METHODS

3.1.1. MATERIALS

Of the solvents used:-

Octanol was puriss grade from Koch-Light.

Cyclohexane was spectroscopic grade from B.D.H.

Chloroform was spectroscopic grade from B.D.H.

The solutes studied are now listed, along with their respective grades and suppliers:-

i)	p-n-propylphenol	Koch-Light
ii)	phenol	B.D.H.; Analargrade
iii)	quinalbarbitone	B.D.H.; B.P. grade.
iv)	propranolol hydrochloride (Inderal)	I.C.I.
v)	quinoline	I.C.I.
vi)	p-cresol	B.D.H. Laboratory reagent
vii)	p-ethylphenol	Koch-Light
viii)	amoxycillin	Beechams
ix)	sodium cromoglycate	Fisons
x)	m-cresol	B.D.H.; Lab. reagent
xi)	o-cresol	B.D.H.; Lab reagent.
xii)	3,5-xylenol	recrystallised/redistilled from B.D.H. Lab. reagents.
Xiii)	2,4-xylenol	
xiv)	2,6-xylenol	
xv)	2,5-xylenol	
xvi)	2,3-xylenol	
xvii)	3,4-xylenol	
xviii)	2,4,6-trimethylphenol	
xix)	3,5-diethylphenol	
xv)	decylbenzyl dimethylammonium chloride	Sterling Winthrop

Acids and alkali were prepared from B.D.H. laboratory grade reagents. Buffer solutions for standardisation of pH meters etc. were from B.D.H.

3.1.2. APPARATUS:

The spectrophotometers used in the u/v assays were as follows:-

1) Aston University laboratories;

(a) Pye-Unicam S.P.800 recording spectrophotometer for preliminary scanning of the whole u.v/visible range to determine the most suitable λ_{max} for each compound.

(b) Pye-Unicam S.P. 500 for accurate determination of λ_{max} and corresponding absorbance values.

2) Leeds University AKUFVE.

(a) Pye-Unicam SP.1800 recording spectrophotometer.

3) Birmingham University AKUFVE.

(a) Pye-Unicam SP.500 u/v-visible spectrophotometer.

(b) Perkin-Elmer 457 u/v-visible recording spectrophotometer with flow cell attachment.

pH meters were:-

at Aston; Pye model 290

at Leeds)
) Radiometer (Copenhagen) on-line system.
at Birmingham)

3.1.3. SOLVENT EXTRACTION APPARATUS. ,

1) For the shake flask determinations, 250 ml stoppered, conical flasks were used as vessels for the system under study and these were agitated by using a Baird and Tatlock thermostatted, shaking water bath.

2) 3.1.4. JACKETED SEPARATING VESSEL.

This apparatus was built in our laboratories and represents the first step away from the batchwise methods, used in the shake flask studies, towards a continuous mode of operation. Fig. 7. illustrates the construction and operation of this equipment.

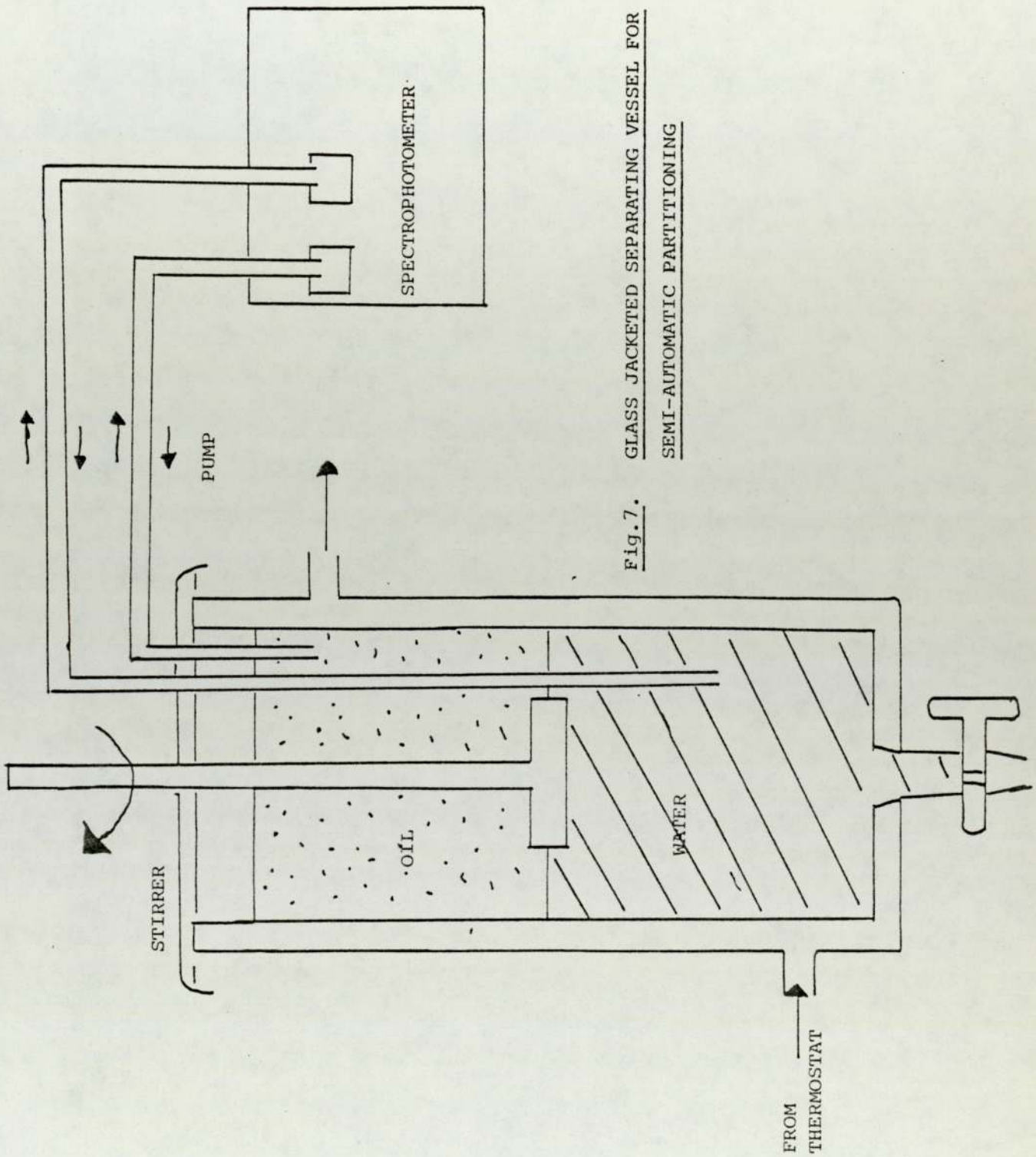


Fig.7. GLASS JACKETED SEPARATING VESSEL FOR SEMI-AUTOMATIC PARTITIONING

With some systems it was found that continuous assays (with a spectrophotometer) were possible when the stirring speed was kept low. Otherwise the stirrer was stopped and the two phases allowed to settle prior to assay.

A number of experiments were carried out on this equipment and it was found to be a useful and cheap method in systems with good settling properties and rapid equilibration attainment e.g. phenol distributed between cyclohexane and water achieved equilibrium after only a few minutes at 25°C.

In one respect this system scored over the final choice in that loss of solvent by evaporation could virtually be eliminated. However, for many systems rapid equilibration and/or separation of the two phases could not be attained and it was necessary to employ a far more sophisticated (and expensive) apparatus which is the subject of our next discussion.

3.1.5. 3) THE AKUFVE APPARATUS

In this apparatus the same basic principles apply as in the conventional method of experimentation, but the procedure has been given the form of a rapid sequence of continuous unit operations: mixing-separation - on line analysis.

Fig. 5. illustrates the essential features of the system:-

In a mixing chamber , equipped for fast and efficient mixing, the solvents used to compose the two phase system are, brought together with the solute and other reagents. Here the partition of the solute takes place according to the conditions in the system (pH, concentrations, temperature etc.)

The two phase mixture then flows into a centrifugal separator designed for fast and absolute phase separation. The two streams of pure phase flow from the centrifuge through measuring cells (or sampling ports) where the concentration of the species of interest can be monitored directly (on-line) or by withdrawing samples for assay.

Finally the pure liquid phases are recirculated to the mixer.

Conditions in the two phase system can be altered by addition of reagents to the mixer, or to one phase via the sampling ports. Temperature conditions can be controlled by circulating a suitable medium (water; brine etc.) through the heat exchanger coil in the mixing chamber. The temperature can be measured either with a thermometer or by electronic means using thermistor sensors.

The mixing chamber is a glass cylinder with top and bottom closed by two titanium lids. It has a volume of 1.4 litres. The contents are agitated by means of a variable speed stirrer before passing through a single peripheral outlet to the centrifuge (gravity fed).

The three, three-way valves over the mixer are used to control the direction of flow of the solutions either to the detectors, drain or mixer. Under the mixer there is a single two-way tap which regulates the inflow of mixture to the centrifuge and, at the sides, two needle valves for the throttling of the two liquid streams from thecentrifuge in order to vary outlet pressure and facilitate control of the interface boundaries inside the centrifuge.

The flow rates of the separated liquids are measured by ball type flowmeters capacity range 10 to 100 l/hour. They are made of glass and through these the phases can be checked visually for turbidity, entrainment or emulsification effects etc.

Fig. 6. shows a section through the H-centrifuge.

The two phase mixture enters the centrifuge through the central connection (1) in the inlet chamber (2) and is almost instantaneously accelerated to the rotational speed of the centrifuge bowl (15). The strong turbulence produces the thorough final mixing of the two phases. If emulsification occurs at this stage then the centrifuge speed is reduced as is also the flow rate.

After acceleration the mixture is forced into eight sector-shaped separation chambers symmetrically arranged around the axis. On the way through the separation chambers droplets follow a zig-zag path imposed by peripheral partition walls with baffle ridges between them. The repeated changes of direction in the movement of the suspended droplets together with the effect of the radial walls between the chamber sectors contribute to the good separation results obtained with the H-centrifuge. Collecting chambers for light phase (4) and heavy phase (7) are located above and below respectively, the separation chambers.

The pure phases are removed by pump wheels (5) and (8) in order to obtain adequate pressure and to avoid excessive frothing of the liquid.

The centrifuge is driven either by a high frequency induction motor (14) or by an air motor, at speeds which can be varied up to a maximum of 18,000 r.p.m. The volume of the bowl is 100 ml. and the maximum flow capacity 300 l/hour.

In our studies two AKUFVE units were used, one at Leeds University and the other at Birmingham University. The basic designs of these two systems are compared in Table 1.

Operation of the AKUFVE.

The centrifuge is brought up to the optimum operating speed for the system under study, before the mixture is run in from the mixing chamber. The flow rates into and out of the centrifuge as well as any necessary back pressure is controlled by the appropriate valve(s) as outlined above. This should achieve absolute phase separation for most systems. Sufficient time must be allowed for equilibrium to be reattained after any changes in the experimental conditions i.e. temperature, pH etc. A few minutes is usually adequate.

Table 1

Details of the two AKUFVE units used

for the experimental determinations

	Leeds AKUFVE'	Birmingham AKUFVE
Centrifuge speed:	0-18,000 r.p.m.	0-18,000 r.p.m.
Air motor:		Electric motor
Centrifuge volume:	100 ml	100 ml
Maximum flow rate:	3001/h.	300 1/h.
Mixing chamber volume:	1.41.	1.41.
Sampling facility:	On-line injection ports.	On-line injection ports.
Temperature measurement:	On-line mercury thermometer.	On-line mercury thermometer and thermo-couple in mixing chamber.
Temperature range:	12°C - 45°C.	20°C - 45°C.
Phase volume ratio:	4:1.	10:1 after slight modification.
Analytical detection:-		
	on-line radiometric	on-line radiometric
	off-line spectrophotometric.	off-line spectrophotometric-aqueous phase.
		off-line spectrophotometric-both phases.
pH monitoring:	on-line	on-line
pH control:	on-line	on-line

PROCEDURES

3.2.1. ASSAY PROCEDURE

Since the majority of our assays utilised u.v. spectroscopy it was first necessary to eliminate any variables within the spectral parameters arising from changes within the aqueous (or organic environments) themselves.

Hence we checked solvent absorbance spectra in order to find the usable (greater than 80% transmission) assaying region.

Our samples of octanol were found to absorb quite extensively below 250 nm, presumably due to trace amounts of alkene residues formed during manufacture. It was subsequently checked by experiment that none of these unsaturated compounds were absorbed into the aqueous phase in a typical partitioning experiment. To further eliminate any possibility of error arising from this source we set 250 nm as a lower limit in the choice of λ_{max} for assay purposes when working with octanol-water systems.

The other solvents were found to be trouble free, above 210 nm, in this respect.

The u.v. absorbance of a compound is dependent upon the solvent in which it is dissolved and in some cases the spectral properties can be modified by quite small changes in solvent composition. All types of "calibration" experiments were performed for a given solvent when saturated with the complementary partitioning phase e.g. octanol-saturated water.

Beer-Lambert plots were used to check for any non-linearities in the absorbance spectra over the range of concentrations to be studied. When working "on location" away from the laboratory, simple calibration checks were performed on the equipment provided for assay.

Extensive experiments were carried out to check for any variation of u.v. spectra/absorbance over the temperature range covered in our experiments. Negligible variations were found for virtually all the compounds studied. Similarly ionic strength variations produced no measurable effect on the u.v. spectra.

One area that required care (and calibration procedures) was in the study of partitioning over a range of pH. The absorbance of the compound in aqueous solution was determined over the range of pH under study and the values were used obtained for determining the actual concentrations during the partitioning experiment.

An elegant solution to this problem is to assay the organic phase as only the non-ionised species is extracted into this phase and thus should give a normal linear relationship between absorbance and concentration over a wide pH range.

3.2.2. SHAKE-FLASK EXPERIMENTS

Using the well established technique of agitating flasks in thermostated water baths we studied the effect of variation of:-

i) chemical structure

ii) ionic strength

iii) pH

iv) mixed organic solvents

v) temperature

vi) solvent system.

on the partition coefficients of the following homologous series of phenols:-

a) phenol

b) p-cresol

c) p-ethylphenol

d) p-n-propylphenol

The solvents used were cyclohexane and octanol.

3.2.3. THE EFFECT OF CHEMICAL STRUCTURE OF THE HOMOLOGUE AND SOLVENT TYPE ON PARTITION COEFFICIENT.

a) solvent system; cyclohexane - water.

Aqueous solutions of the p-n-alkyl-phenols; phenol, p-cresol, p-ethylphenol and p-propylphenol were prepared at concentrations lying within the range 2 to 10 x 10^{-4} moles per litre, using water saturated with cyclohexane.

25 ml aliquots of each solution were equilibrated with an equal volume of cyclohexane, saturated with water, in 100 ml bottles. Initially the bottles were shaken by hand before being transferred to an electrically driven shaking rack contained in a thermostatted water bath. Here they were agitated at a fixed rate for forty-five minutes at the required temperature. After standing for one hour, aliquots of the aqueous phase were carefully withdrawn by a Pasteur pipette and then assayed spectrophotometrically by comparison with a previously prepared calibration graph (λ_{max} - 210.5 nm).

The concentration of solute in the cyclohexane phase was deduced by mass balance and partition coefficients subsequently calculated. Three different concentrations were used to calculate each partition coefficient in order to show that, within the limit of experimental error, there was no variation with concentration for this system under these conditions.

In all subsequent experiments the same general procedure was adopted i.e. the aqueous phase was assayed spectrophotometrically the solute concentration in the organic phase being deduced from the mass balance relationship.

The temperature was 22°C $\pm$ 1°C throughout, unless otherwise stated, and the pH of the aqueous phase was monitored at 5.5, except of course in the pH studies. In all cases the two phases used were mutually saturated with each other prior to use. In most cases 20 ml of each phase were used.

b) solvent system; octanol-water

Partition coefficients of the four p-n-alkyl phenols were determined using octanol as the organic phase, the initial solute concentrations in the aqueous phase falling within the range 2 to 9 x 10^{-2} molar for phenol, p-cresol, p-ethylphenol and 1 to 4 x 10^{-1} molar for p-n-propylphenol.

Fresh calibration graphs were prepared at λ_{max} = 270 nm, 278 nm, 276 nm, 276nm for phenol, p-cresol, p-ethyl-phenol and p-n-propylphenol respectively, as there was interference from the octanol layer at the previously used λ_{max} of 210.5 nm.

The results of these experiments are presented in Tables 2 and 3 and Figures 8 and 9.

3.2.4. EFFECT OF IONIC STRENGTH.

Using sodium chloride as electrolyte, aqueous solutions of different ionic strengths ranging from 0.3 to 5.0 were prepared.

Solutions of (i) phenol and (ii) p-cresol in cyclohexane were prepared at concentrations of 4 to 11 x 10^{-5} molar. 20 ml aliquots of this organic phase were then equilibrated with 20 ml of the aqueous phase, at different ionic strengths, in 100 ml bottles using the procedure described above.

The aqueous phase was assayed at λ_{max} = 210.5 nm and the partition coefficients calculated as before.

A similar procedure was adopted using octanol as the organic phase and a concentration range of 4 to 9 x 10^{-2} molar and assaying at 270 nm and 278 nm respectively.

The results of these experiments are presented in Tables 5, 6, 7 and 8 and Figures 10,11, 12 and 13.

Table 2.

Partitioning of alkyl-phenol homologues between water and cyclohexane

Temperature 22°C ± 1°C Log K_D values

Compound	Classical	Glass Vessel	AKUFVE	Lit.value.
Phenol	- 0.65	- 0.78	- 0.65	-0.72
p-cresol	- 0.06	- 0.37	- 0.36	-0.19
p-ethylphenol	0.42	-	-	0.44
p-propylphenol	1.10	-	1.02	0.86

Table 3.

Partitioning of alkyl-phenol homologues between water and octanol

Temperature 22°C ± 1°C Log K_D values

Compound	Classical	Glass Vessel	AKUFVE	Lit.value.
Phenol	1.53	1.50	1.54	1.48
p-cresol	1.90	2.04	1.92	1.92
p-ethylphenol	2.12	-	-	2.40
p-propylphenol	2.75	-	-	

In both of the above experiments the results for the two non-classical procedures have been "cross referenced" from studies primarily involved with other parameters i.e. temperature and ionic strength effects. However, it can be seen that where results are available for comparison those obtained on the AKUFVE give a good (in the octanol-water system an excellent) correlation with the classically derived results. Those for the glass, jacketed, separating vessel are not as successful for the cyclohexane - water system, but the results obtained for the octanol-water system correlate very closely

to those obtained by the other methods as well as with available literature values. In both systems, however, the same trends are observed with the glass separating vessel.

In view of these findings tables were constructed showing the variation of partition coefficients within the homologues of the alkyl substituted phenols and some of their isomeric forms. All results were obtained on the AKUFVE for the aqueous-cyclohexane system.

Table 4.

Partitioning of di-; tri-; and tetrasubstituted analogues of phenol.

Temperature	25°C, ± 1°C
Compound	log K_D
Phenol	-0.65
3,5-xylenol	0.38
3,5-diethylphenol	1.31
o-cresol	0.12
m-cresol	-0.25
p-cresol	-0.36
2,3-xylenol	0.57
2,4-xylenol	0.67
2,5-xylenol	0.69
2,6-xylenol	0.99
3,4-xylenol	0.29
3,5-xylenol	0.38
2,4,6-trimethylphenol	1.51

Fig.8. Partitioning of alkyl-phenol homologues between water and cyclohexane.

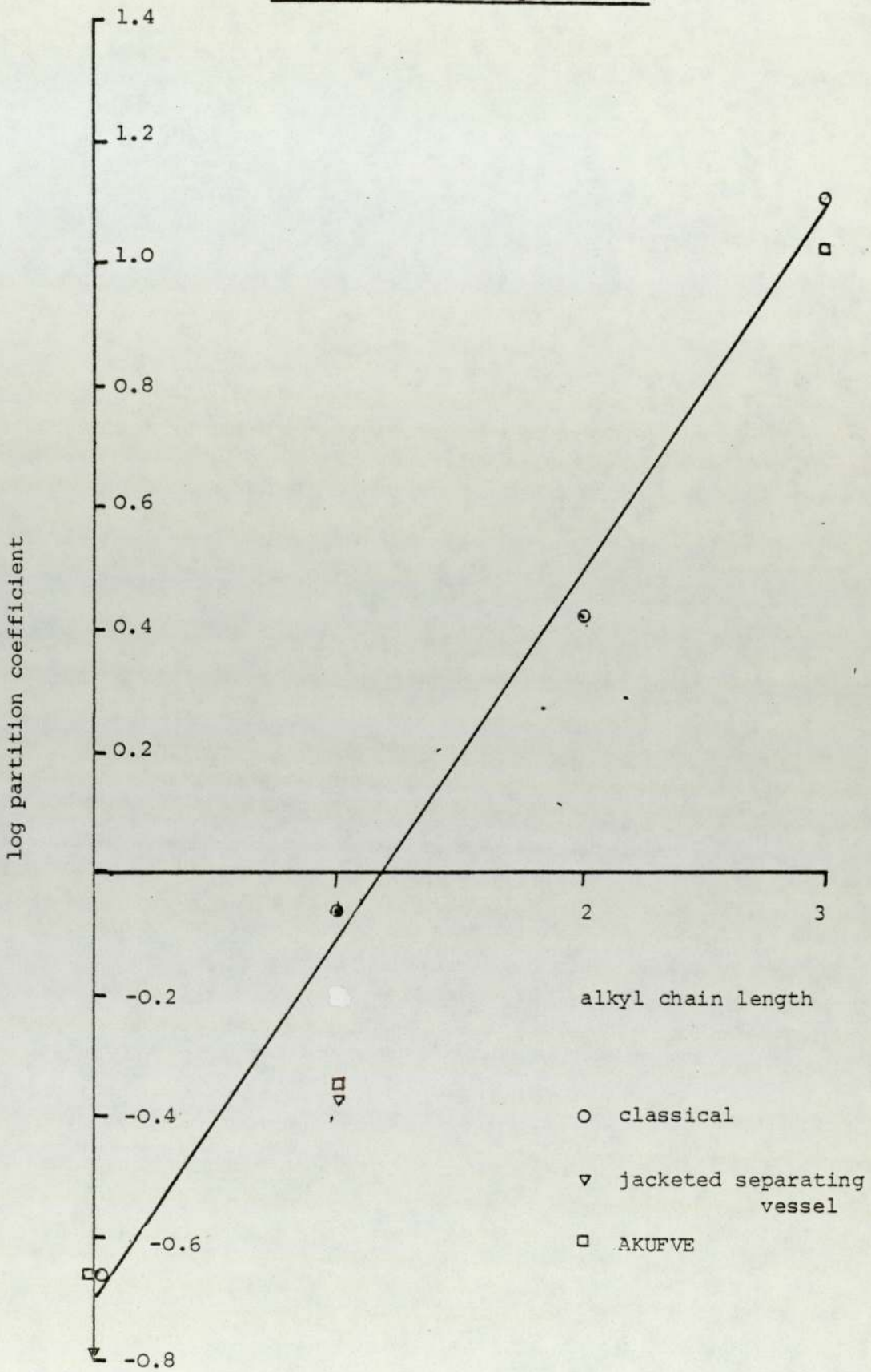


Fig. 9. Partitioning of alkyl-phenol homologues between Water and Octanol

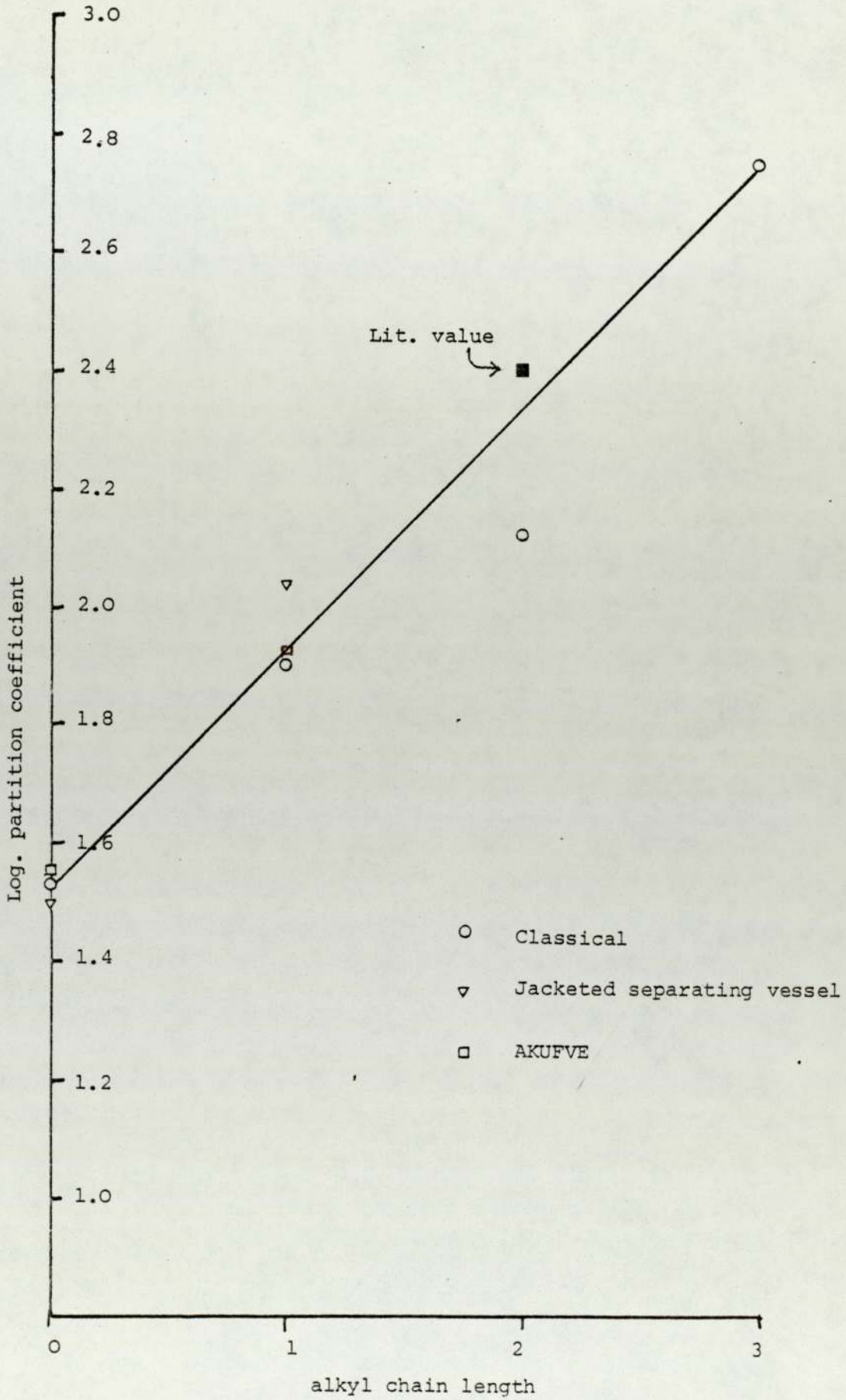


Table 5.

The effect of ionic strength on the partitioning of phenol between water and cyclohexane

Log K_D values

Ionic Strength	Classical	Glass vessel	AKUFVE
0	- 0.65	- 0.78	- 0.64
0.34	- 0.64	- 0.78	- 0.58
0.68	- 0.64	- 0.78	- 0.52
1.36	- 0.56	- 0.64	- 0.39
2.04	- 0.52	- 0.58	- 0.26
2.72	- 0.42	- 0.43	- 0.18 at I = 2.50

Table 6.

The effect of ionic strength on the partitioning of p-cresol between water and cyclohexane

Log K_D values

Ionic Strength	Classical	Glass vessel	AKUFVE
0	0.05	- 0.35	- 0.36
0.34	0.06	- 0.09	- 0.34
0.68	0.06	- 0.09	- 0.24
1.36	0.09	0.06	- 0.15
2.04	0.13	0.21	- 0.02
2.72	0.25	0.37	+ 0.05

Table 7

The effect of ionic strength on the partitioning of phenol between water and octanol

Ionic Strength	Log. K_D values		
	Classical	Glass Vessel	AKUFVE
0	1.40	1.50	1.42
0.34	1.70	1.56	1.46
0.68	1.55	1.63	1.56
1.36	1.64	1.70	1.59
2.04	1.80	1.81	1.65
2.72	1.97	1.87	1.72

Table 8.

The effect of ionic strength on the partitioning of p-cresol between water and octanol

Ionic Strength	Log. K_D values		
	Classical	Glass vessel	AKUFVE
0	1.92	2.04	1.92
0.34	2.00	2.12	1.99
0.68	2.07	2.20	2.06
1.36	2.22	2.36	2.12
2.04	2.37	2.52	2.24
2.72	2.52	2.67	2.33

Fig. 10. Effect of ionic strength on the phenol-cyclohexane/water system

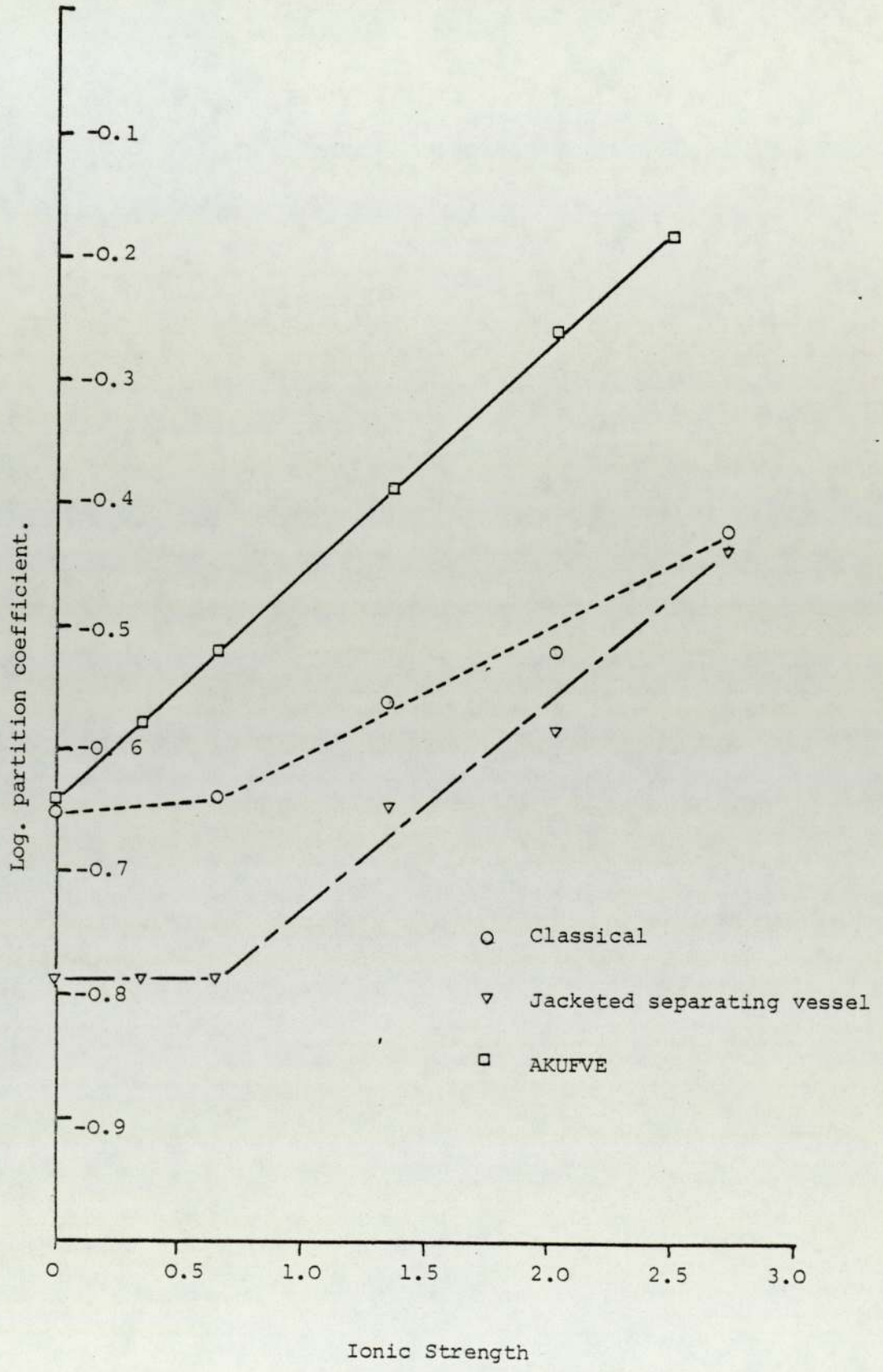


Fig. 11. Effect of ionic strength on p-cresol-cyclohexane/water system.

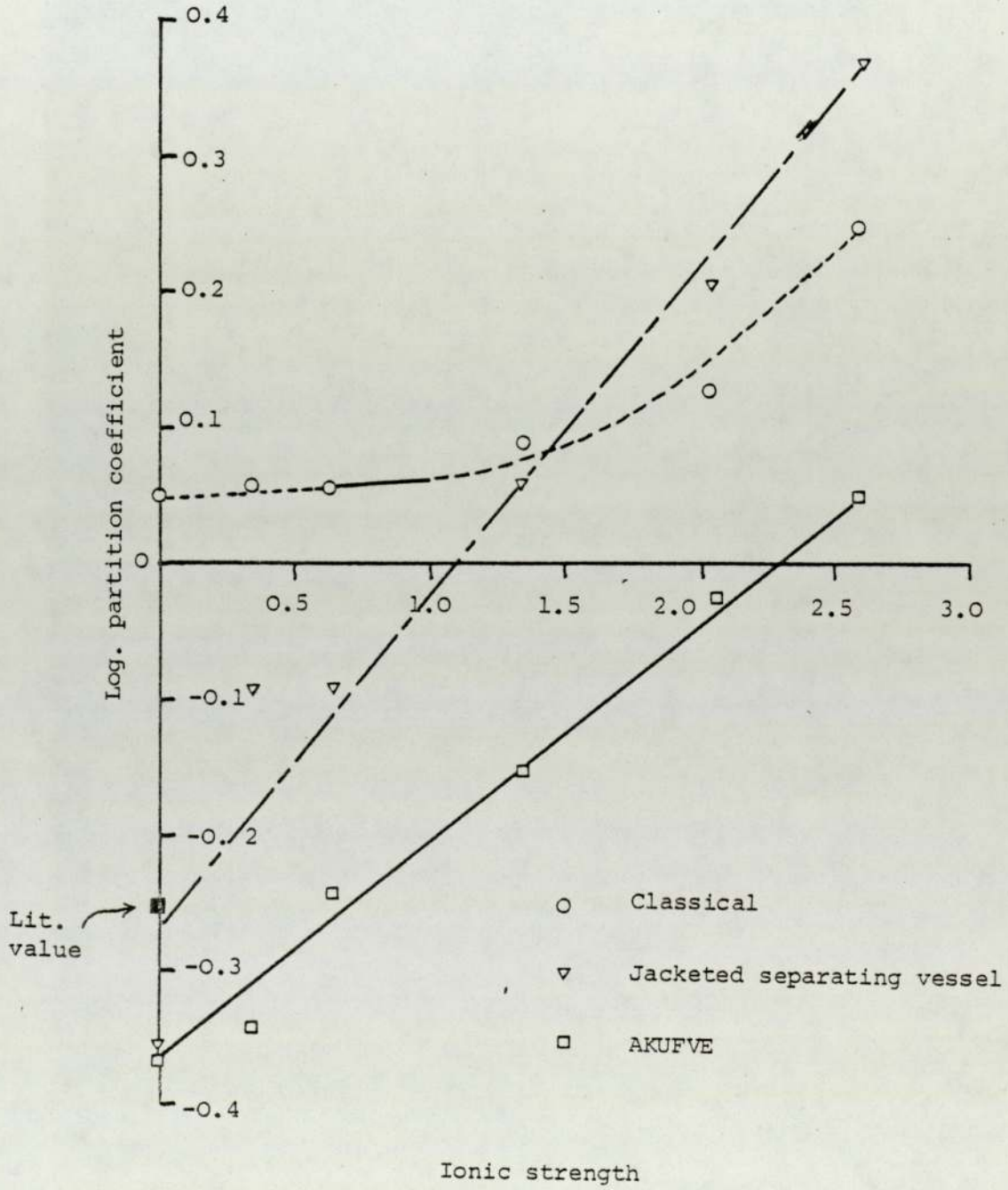


Fig. 12. Effect of ionic strength on the phenol-octanol/water system.

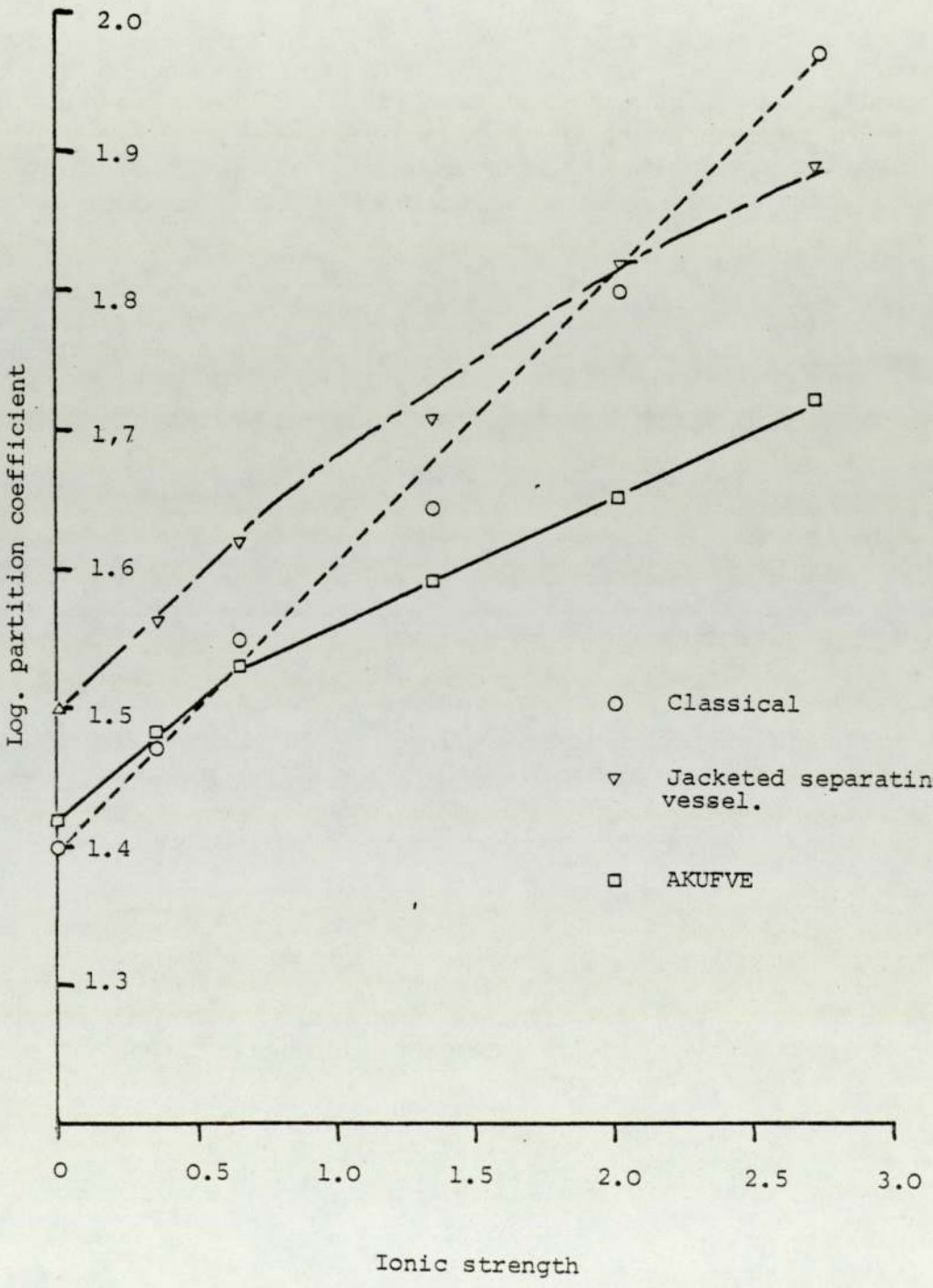
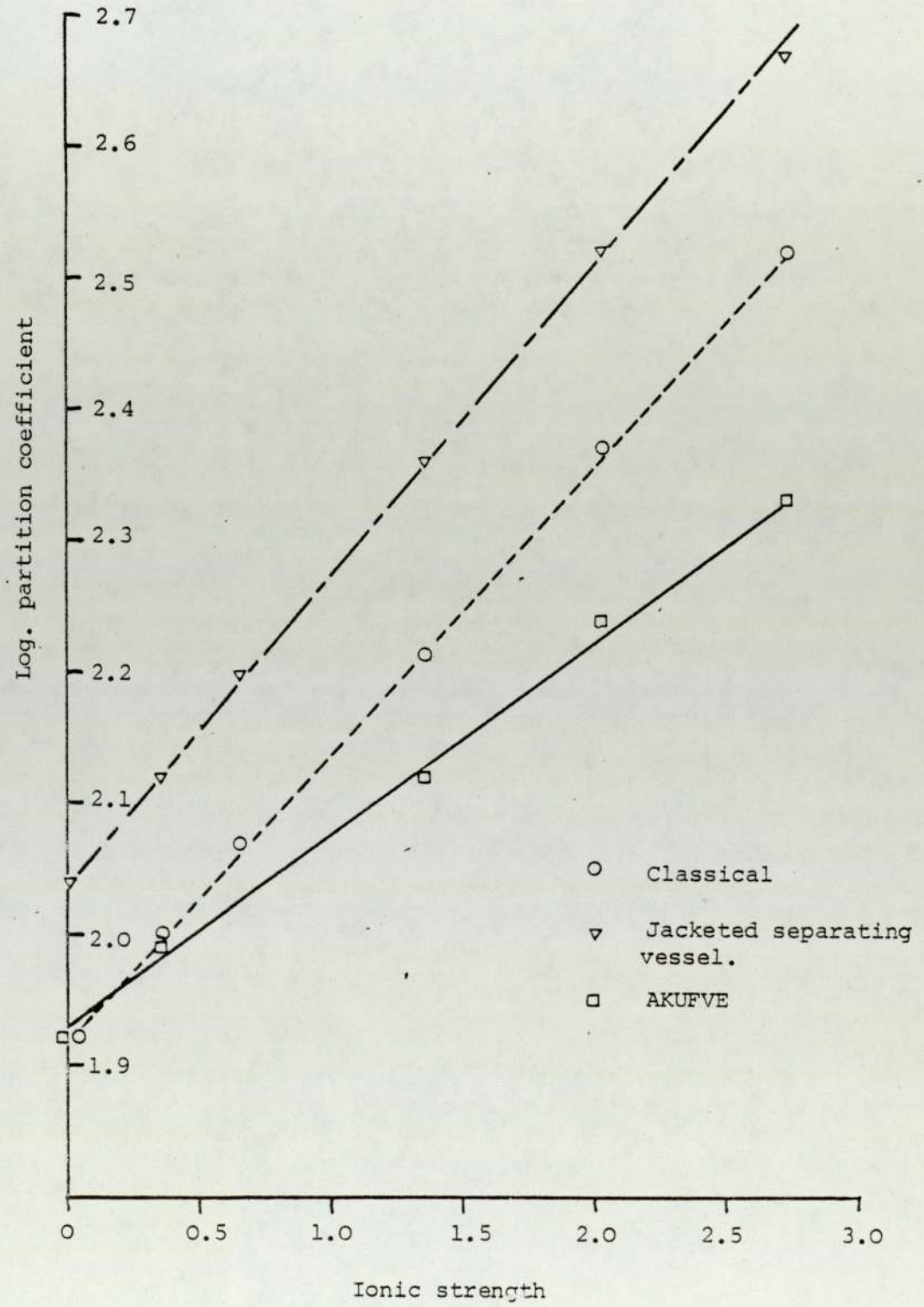


Fig. 13. Effect of ionic strength on the p-cresol-octanol/water system.



3.2.5. EFFECT of pH

Since the absorbance of phenols was found to shift to longer wavelengths in alkaline solution standard calibration curves were prepared for phenol and p-cresol at pH 11.3. The peak at 235 nm was used for assaying.

Buffer solutions were not used in the pH studies in order to avoid "ionic strength" effects, but a very close check was kept on the pH of the aqueous phase throughout the experiment.

The results obtained in these studies are given in Figures 14, 15, 16 and 17. They have all been corrected, using the Henderson-Hasselbach equation, to values for the undissociated species.

3.2.6. EFFECT OF MIXED ORGANIC SOLVENTS

In these experiments the effect of using solvent mixtures of between 20% octanol in cyclohexane and 80% octanol in cyclohexane as the organic phase, on the partition coefficients of p-n-propylphenol and p-cresol were studied.

The results obtained were compared with those for the pure solvents under the same conditions and are to be found in Figures 18 and 19.

The concentrations of the aqueous solutions of cresol and p-n-propylphenol lay in the range 4 to 11 x 10^{-4} molar and 7 x 10^{-2} molar respectively. The wavelength peaks used for assay were 278 nm for cresol and 276 nm for p-n-propylphenol, the absorbance - concentration values being read from the calibration curves for the octanol-water system.

3.2.7. EFFECT OF TEMPERATURE

Aqueous solutions containing 2 to 9 x 10^{-2} moles per litre of p-cresol were equilibrated with an equal volume of octanol and shaken at temperatures of 25°C, 35°C and 45°C. Partition coefficients were calculated as before, the results being given in Fig. 20.

3.3.1. STUDIES WITH A JACKETED SEPARATING VESSEL

The experiments carried out on this equipment took a great deal longer to perform than on the AKUFVE, although they did, however, offer a greater degree of flexibility than when using the classical shake flask technique.

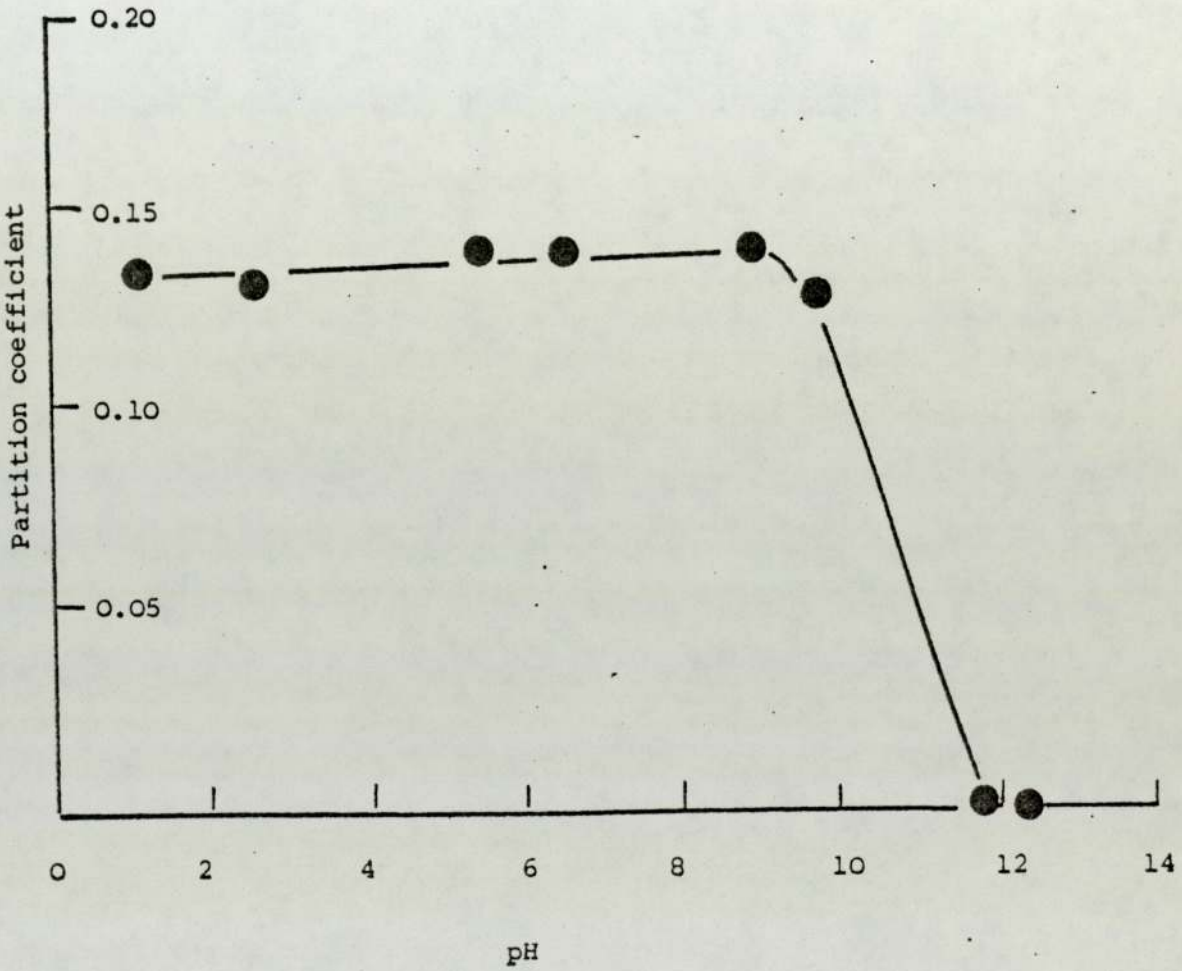
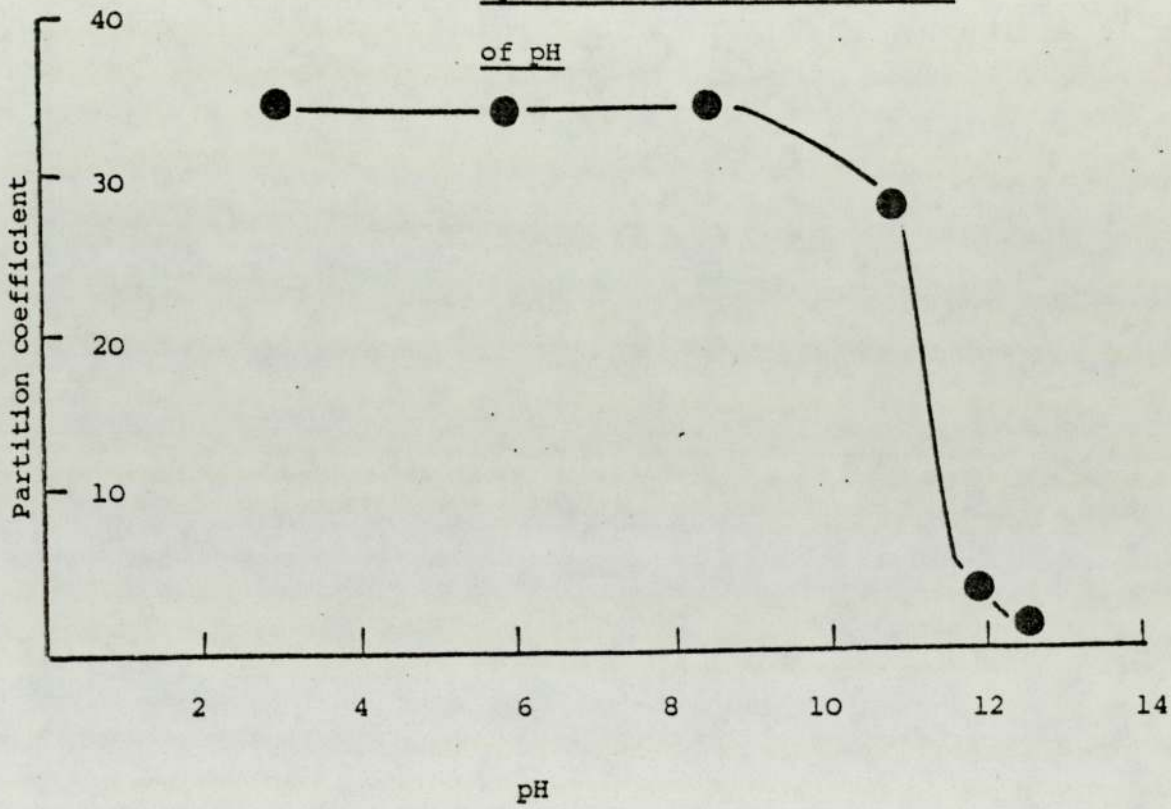


Figure 14. Partition coefficient Phenol. System cyclohexane/ water. Effect of pH

Figure 15. Partition coefficient Phenol, System octanol/water. Effect of pH



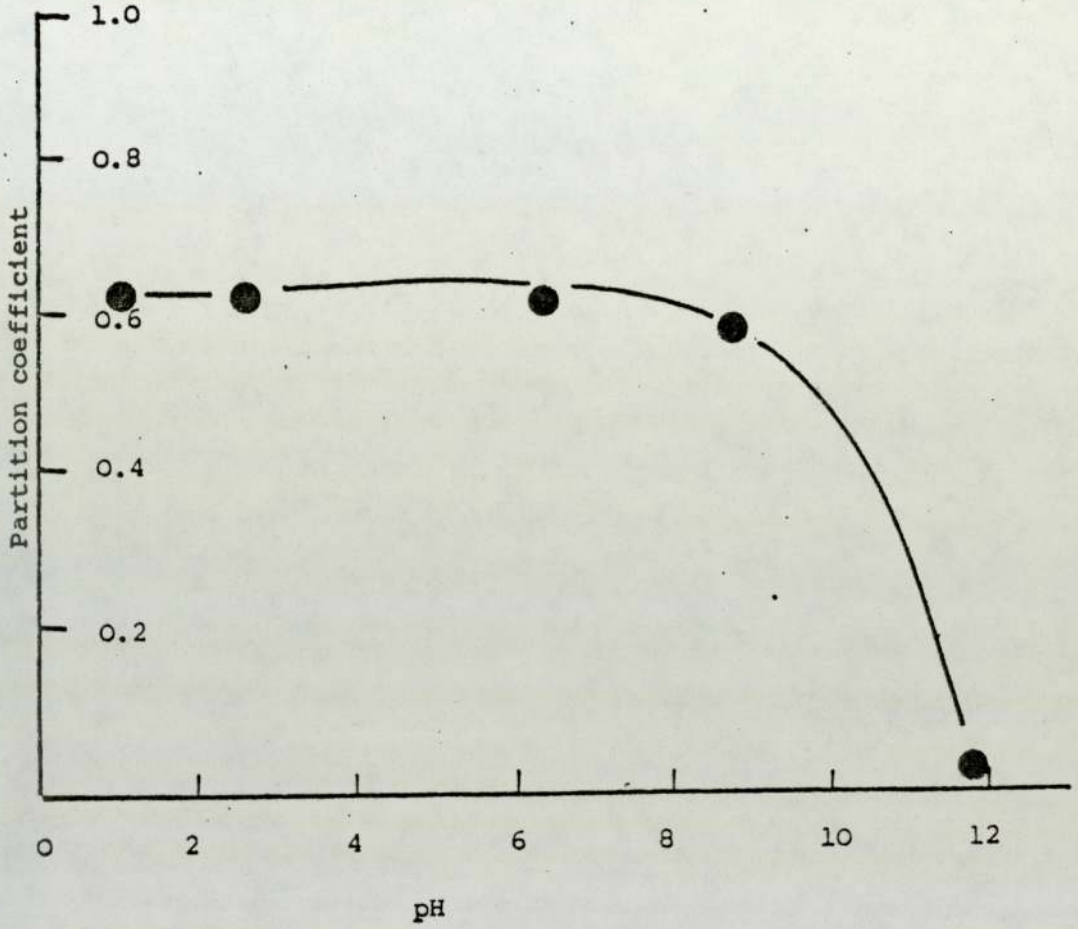


Figure 16. Partition coefficient p-cresol. System cyclohexane/water. Effect of pH

Figure 17. Partition coefficient p-cresol. System octanol/water. Effect of pH

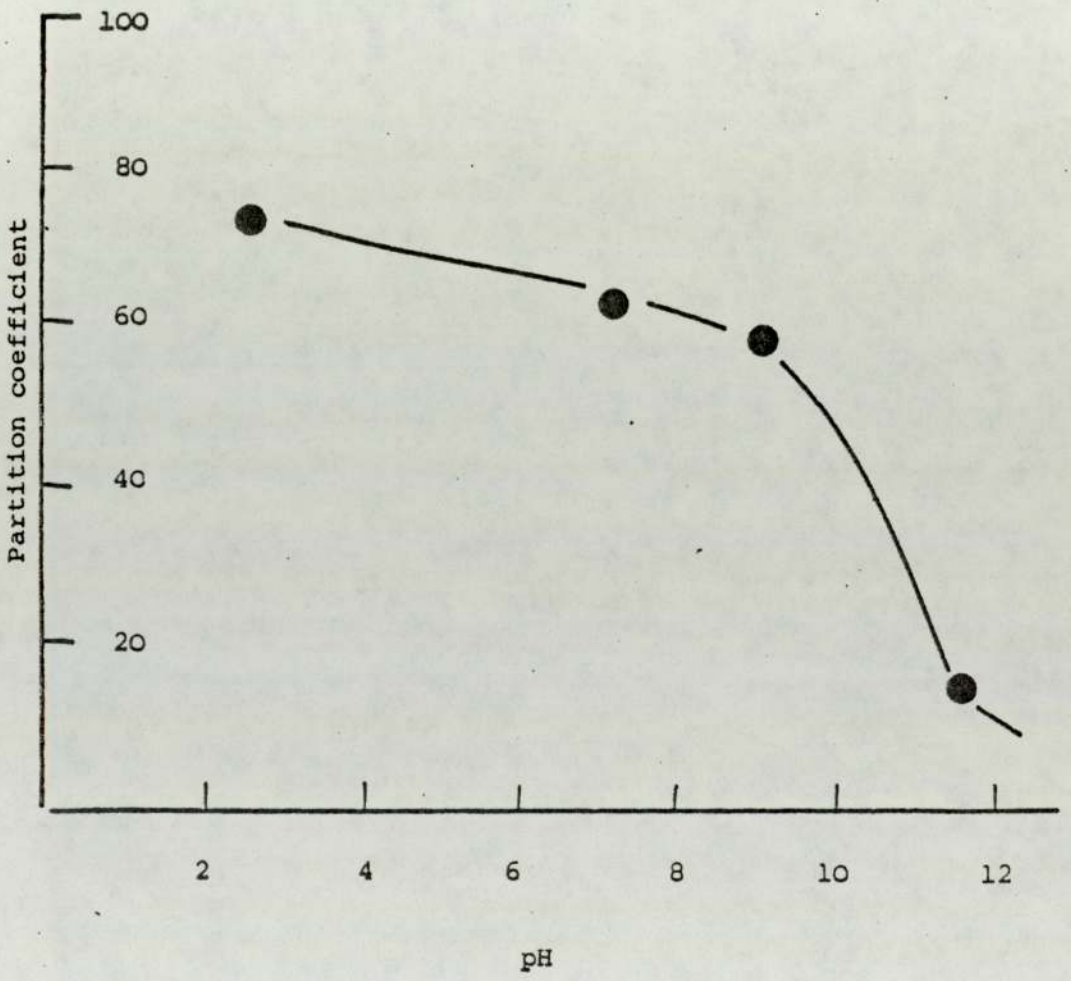
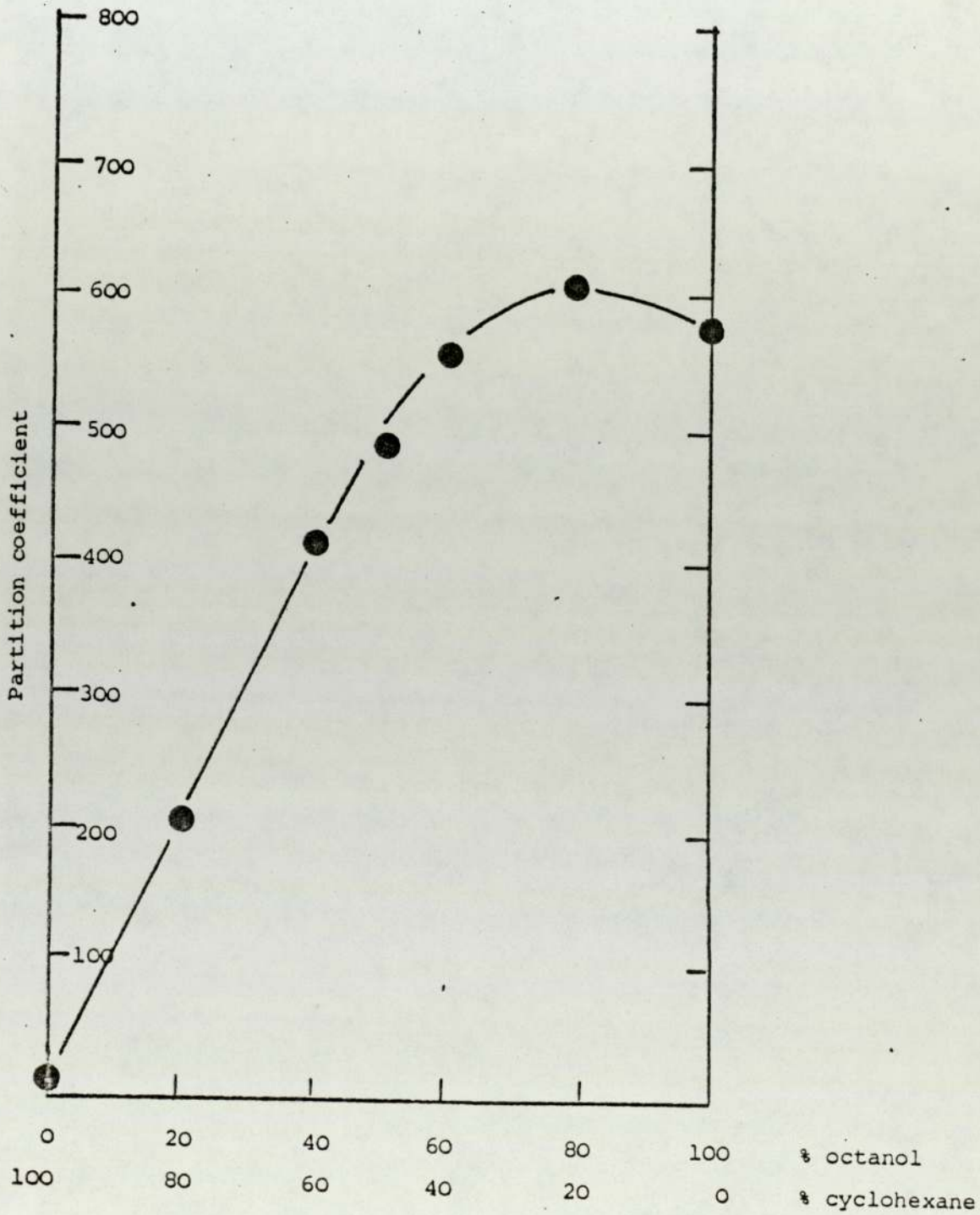


Figure 18. Partition coefficient n-propyl Phenol - mixed solvent system octanol/cyclohexane



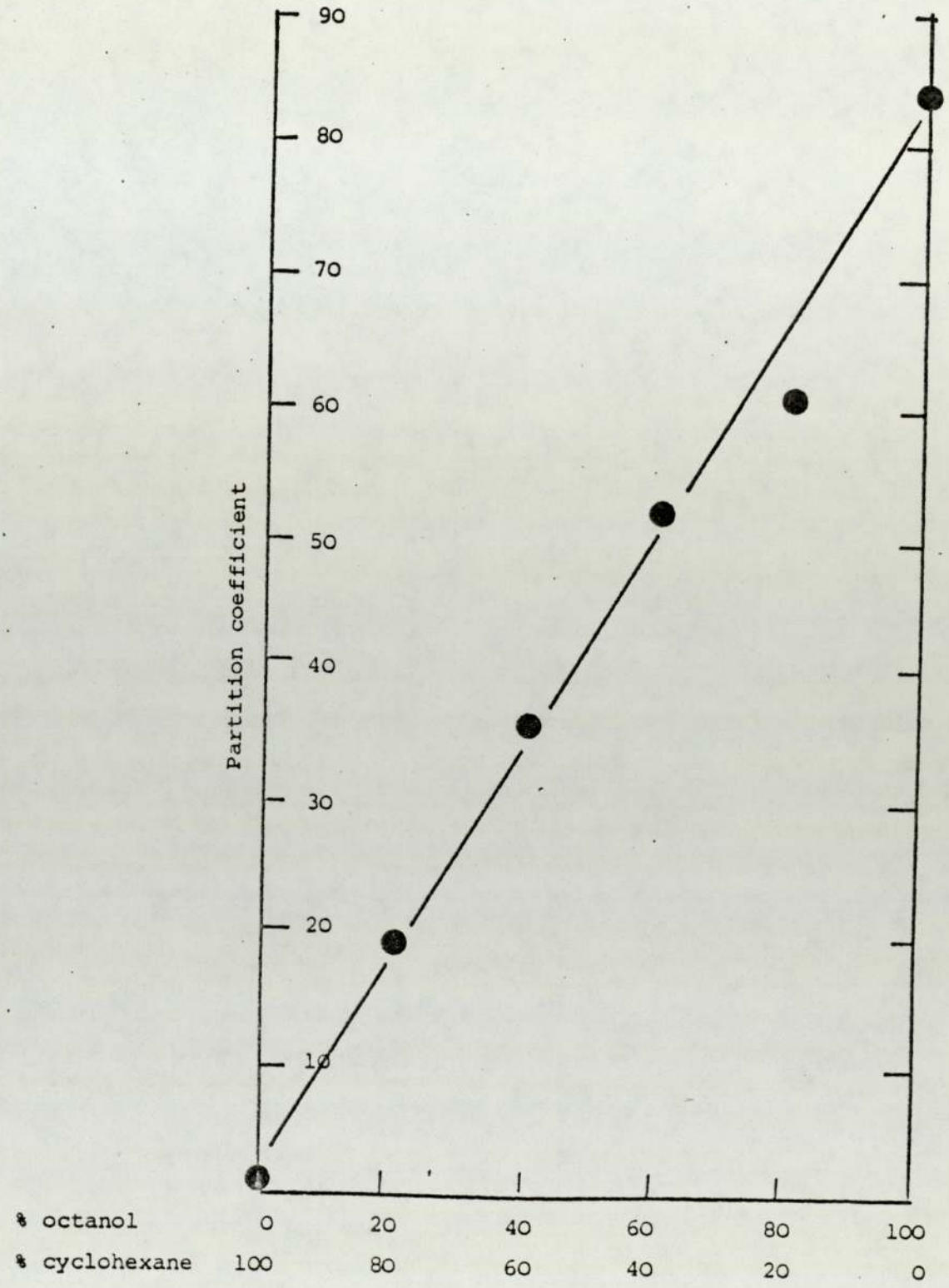
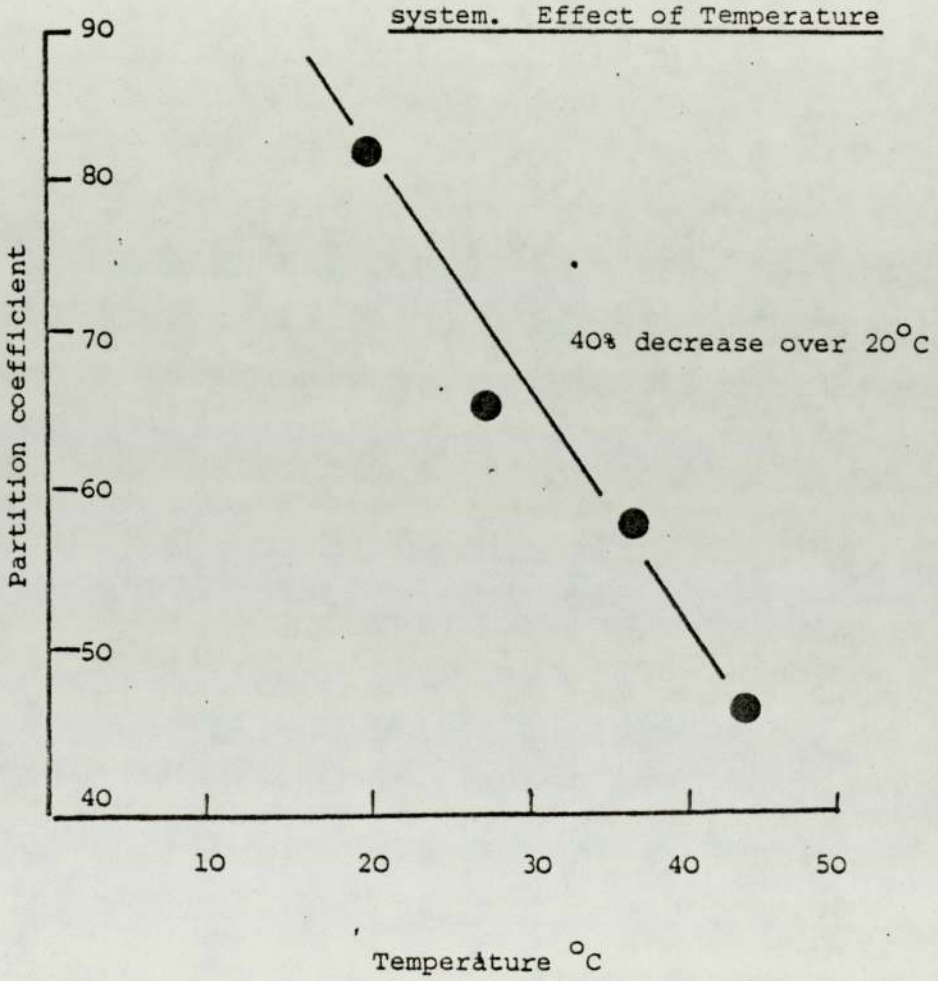


Figure 19 Partition coefficient p-cresol mixed solvent system. Octanol/cyclohexane

Figure 20 Partition coefficient cresol - octanol/water system. Effect of Temperature



For the sake of comparison, systems which were also studied on either (or both) of the classical and AKUFVE apparatus were investigated viz

Solute	Solvent system
Phenol	Cyclohexane/water
p-cresol	" "
Phenol	Octanol/water
p-cresol	" "

The partition coefficients were determined and the effect of varying the temperature and ionic strength (aqueous phase) were also investigated.

The results of these experiments are given in Tables 5, 6, 7, 8, 9, 10, 11, and 12 and Figs. 10, 11, 12, 13, 21, 22, 23 and 24.

3.4.1. STUDIES WITH THE AKUFVE APPARATUS

For all of the experiments carried out on this equipment equal volumes (500 mls.) of solvent and water were used, except for the large phase volume ratio studies where an external stirred reservoir was incorporated into the aqueous line.

Initially emulsification and solvent loss (by evaporation) proved to be a problem, although the former trouble was subsequently largely overcome by careful control of the centrifuge flow rates and rotational speeds.

Solvents with high vapour pressures, such as cyclohexane and chloroform, are able to escape from the AKUFVE unless precautions are taken to provide a closed system. For example in some of our detailed temperature studies, with cyclohexane as organic solvent up to ten per cent of the solvent could be lost. As a result in these investigations a cyclic procedure was adopted (low temperature to high temperature back to low temperature) and any hysteresis effects were noted and a correction made. Without doubt a totally closed system (preferably of much smaller volume) could be made without undue difficulty.

The important variables that can influence the partition of a solute between organic and aqueous phases were listed in the introduction (c.f. methodology section p.43) and each of these have been examined in turn.

Table 9.

The effect of temperature on the partition coefficient of phenol distributed between cyclohexane and water as determined in the glass, jacketed separating vessel.

Temperature	Log. K_D.
20°C	- 0.86
25°C	- 0.78
30°C	- 0.78
35°C	- 0.78
40°C	- 0.71

Table 10.

The effect of temperature on the partition coefficient of p-cresol distributed between cyclohexane and water as determined in the glass, jacketed separating vessel.

Temperature	Log. K_D.
20°C	- 0.39
27°C	- 0.35
30°C	- 0.32
35°C	- 0.09
40°C	- 0.06

Table 11.

The effect of temperature on the partition coefficient of phenol distributed between octanol and water as determined in the glass jacketed separating vessel.

Temperature	Log. K_D.
20°C	1.53
25°C	1.50
27°C	1.50
30°C	1.48
35°C	1.47
40°C	1.45
45°C	1.42

Table 12.

The effect of temperature on the partition coefficient of p-cresol distributed between octanol and water as determined in the glass, jacketed separating vessel.

Temperature	Log. K_D.
23°C	2.04
27°C	2.02
31°C	2.01
36°C	1.98
41°C	1.96

Fig. 21. Plot of log. K_D. vs. $(1/T)\ ^{o}K^{-1}$ for phenol distributed between cyclohexane and water as determined in the glass, jacketed separating vessel.

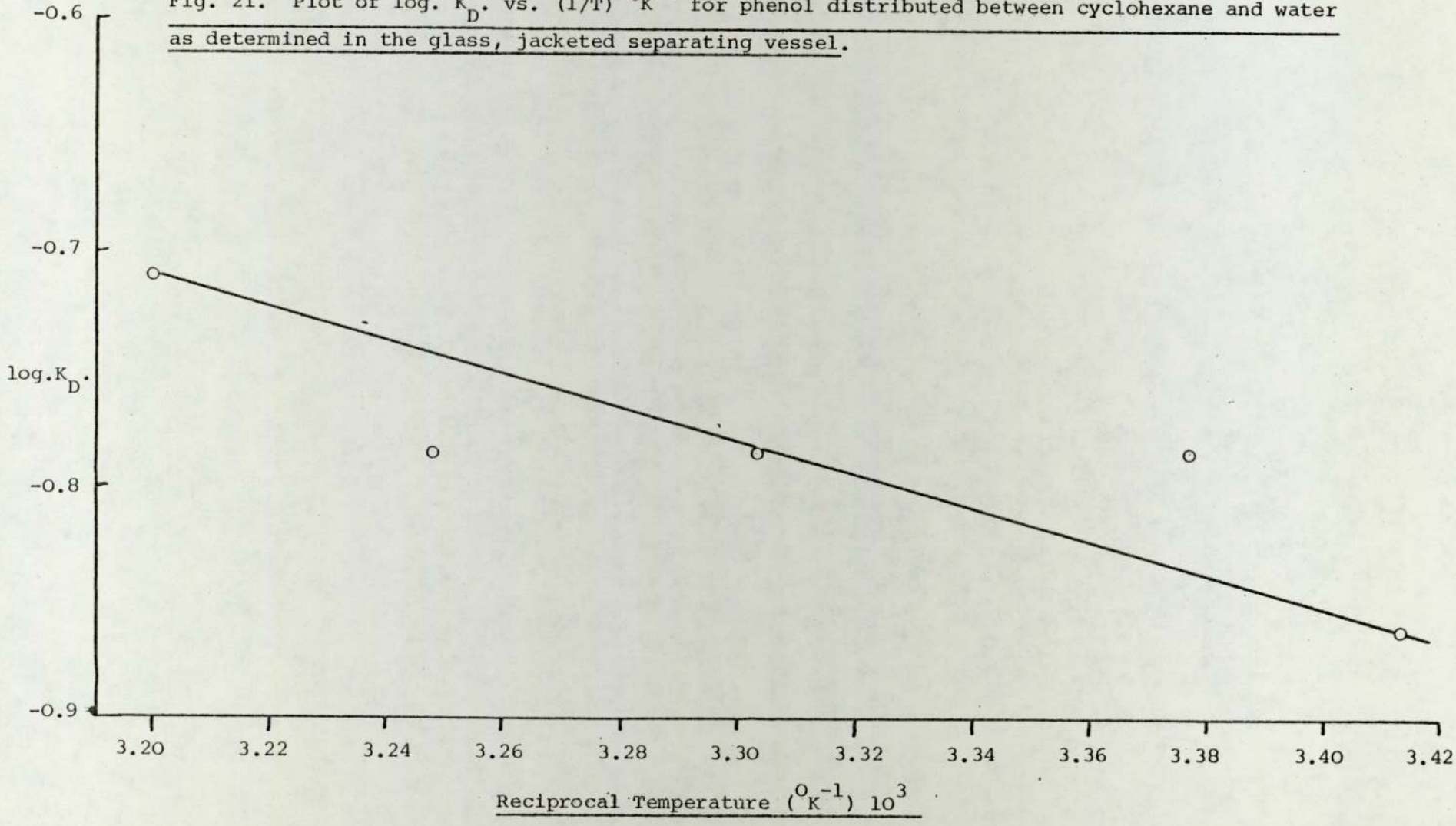


Fig. 22. Plot of $\log.K_D$. vs. $(1/T)^{\circ}K^{-1}$ for p-cresol distributed between cyclohexane and water as determined in the glass, jacketed separating vessel.

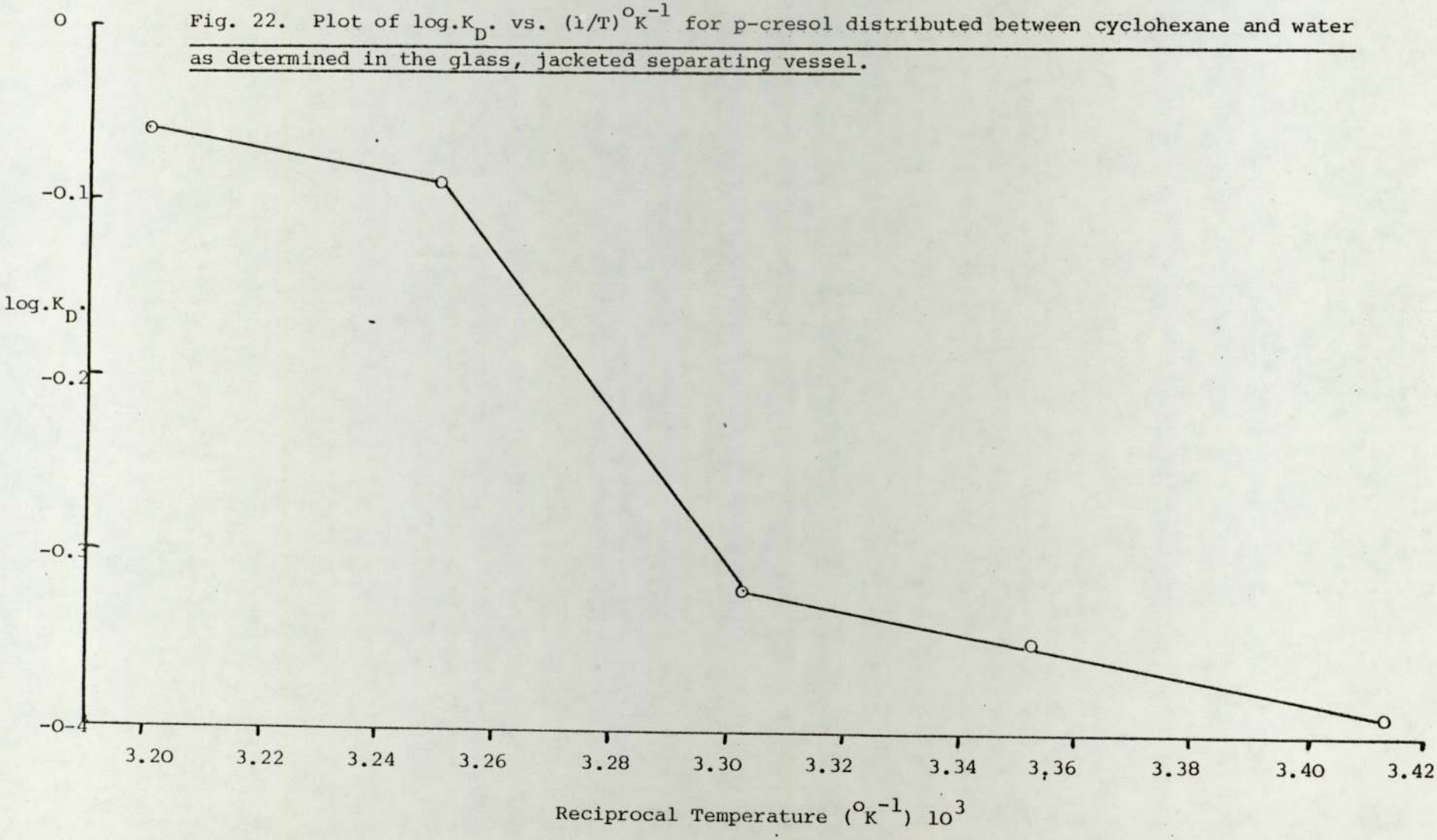
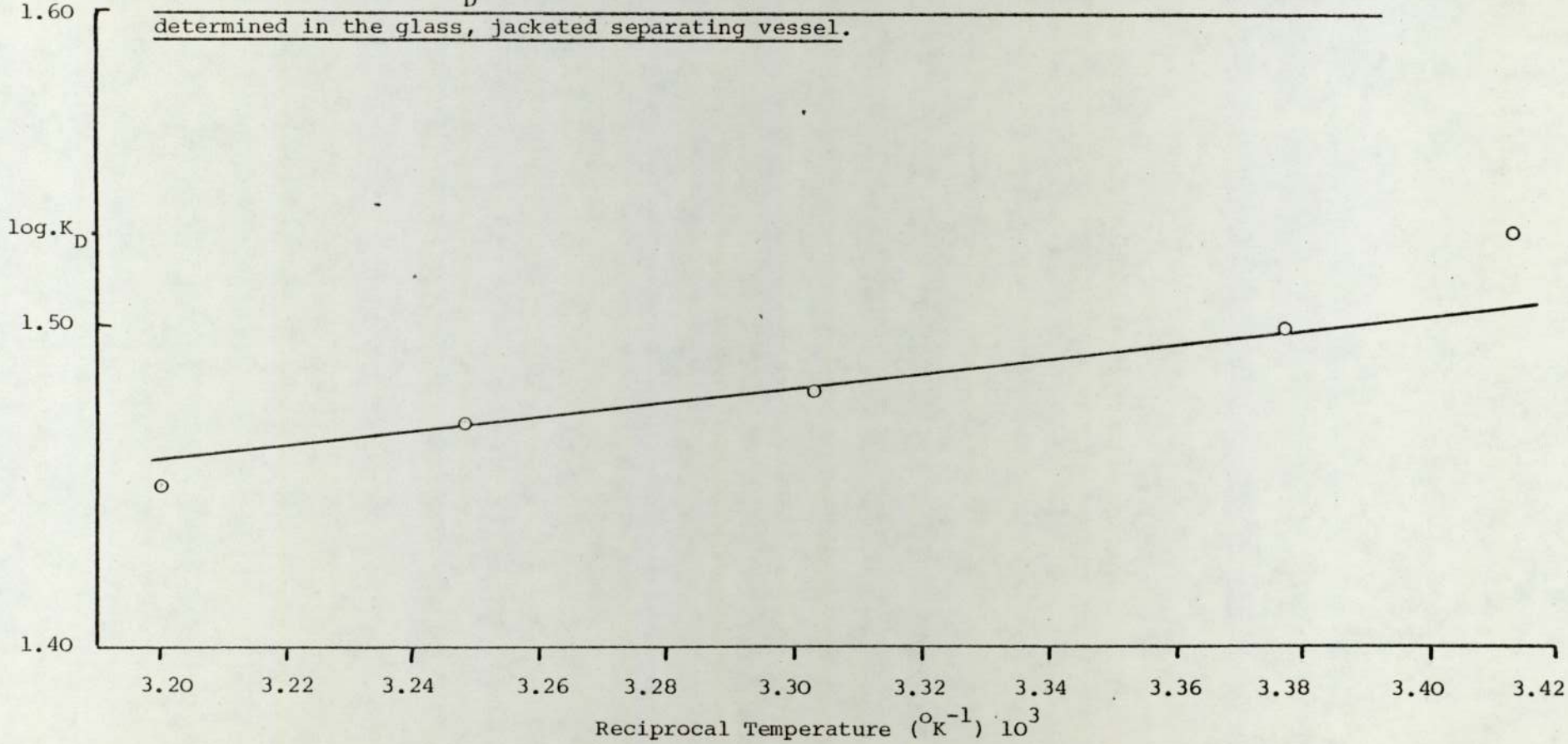


Fig. 23. Plot of log. K_D. vs. $(1/T)^{\circ}K^{-1}$ for phenol distributed between octanol and water as determined in the glass, jacketed separating vessel.



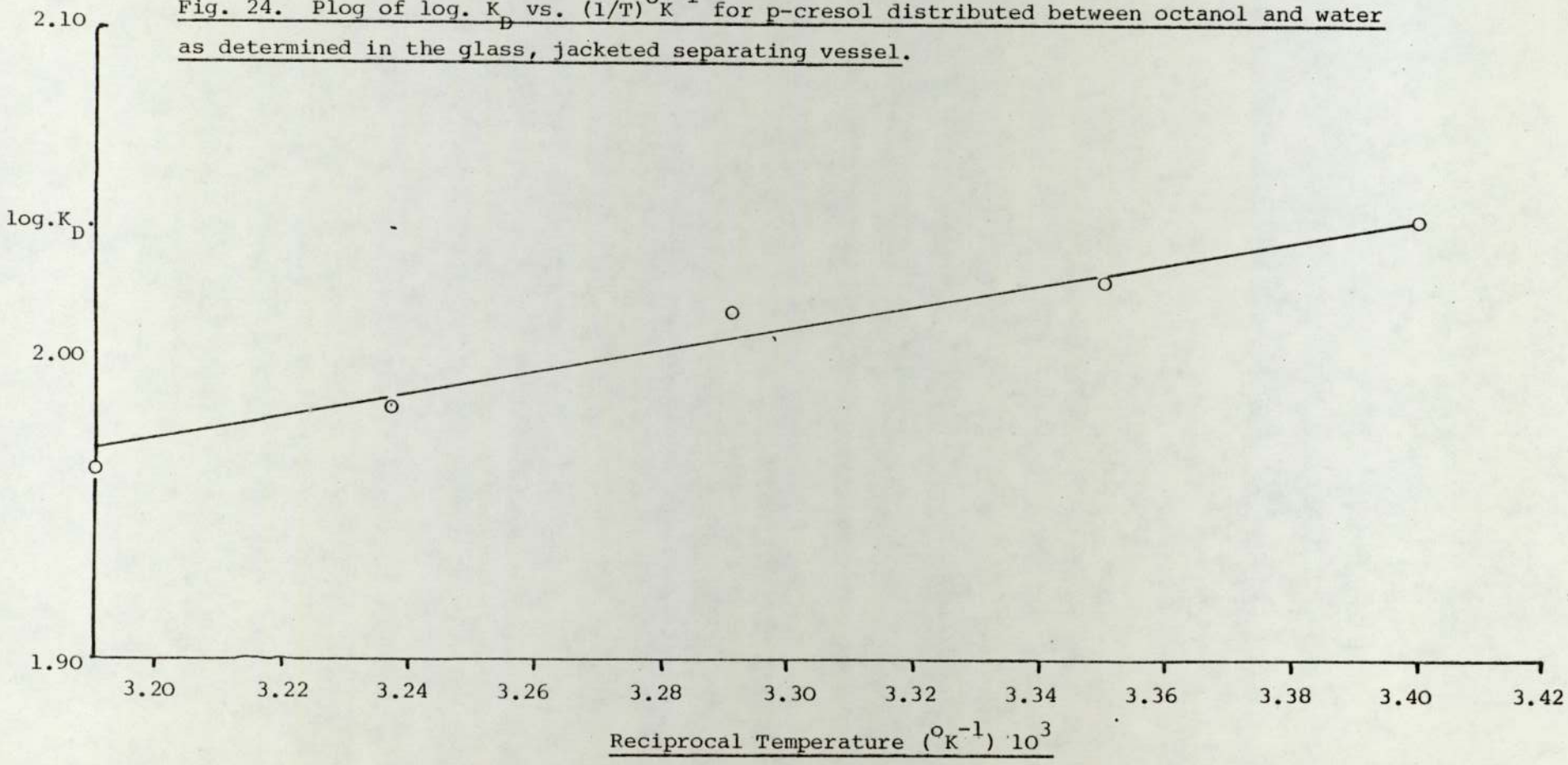


Fig. 24. Plog of log. K_D vs. $(1/T)^{\circ}K^{-1}$ for p-cresol distributed between octanol and water as determined in the glass, jacketed separating vessel.

3.4.2. a) SOLUTE CONCENTRATION.

The effect of concentration for the systems phenol distributed between (i) cyclohexane and water; (ii) octanol and water were studied, the results being given in table 13.

3.4.3. b) IONIC STRENGTH

The effect of varying the ionic strength of the aqueous phase by the addition of sodium chloride, was investigated for the systems:-

i) phenol distributed between cyclohexane and water.

ii) " " " octanol " "

iii) p-cresol " " " " "

iv) " " " cyclohexane " "

The results are tabulated in Tables 5, 6, 7 and 8 and plotted in Figs. 10, 11, 12 and 13.

3.4.4. c) TEMPERATURE

The following systems were studied with respect to this parameter:

Solute	Solvent system
Phenol	Octan-1-ol/Water
p-cresol	" "
Phenol	Cyclohexane/Water
o-cresol	" "
m-cresol	" "
p-cresol	" "
p-n-propylphenol	" "
3,4-xylenol	" "
3,5-xylenol	" "
2,3-xylenol	" "
2,4-xylenol	" "
2,5-xylenol	" "
2,6-xylenol	" "
3,5- diethyl phenol	" "
2,4,6-trimethyl phenol	" "
Quinalbarbitone	" "

Table 13.

The Effect of Solute Concentration and Nature of the Solvent on the Partition Behaviour of Phenol at 25°C

AKUFVE

SOLUTE CONCENTRATION

Phenol 25°C

Concentration added	P
Cyclohexane-Water	
5×10^{-5} M	0.28
6×10^{-5} M	0.23
7×10^{-5} M	0.21
8×10^{-5} M	0.23
9×10^{-5} M	0.22
10×10^{-5} M	0.22
Octanol-Water	
0.5×10^{-3} M	46
1.0×10^{-3} M	38.3
1.5×10^{-3} M	35.6
2.0×10^{-3} M	34.5
2.5×10^{-3} M	34.5

The results of these experiments are given in Tables 14 to 28 and derived thermodynamic data (i.e. enthalpy, entropy, free energy) are also given in Table 29. Graphical plots are given in figs. 25 to 40.

3.4.5. d) HOMOLOGOUS SERIES

As explained in the results section p.80 table 4 has been "constructed" to show the variation of partition coefficients within the homologues of the alkyl substituted phenols and some of their isomeric forms.

3.4.6. e) EFFECT of pH.

The pH partition profile for the weak base Propranololwas investigated. This drug was selected because its molar absorptivity in the aqueous phase is independent of pH. Both the apparent partition coefficients and the free base values are given in Table 30. These results are plotted in fig. 41.

Similar trends were observed for the solute quinoline in the octanol-water system but the inherent uncertainty in assaying this compound on-line over a range of pH renders only qualitative results.

3.4.7. f) EFFECT OF SOLVENT DENSITY AND PHASE VOLUME RATIO.

The effect of solvent density (ρ) on the efficiency of the centrifugal separator was studied using chloroform/cyclohexane mixtures. It was found that the AKUFVE gave unsatisfactory separation between ρ-solvent values of 0.88 to 1.05.

The AKUFVE is normally operated at a phase ratio of 1:1, however, we were able to perform experiments with water/oil ratios of up to 4:1. Since higher ratios may be required when dealing with very high or very low partition coefficients a modification to the normal experimental arrangement was devised and this is outlined in fig 42.

With this arrangement it was found that phase volume ratios of 10:1 and above could be employed, albeit at a slower equilibrium rate.

The effect of temperature on partition coefficients as determined on the AKUFVE, for the following systems:-

Table 14.

solute: p-cresol

solvent system: water-octanol

Temperature	K_D.	Log. K_D.
22.5°C	93.5	1.97
26.0°C	90.6	1.96
29.5°C	87.8	1.94
33.5°C	83.9	1.92
36.5°C	80.6	1.91
40.0°C	76.9	1.88
43.5°C	74.0	1.87

Table 15.

solute: phenol

solvent system: water-cyclohexane

Temperature	K_D.	Log. K_D.
20.4°C	0.19	- 0.72
24.2°C	0.22	- 0.65
27.4°C	0.24	- 0.63
30.5°C	0.26	- 0.58
33.9°C	0.30	- 0.53
36.8°C	0.31	- 0.51
40.0°C	0.33	- 0.48

Table 16

solute: o-cresol

solvent system: water-cyclohexane

Temperature	K_D.	Log. K_D.
24.9°C	1.33	0.12
28.7°C	1.48	0.17
32.8°C	1.56	0.19
35.5°C	1.65	0.22
37.9°C	1.81	0.26
40.0°C	1.86	0.27

Table 17

solute: m-cresol

solvent system: water-cyclohexane

Temperature	K_D.	Log. K_D.
22.9°C	0.56	- 0.25
26.8°C	0.63	- 0.20
29.3°C	0.71	- 0.15
32.2°C	0.83	- 0.08
35.5°C	0.94	- 0.03
41.1°C	1.01	- 0.00

Table 18.

solute: p-cresol

solvent system: water-cyclohexane

Temperature	K_D.	Log. K_D.
20.0°C	0.44	-0.36
23.2°C	0.64	-0.19
28.2°C	0.92	-0.04
31.7°C	0.92	-0.04
35.1°C	0.92	0.00
39.6°C	1.01	0.05
44.9°C	1.11	0.07

Table 19

solute: 2,3-xylenol
solvent system: water-cyclohexane

Temperature	K_D.	Log. K_D.
21.0°C	3.29	0.52
25.0°C	3.69	0.57
27.7°C	3.98	0.60
30.3°C	4.17	0.62
34.5°C	4.37	0.64
38.6°C	4.73	0.67
41.6°C	5.00	0.70

Table 20

solute: 2,4-xylenol
solvent system: water-cyclohexane

Temperature	K_D.	Log. K_D.
23.2°C	4.21	0.67
26.7°C	4.50	0.65
30.5°C	4.86	0.69
32.9°C	5.08	0.71
36.1°C	5.42	0.73

Table 21

solute: 2,5-xylenol
solvent system: water-cyclohexane

Temperature	K_D.	Log. K_D.
20.8°C	4.48	0.65
24.7°C	4.94	0.69
27.7°C	5.28	0.72
31.0°C	5.69	0.75
34.1°C	5.90	0.77
37.0°C	6.22	0.79
39.8°C	6.42	0.81

Table 22.

solute: 2,6-xylenol
solvent system: water-cyclohexane

Temperature	K_D.	Log K_D.
21.4°C	9.52	0.98
23.7°C	9.80	0.99
26.1°C	10.71	1.03
30.5°C	10.91	1.04
33.9°C	11.45	1.06
37.2°C	11.87	1.07
40.6°C	12.29	1.09

Table 23.

solute: 3,4-xylenol
solvent system: water-cyclohexane

Temperature	K_D.	Log K_D.
20.9°C	1.75	0.24
25.6°C	1.94	0.29
28.0°C	2.01	0.30
30.5°C	2.14	0.33
33.3°C	2.23	0.35
35.9°C	2.34	0.37
38.6°C	2.44	0.39
41.1°C	2.54	0.40

Table 24.

solute: 3,5-xylenol
solvent system: water-cyclohexane

Temperature	K_D.	Log K_D.
20.4°C	2.10	0.32
25.4°C	2.40	0.38
28.3°C	2.62	0.42
30.6°C	2.79	0.45
33.9°C	2.98	0.48
37.0°C	3.21	0.51
39.8°C	3.36	0.53

Table 25.

solute: 2,4,6-trimethylphenol

solvent system: water-cyclohexane

Temperature	K_D.	Log K_D.
20.7°C	30.75	1.49
24.3°C	32.3	1.51
27.4°C	34.5	1.54
30.5°C	34.5	1.54
34.1°C	35.9	1.55
39.3°C	37.9	1.58

Table 26.

solute: 3,5-diethylphenol

solvent system: water-cyclohexane

Temperature	K_D.	Log. K_D.
20.8°C	19.6	1.29
25.0°C	20.2	1.31
28.7°C	21.4	1.33
33.5°C	24.5	1.39
36.1°C	26.2	1.42
39.3°C	26.2	1.42

Table 27.

solute: phenol

solvent system: water-octanol

Temperature	K_D.	Log. K_D.
12°C	37.8	1.58
16°C	37.8	1.58
20°C	36.6	1.56
22°C	35.8	1.55
25°C	34.5	1.54
26°C	34.7	1.54
27°C	34.0	1.53
29°C	33.6	1.53
31°C	33.6	1.53
33°C	32.4	1.51

Table 28.

solute: p-n-propyl phenol

solvent system: water-cyclohexane

Temperature	K_D.	Log. K_D.
12°C	9.8	0.99
14°C	9.8	0.99
17°C	10.2	1.01
20°C	10.8	1.03
23°C	10.5	1.02
25°C	11.1	1.05
26°C	11.4	1.06
28°C	11.8	1.07
30°C	12.6	1.10
32°C	13.7	1.14

Table 29.

The Thermodynamic Parameters for the Transfer of various Phenolic Compounds between Water and Cyclohexane

(values of P were calculated using mole fraction concentration scale).

Compound	ΔG(kj/mole) at 25°C	ΔH/kj/mole)	ΔS(j/mole. deg.$^{-1}$)
Phenol	-0.48	+22.60	+77.4
o-Cresol	-5.00	+18.56	+78.9
m-Cresol	-3.65	+18.99	+75.9
2,3-Xylenol	-7.80	+15.60	+78.4
2,4-Xylenol	-8.06	+16.09	+81.0
3,4-Xylenol	-6.05	+17.82	+80.0

Fig. 25. Plot of log. K_D vs. $(1/T)^{\circ}K^{-1}$ for p-cresol distributed between octanol and water.

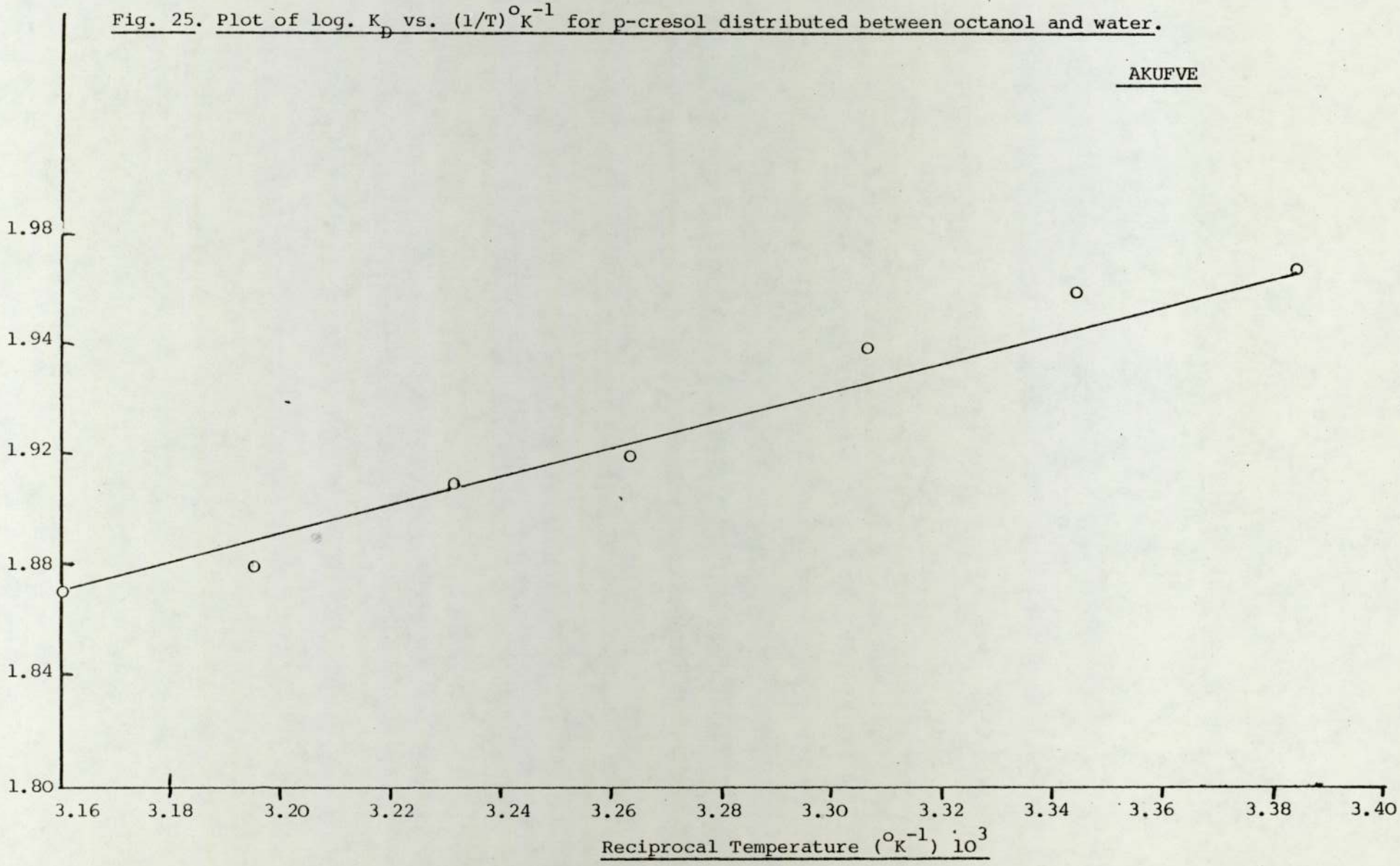


Fig.26.

Plot of log K_D. vs. $(1/T)^{o}K^{-1}$ for phenol distributed between cyclohexane and water.

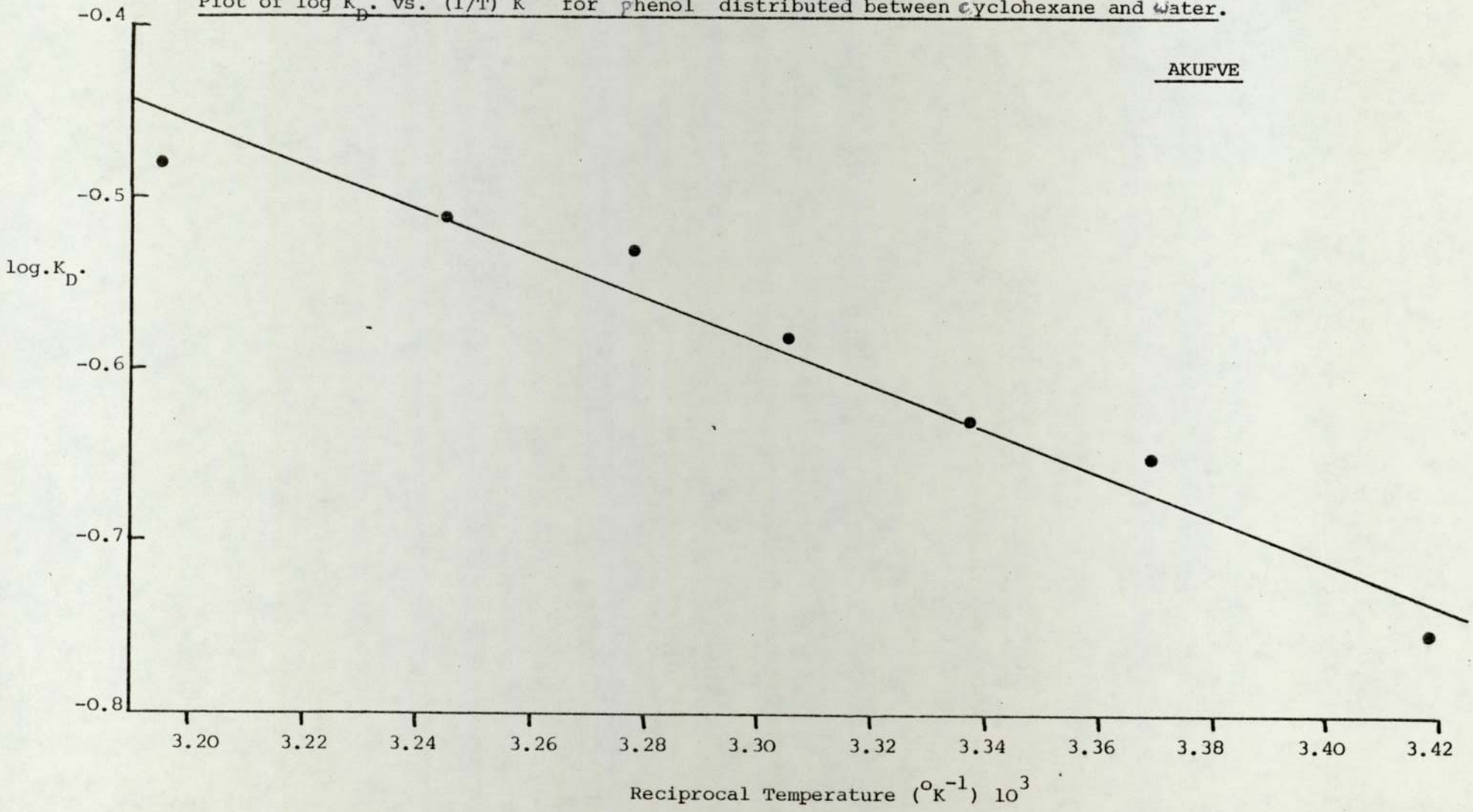


Fig.27.

Plot of log K_D.vs (1/T) $^oK^{-1}$ for o-Cresol distributed between Cyclohexane and Water

AKUFVE

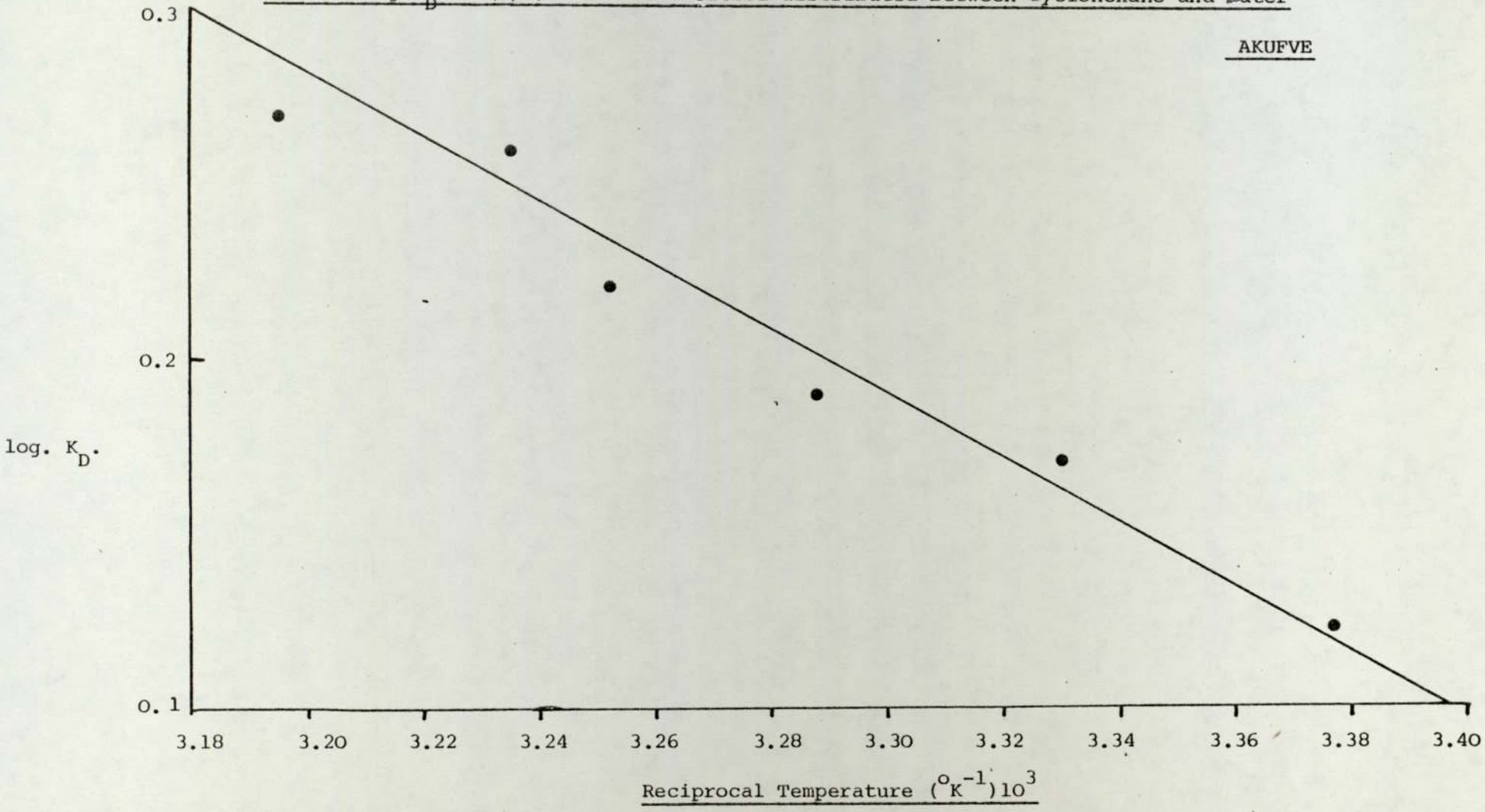


Fig. 28 Plot of $\log.K_D$. vs. $(1/T)^{o}K^{-1}$ for m-cresol distributed between cyclohexane and water.

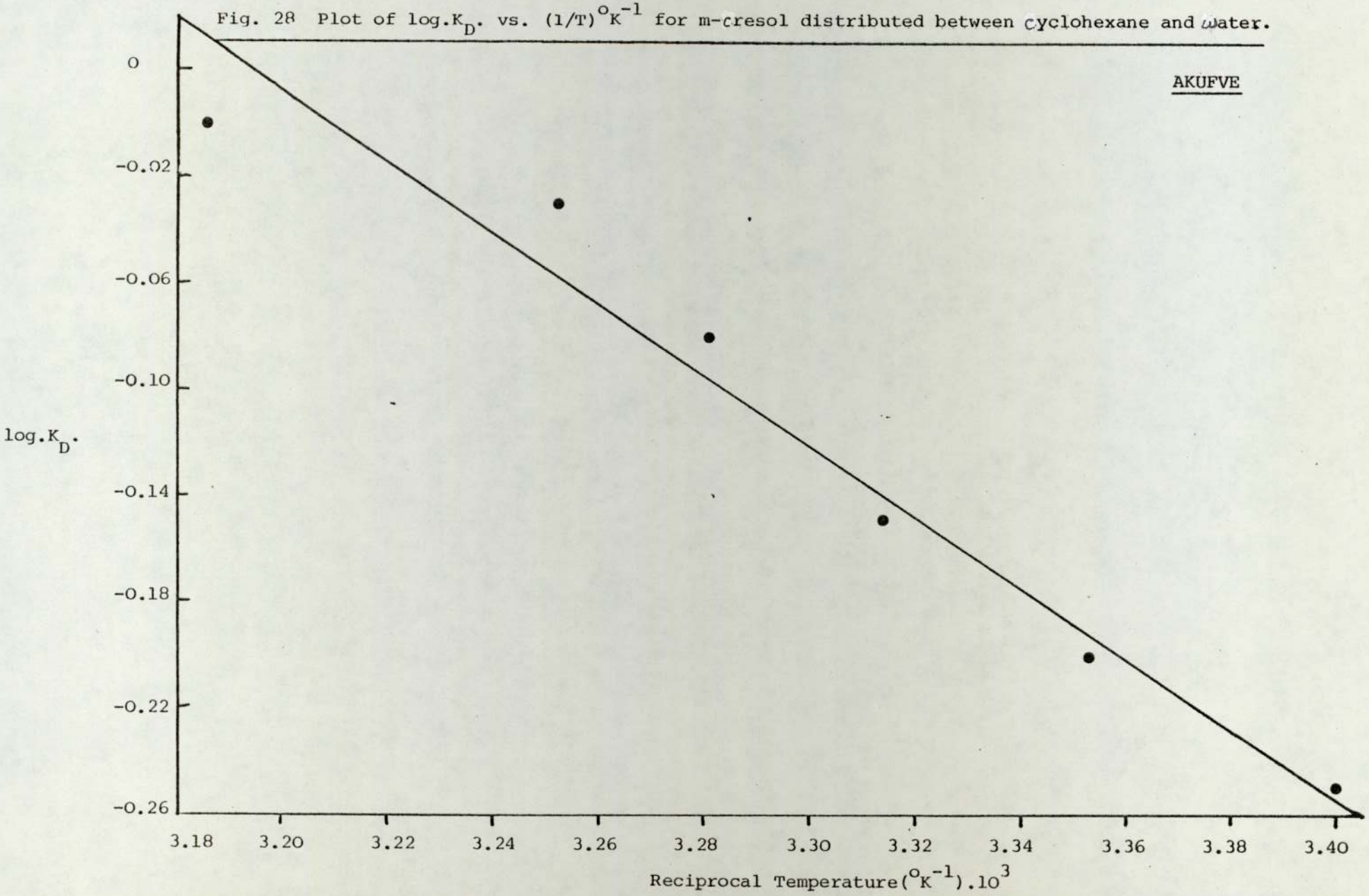


Fig. 29. Plot of log. K_D. vs. $(1/T)^{o}K^{-1}$ for p-cresol distributed between cyclohexane and water

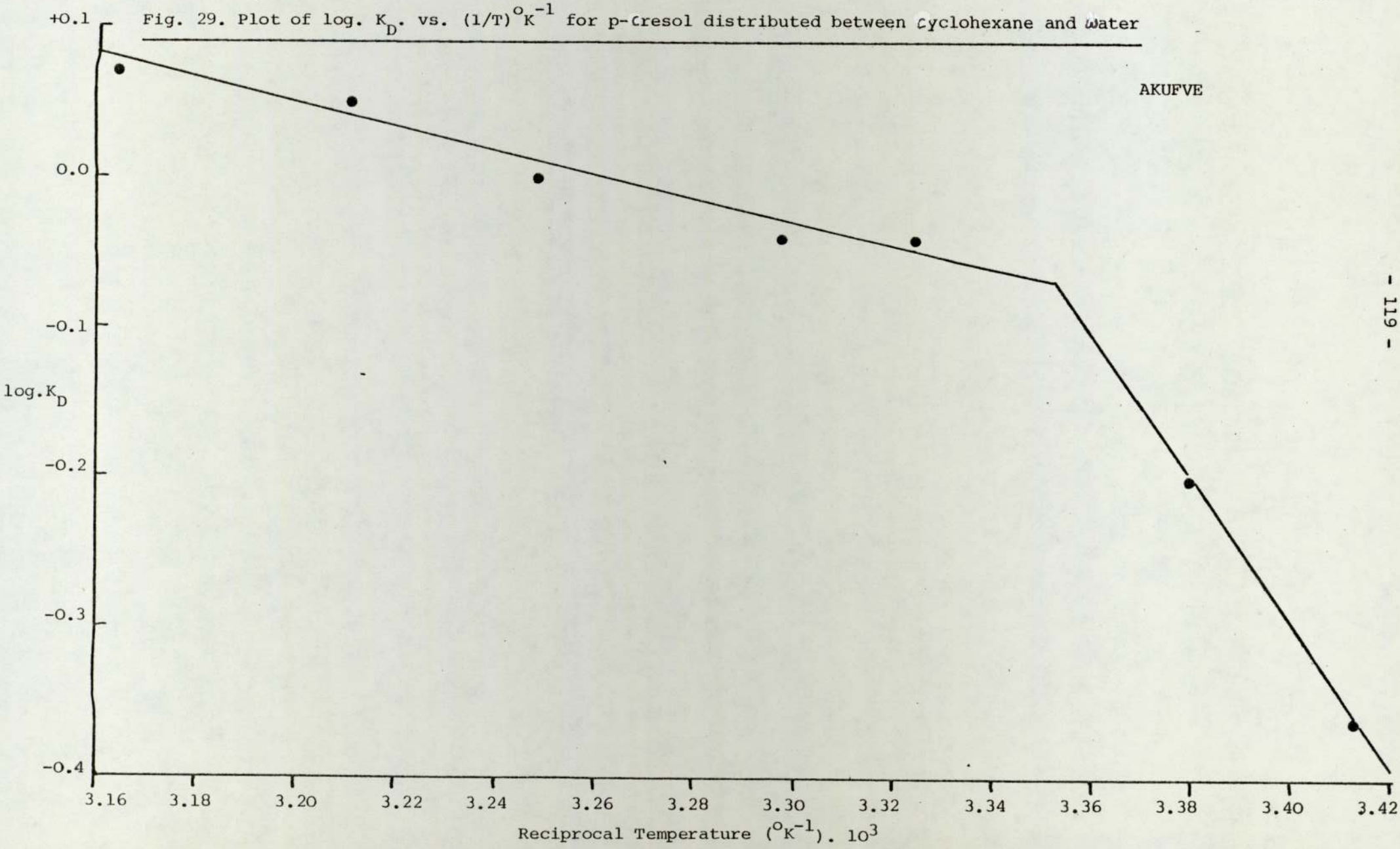


Fig. 30. Plot of log K_D. vs. $(1/T)^{\circ}K^{-1}$ for 2,3-xylenol distributed between cyclohexane and water.

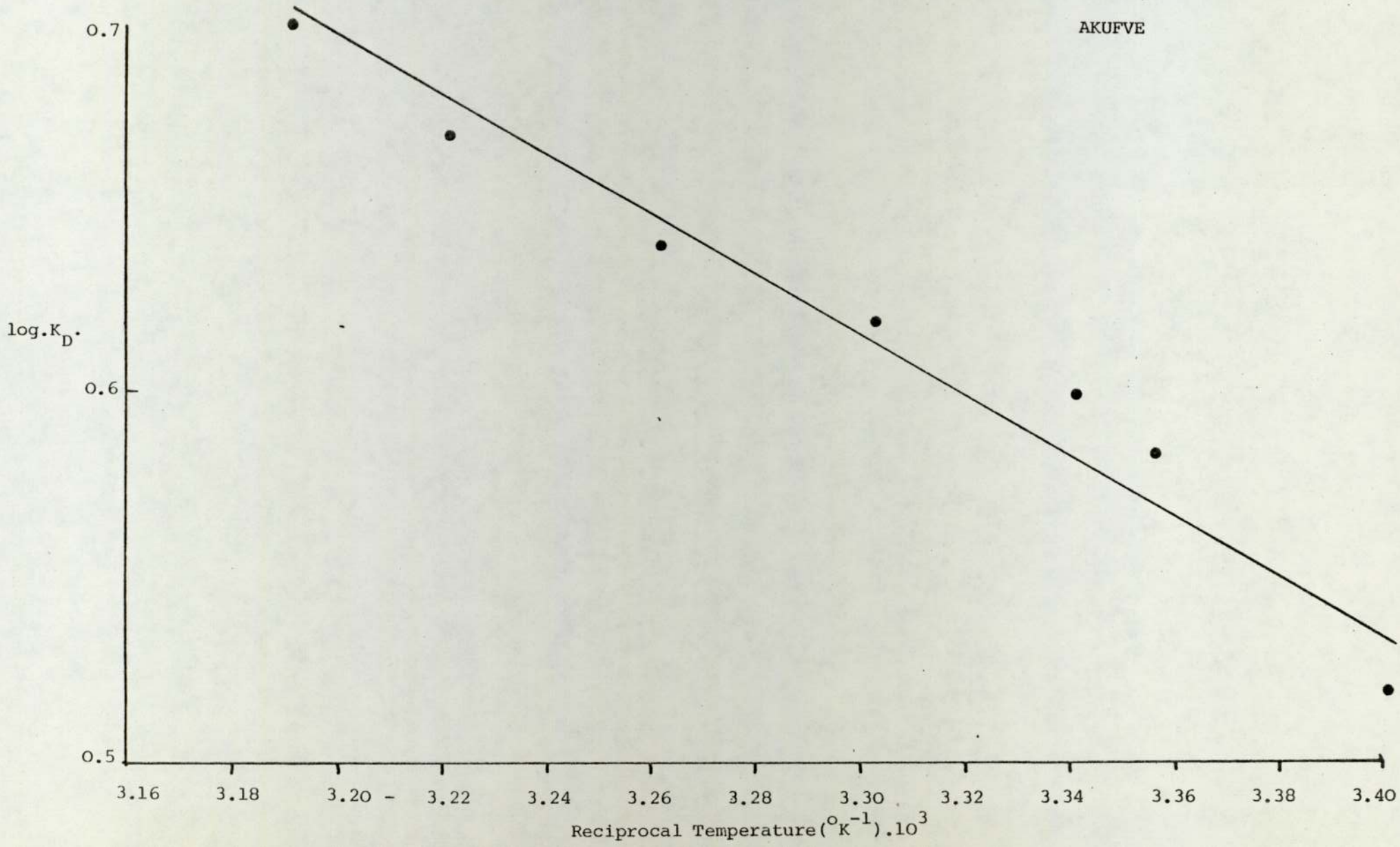


Fig. 31. Plot of log K_D. vs. $(1/T)^{\circ}K^{-1}$. for 2,4-xylenol distributed between cyclohexane and water

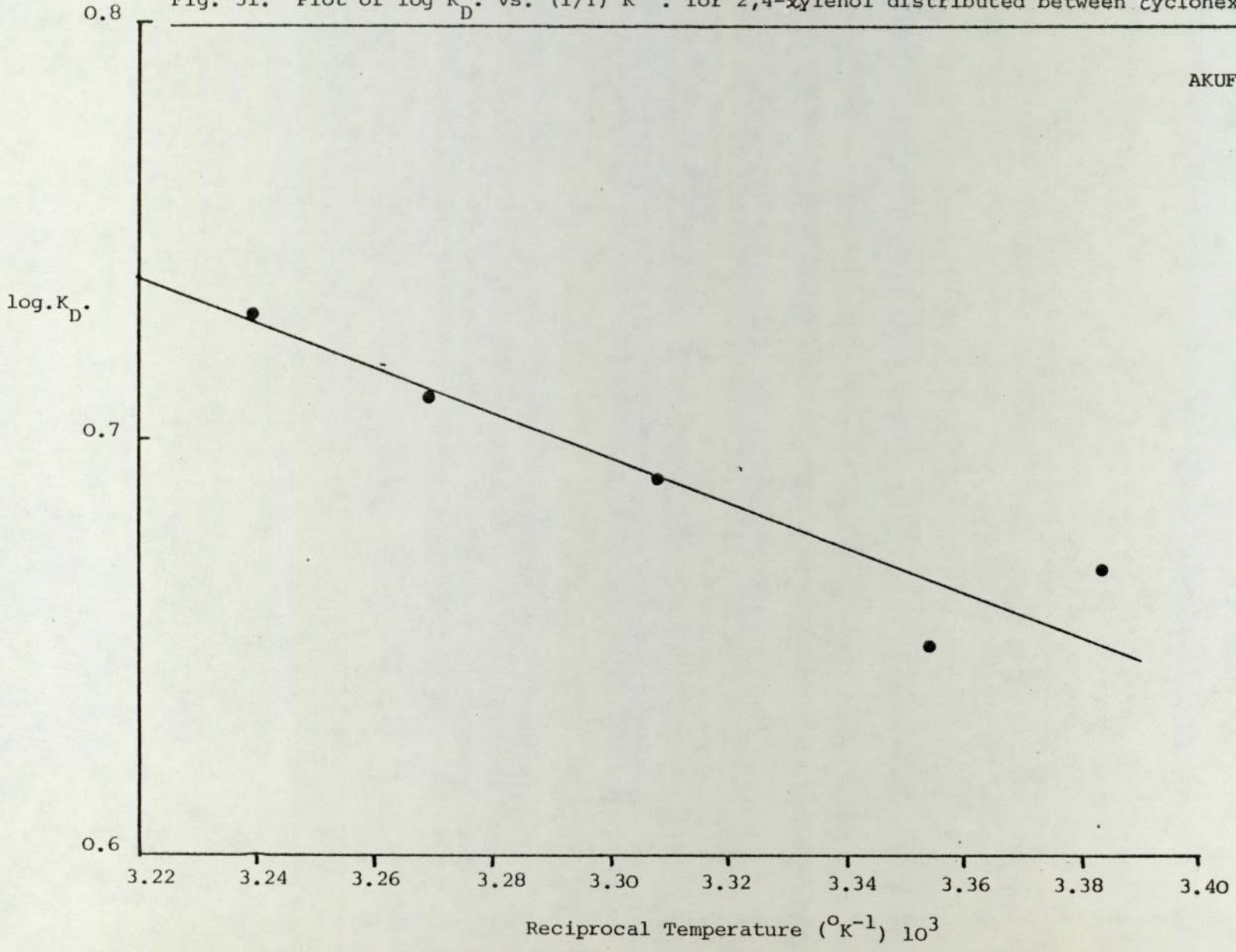


Fig. 32. Plog of log. K_D. vs (1/T) $^{o}K^{-1}$ for 2,5-xylenol distributed between cyclohexane and water

AKUFVE

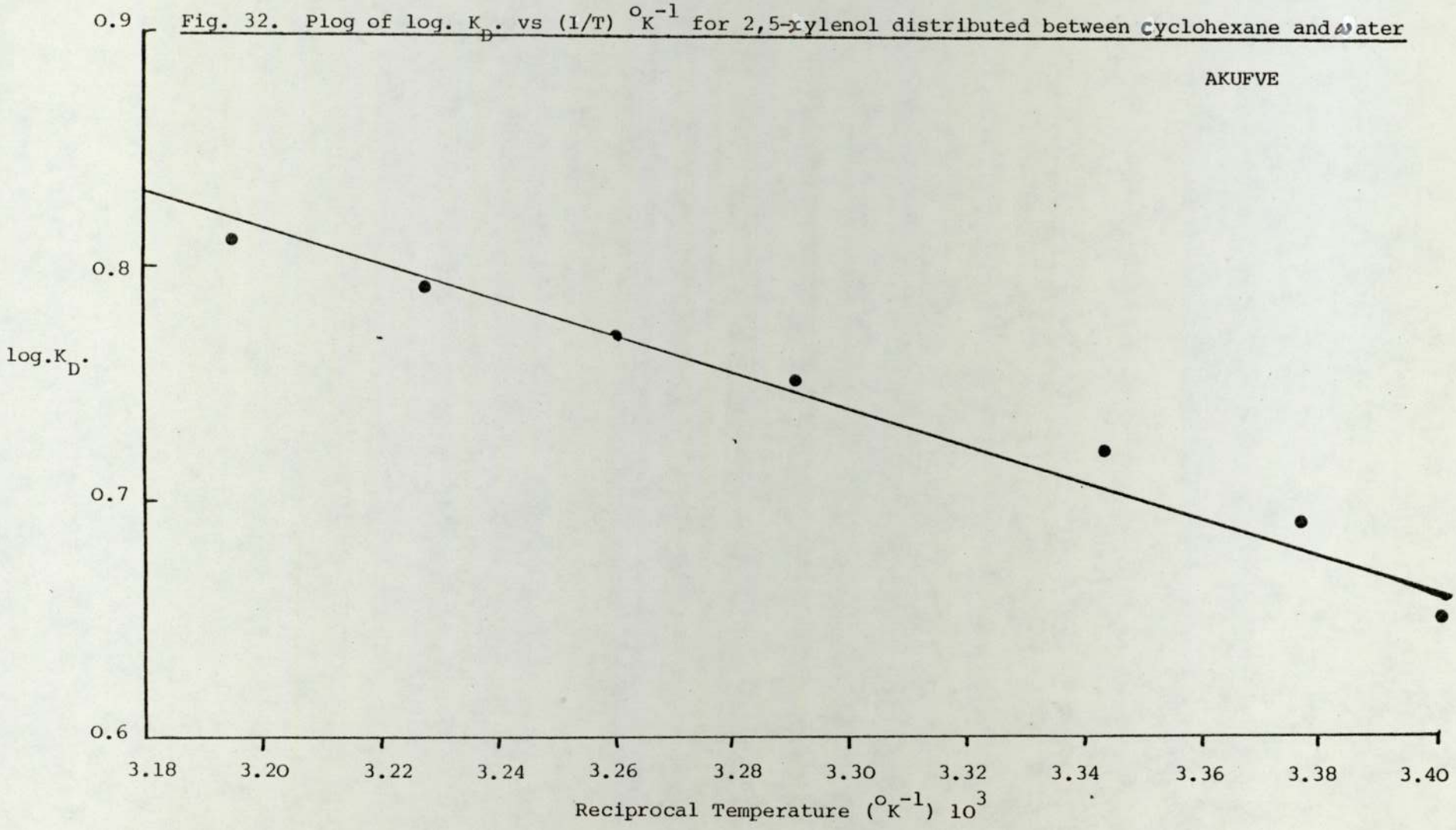


Fig.33. Plot of log K_D. vs. $(1/T)^{\circ}K^{-1}$ for 2,6-xylenol distributed between cyclohexane and water

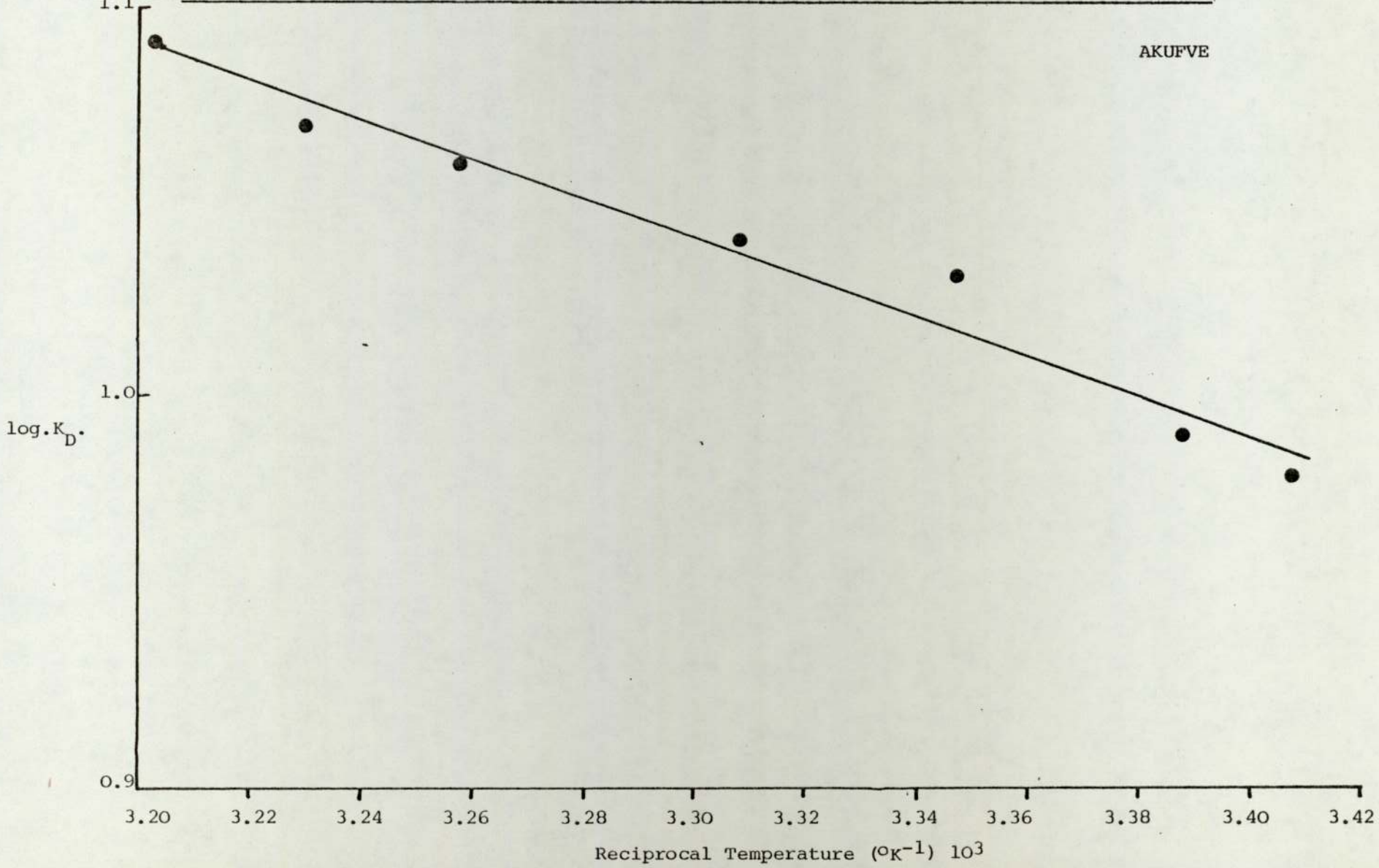


Fig.34. Plot of log K_D vs. $(1/T)^{\circ}K^{-1}$ for 3,4-xylenol distributed between cyclohexane and water

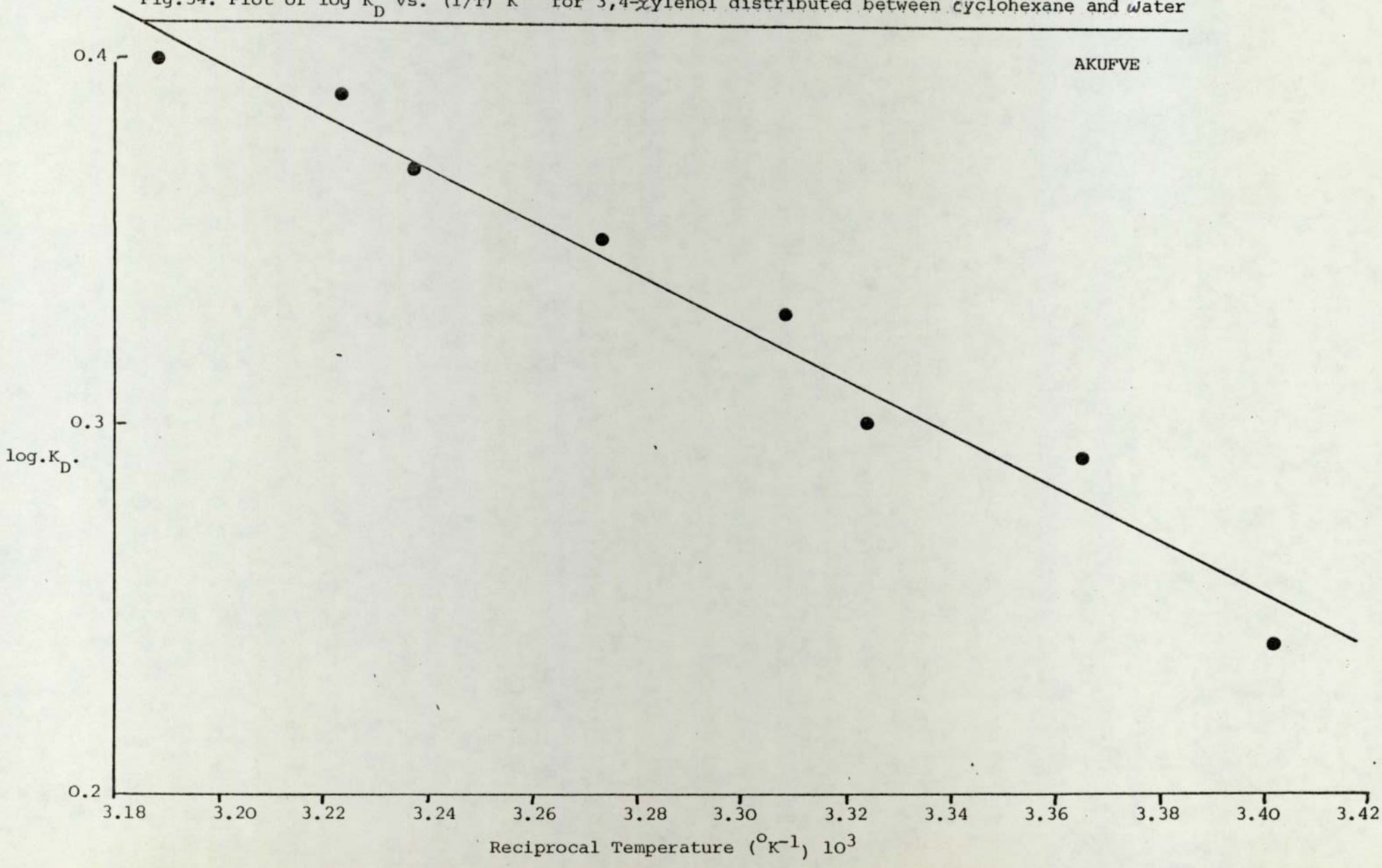


Fig. 35. Plot of log. K_D. vs. $(1/T)^{o}K^{-1}$ for 3,5-xylenol distributed between cyclohexane and water

AKUFVE

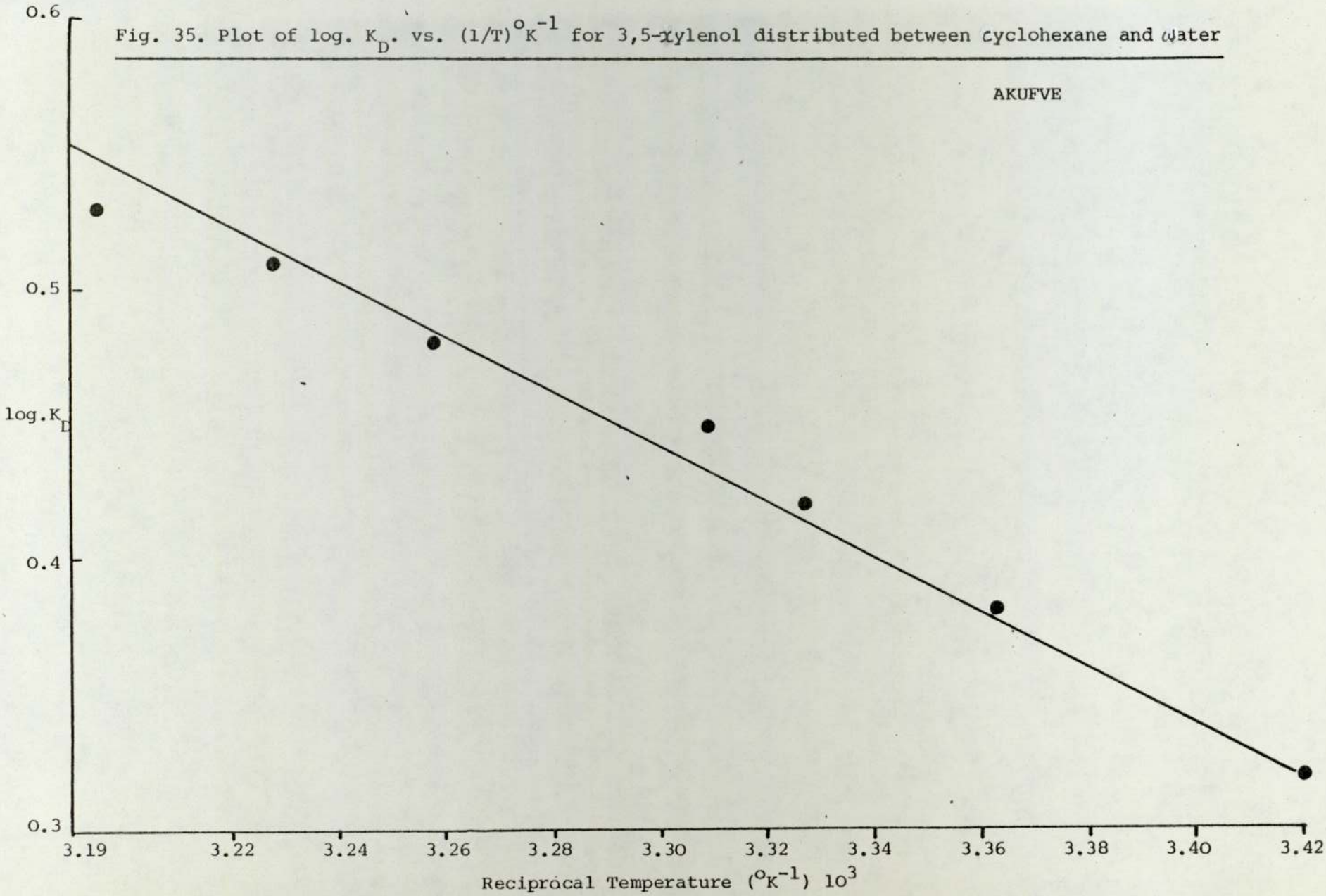


Fig. 36. Plot of log. K_D. vs. $(1/T)^{\circ}K^{-1}$ for 2,4,6-trimethylphenol distributed between cyclohexane and water

AKUFVE

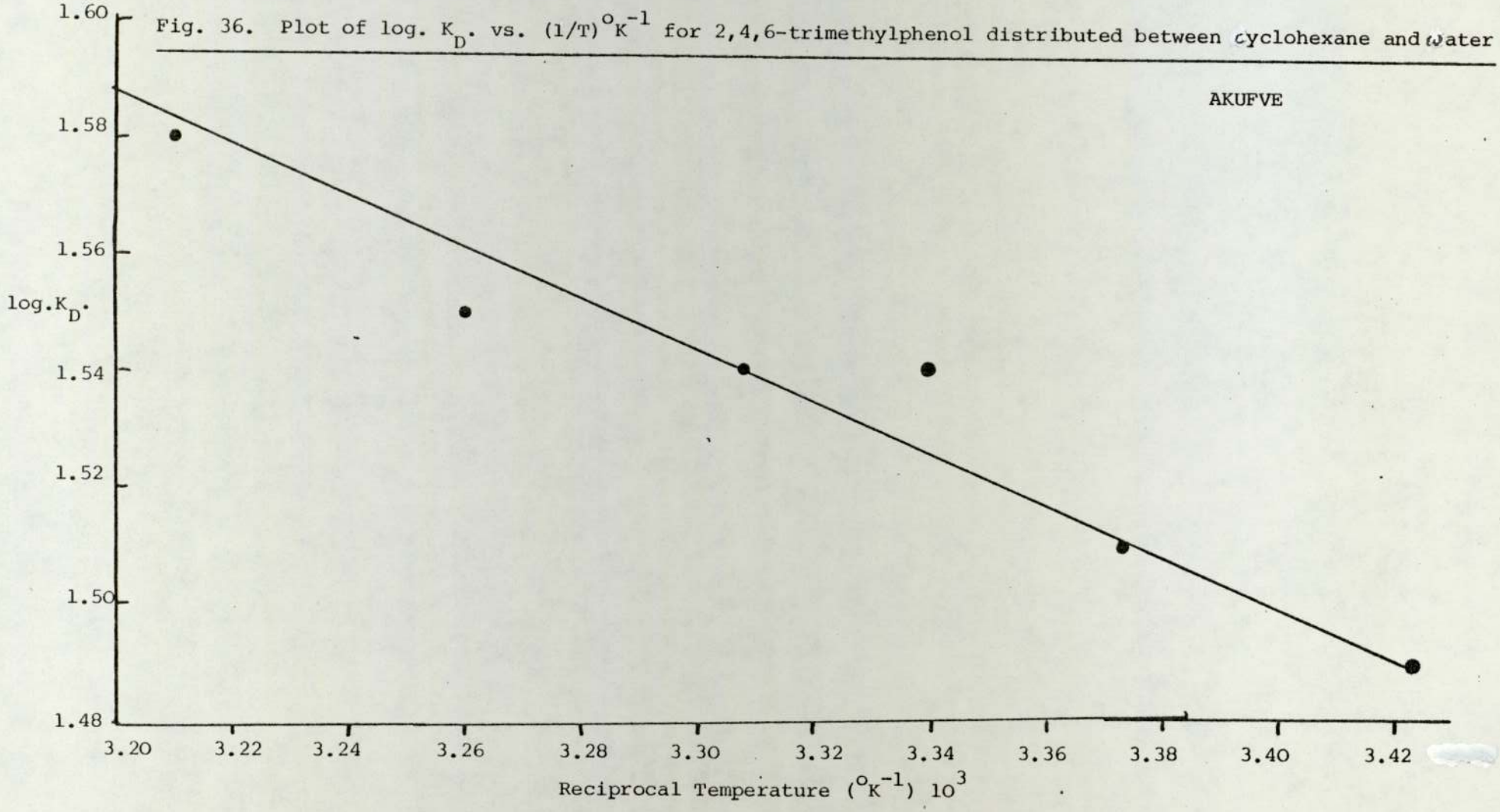


Fig. 37. Plot of log. K_D vs. (1/T) $^{o}K^{-1}$ for 3,5-diethyl phenol distributed between cyclohexane and water

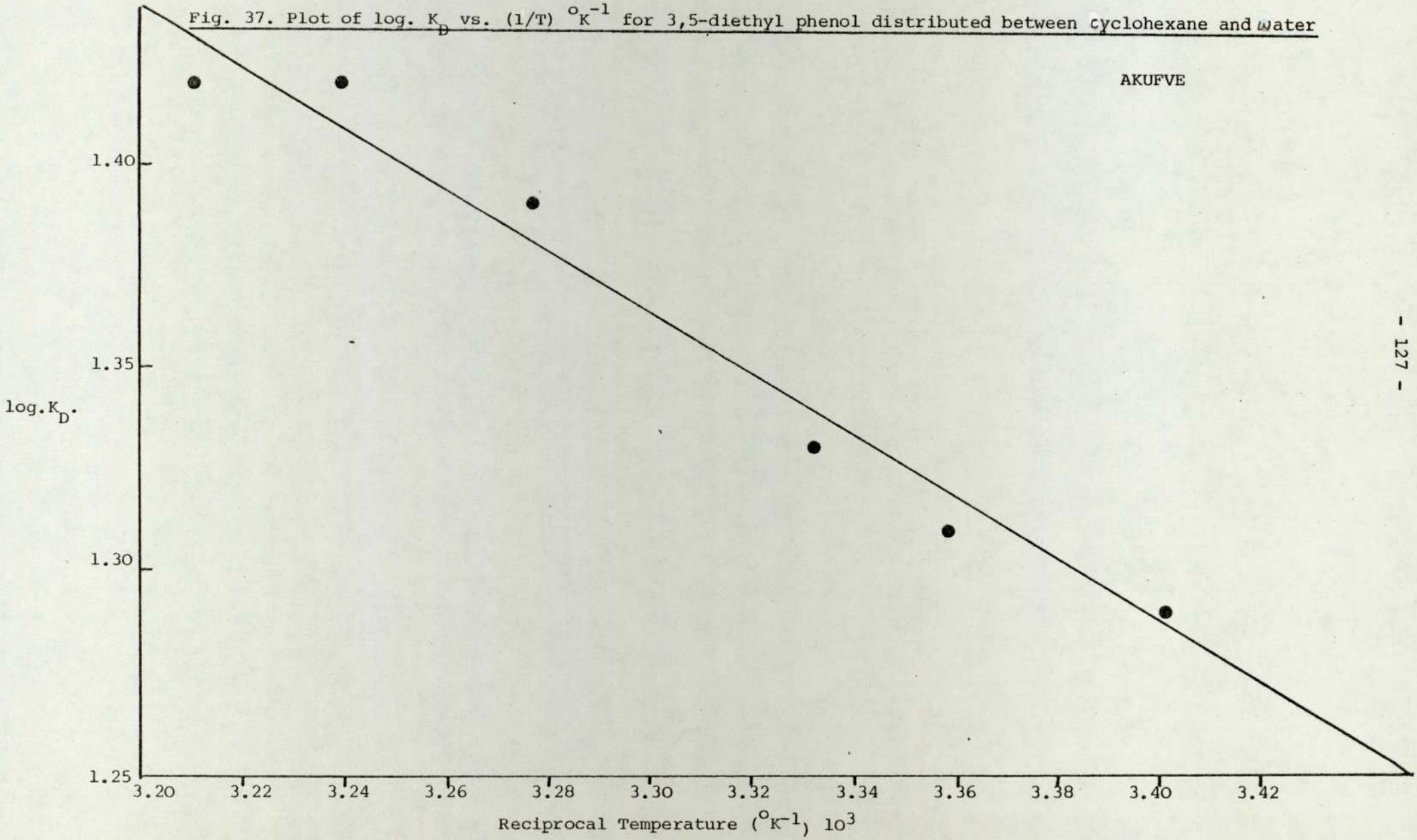


Fig.38. Composite plot of the effect of temperature on a homologous series of phenols distributed between water and cyclohexane, as determined on the AKUFVE apparatus.

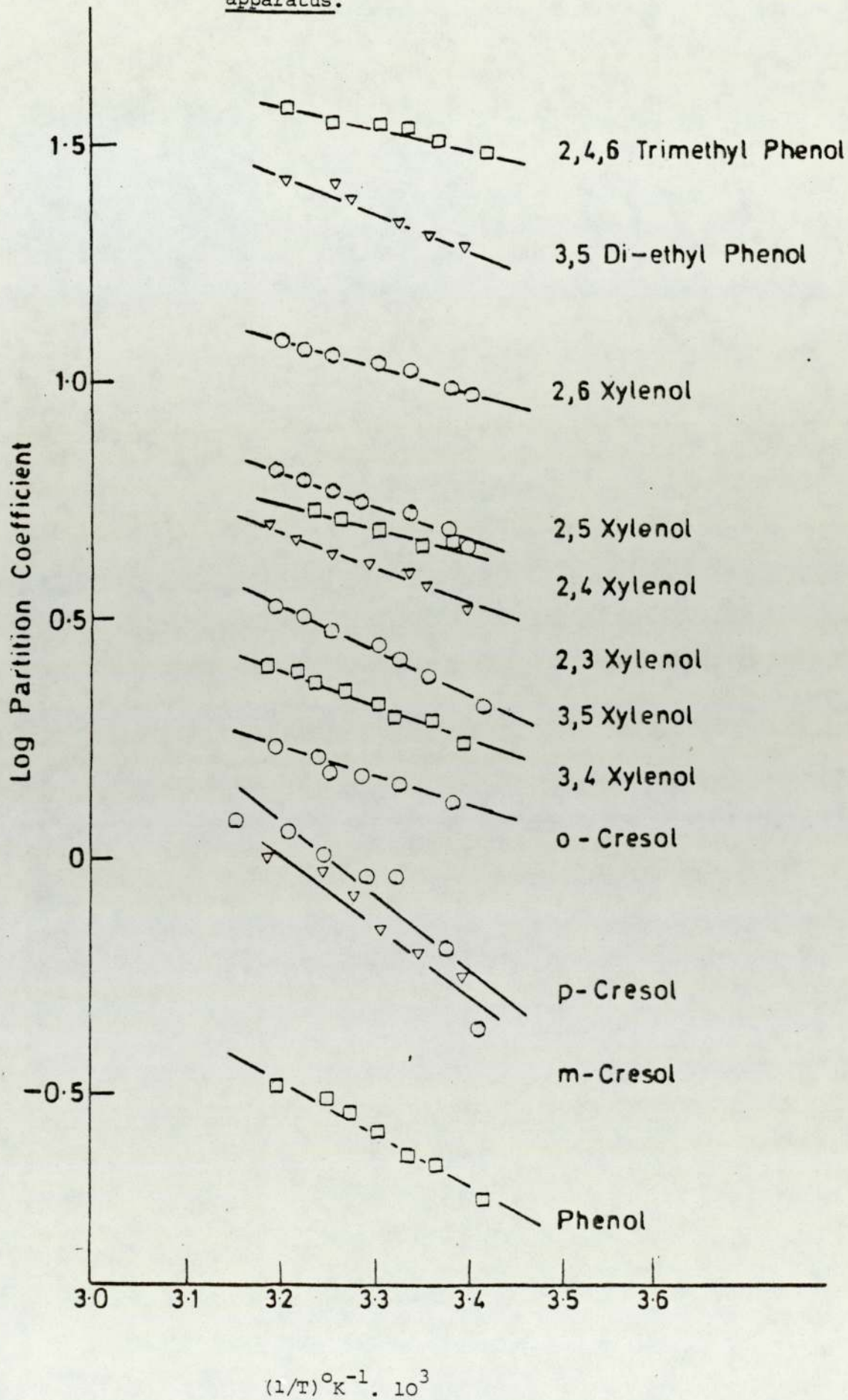


Fig. 39. The effect of temperature on the distribution of phenol between water and octanol as determined on the AKUFVE

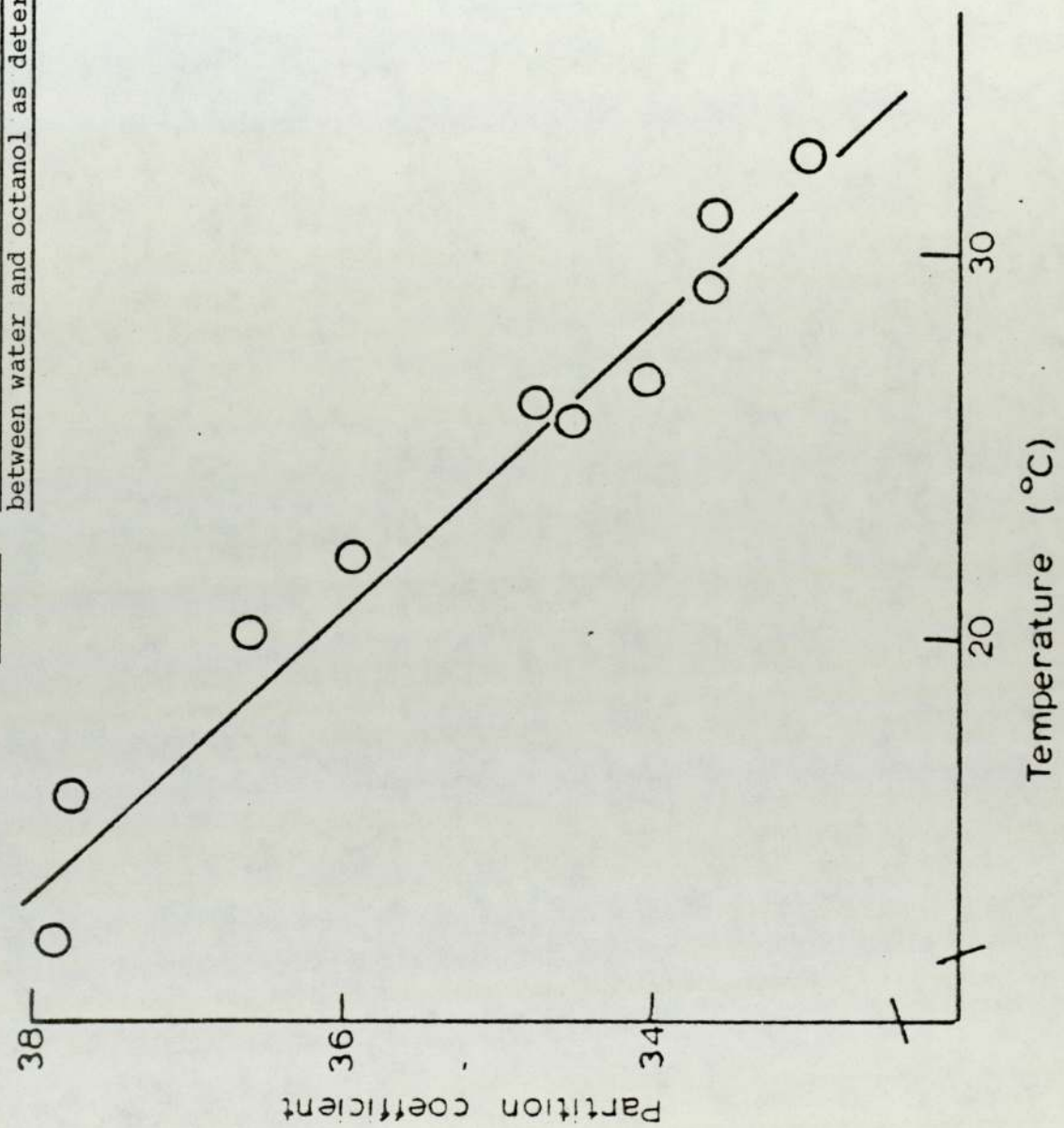


Fig.40. The effect of temperature on the distribution of p-n-propyl phenol between water and cyclohexane as determined on the AKUFVE

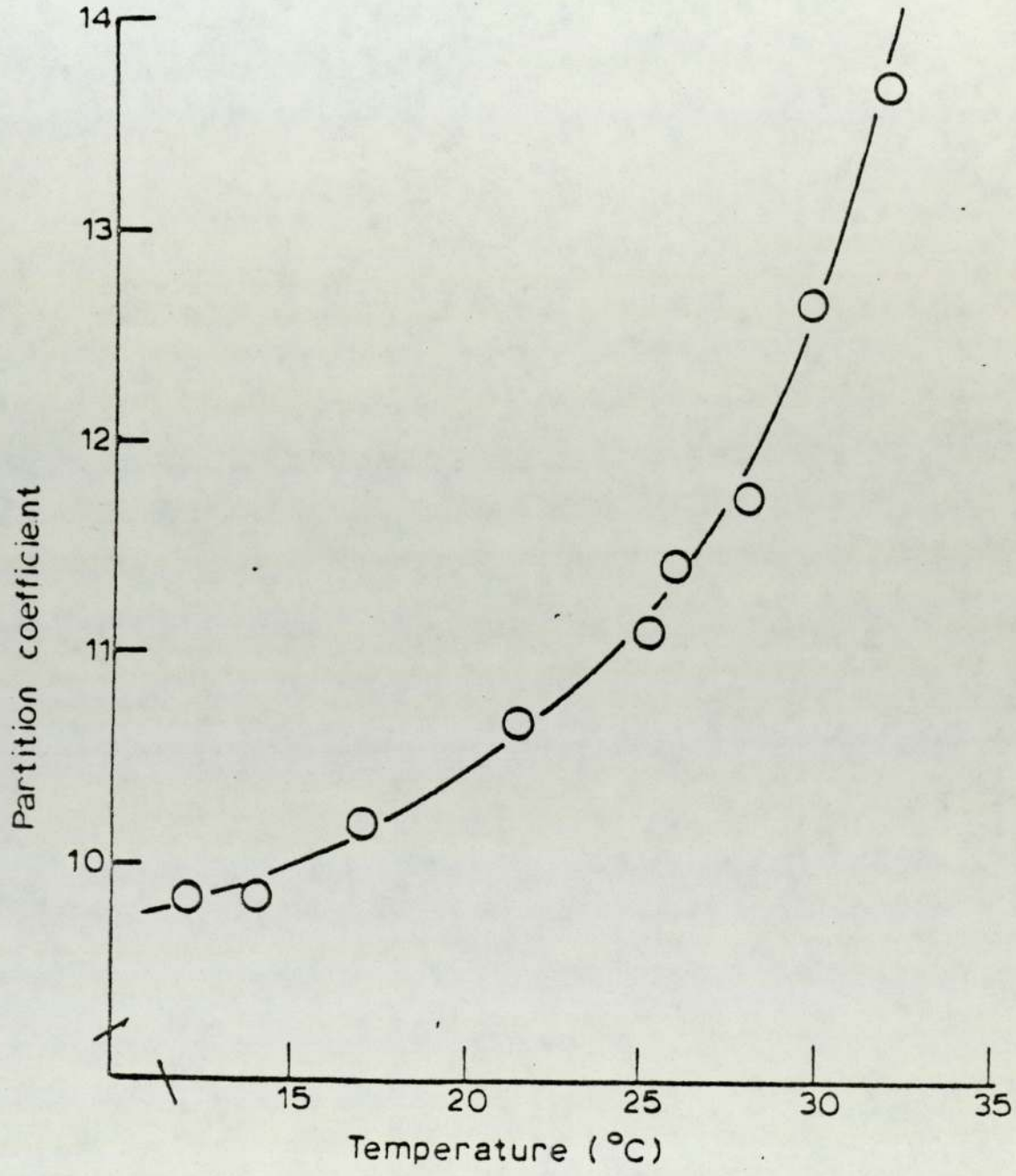


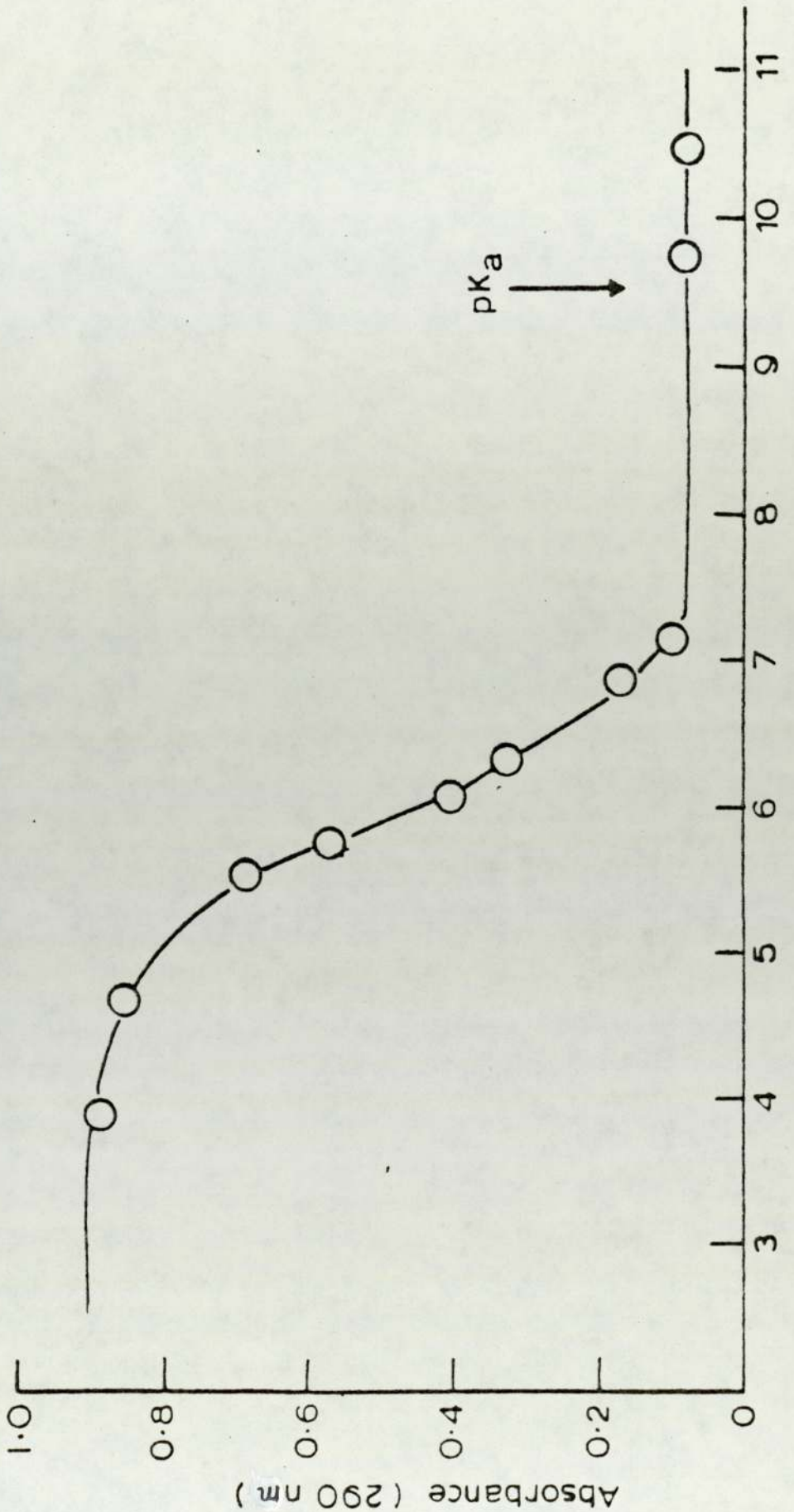
Table 30.

The Distribution of Propranolol between Water and 1-Octanol at 22.5°C

pH	log P apparent	log P (free Base)
4.65	-1.39	3.61
5.53	-0.41	3.52
5.75	-0.18	3.52
6.06	0.22	3.59
6.32	0.33	3.47
6.76	0.95	3.57
7.12	1.54	3.63
		3.56 ± 0.06
		P = 3631

Literature Values (Shake Flask)
log P = 3.33, 3,65

Fig.41. The effect of pH on the distribution of propranolol between water and octanol as determined on the AKUFVE.



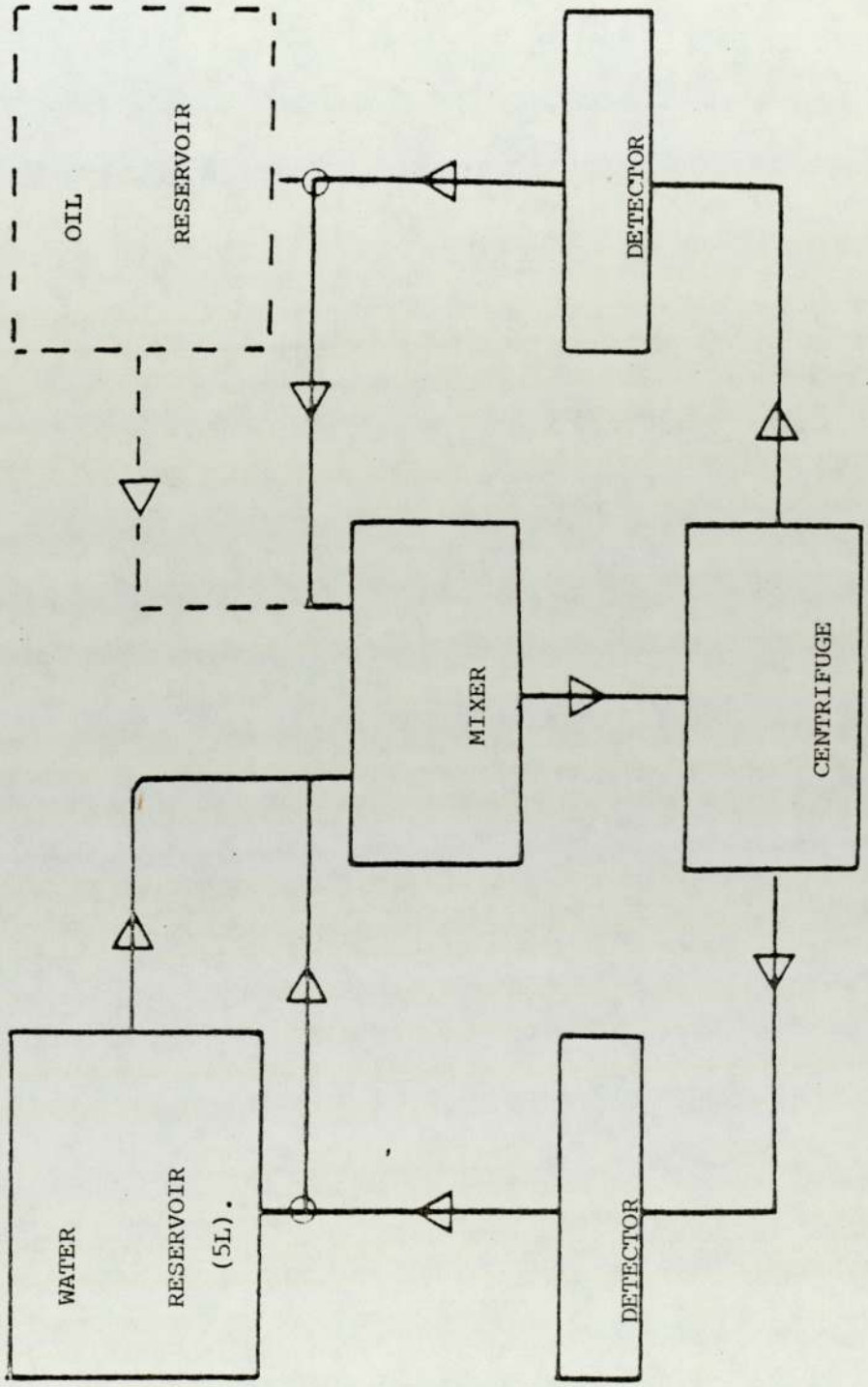


Fig. 42. AKUFVE system for high and low partition coefficients (Phase ratio 1:10 and above).

3.4.8. g) ION-PAIR EXTRACTION

The AKUFVE was also used to follow the extraction of the anionic drug, sodium cromoglycate, from aqueous phase into chloroform, by decylbenzyl dimethylammonium chloride at 25°C by varying the ionic strength of the aqueous phase using sodium chloride.

The phases were allowed to equilibrate for one hour at 25°C in the AKUFVE prior to the addition of any reagents. 100 ml of the saturated water phase was removed and used to make a 5×10^{-3}M solution of decylbenzyldimethylammonium chloride. 10 ml of the aqueous phase was then expelled and 10 ml of 5×10^{-3}M sodium cromoglycate added, the uv absorbance at 326 nm being recorded.

The 100 ml of 5×10^{-3}M decylbenzyldimethylammonium chloride was then added to the mixing chamber of the AKUFVE and the absorbance of the aqueous phase again noted.

Aliquots of sodium chloride were then added, the aqueous uv absorbance being noted after each addition, to produce ionic strengths over the range of zero to 0.065.

The results for this experiment are given in Fig. 43.

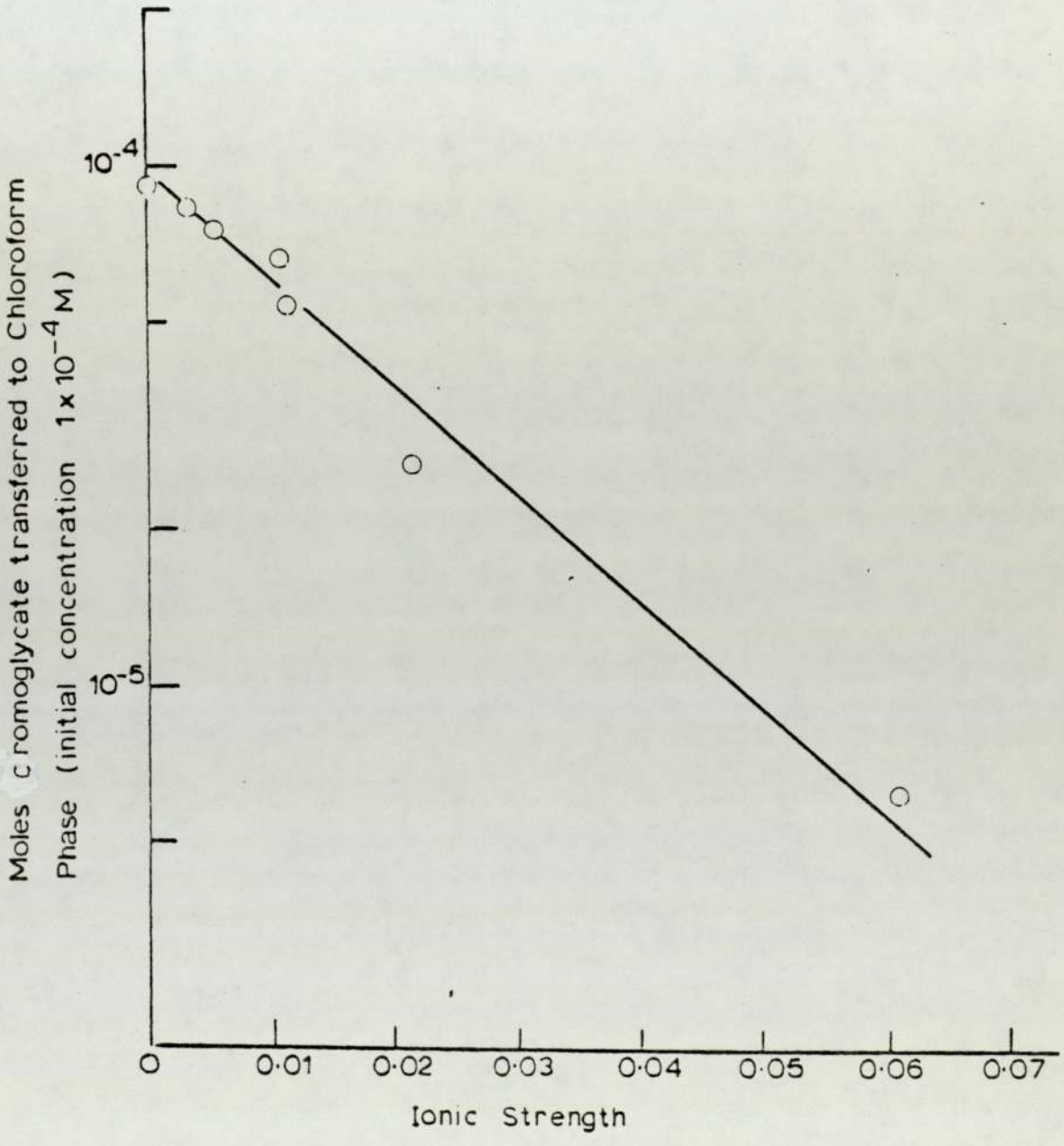
3.4.9. h) EXTRACTION OF UNSTABLE SPECIES.

Finally it was decided to study a kinetic system, one which like almost all such systems involving unstable species is impossible to study by normal shake flask procedures.

It has recently been shown, (Tomlinson, 1976) that the partition coefficients of such drugs may be obtained by a graphical analysis of time profiles of partitioning using a simple two phase diffusion cell model (c.f. Fig 44.)

The experimental system used for the AKUFVE system was the hydrolysis of the drug amoxycillin in 0.5N hydrochloric acid, and in situ partitioning between the aqueous and an isobutanol phase at 37°C.

Fig. 43. The ion-pair extraction of sodium cromoglycate into chloroform by an alkyl-ammonium chloride AKUFVE



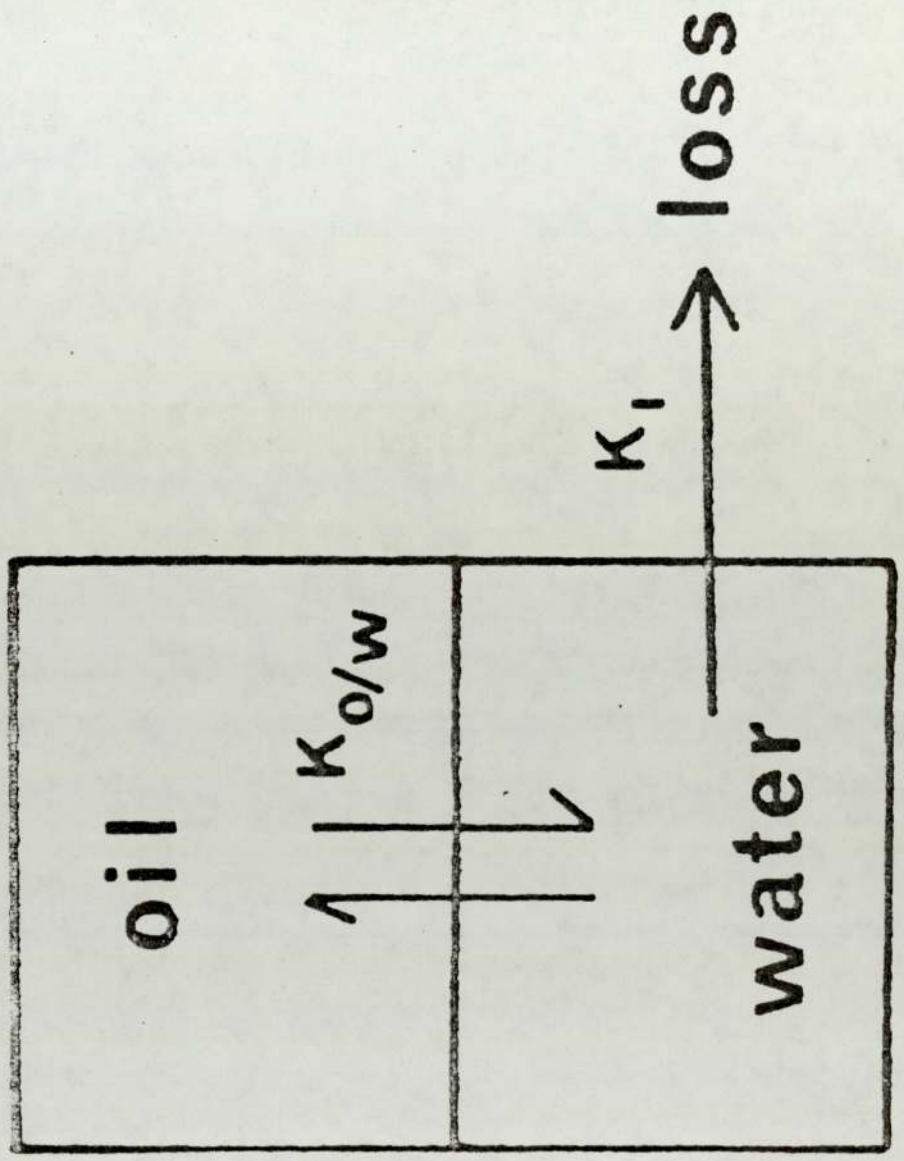


Fig. 44. The distribution of an unstable species between water and an oil phase.

The two phases were saturated and equilibrated at 37°C, in the AKUFVE overnight. 100 ml of the aqueous phase were removed and used to prepare a 0.4% w/v solution of amoxycillin. This was then returned to the mixing chamber and after two minutes a 1 ml sample was taken from the aqueous phase and assayed by the following colourimetric procedure which is specific for the original non-hydrolysed species. Samples of the aqueous phase were assayed at regular intervals over a period of 170 mins. The results are given in Fig. 45.

Assay procedure:

Reagents (prepared on the day to be used).

Reagent A.

2 parts 5 M hydroxylamine hydrochloride solution.

+ 2 parts ammonium acetate - sodium hydroxide buffer.

+ 8 parts of 95% ethanol.

+ 1 part of distilled water.

Reagent B.

200 g of ferric ammonium sulphate dissolved in 700 ml of distilled water + 96.4 ml of conc.sulphuric acid and then make up to one litre with distilled water.

Method.

1 ml of sample added to 4 ml of reagent A and allowed to equilibrate for 30 mins. 1 ml of reagent B then added and the optical absorbance at 485 nm. measured immediately after mixing.

A duplicate "blank" assay is simultaneously carried out on a 1 ml sample of the 0.5M hydrochloric acid being used as the aqueous phase.

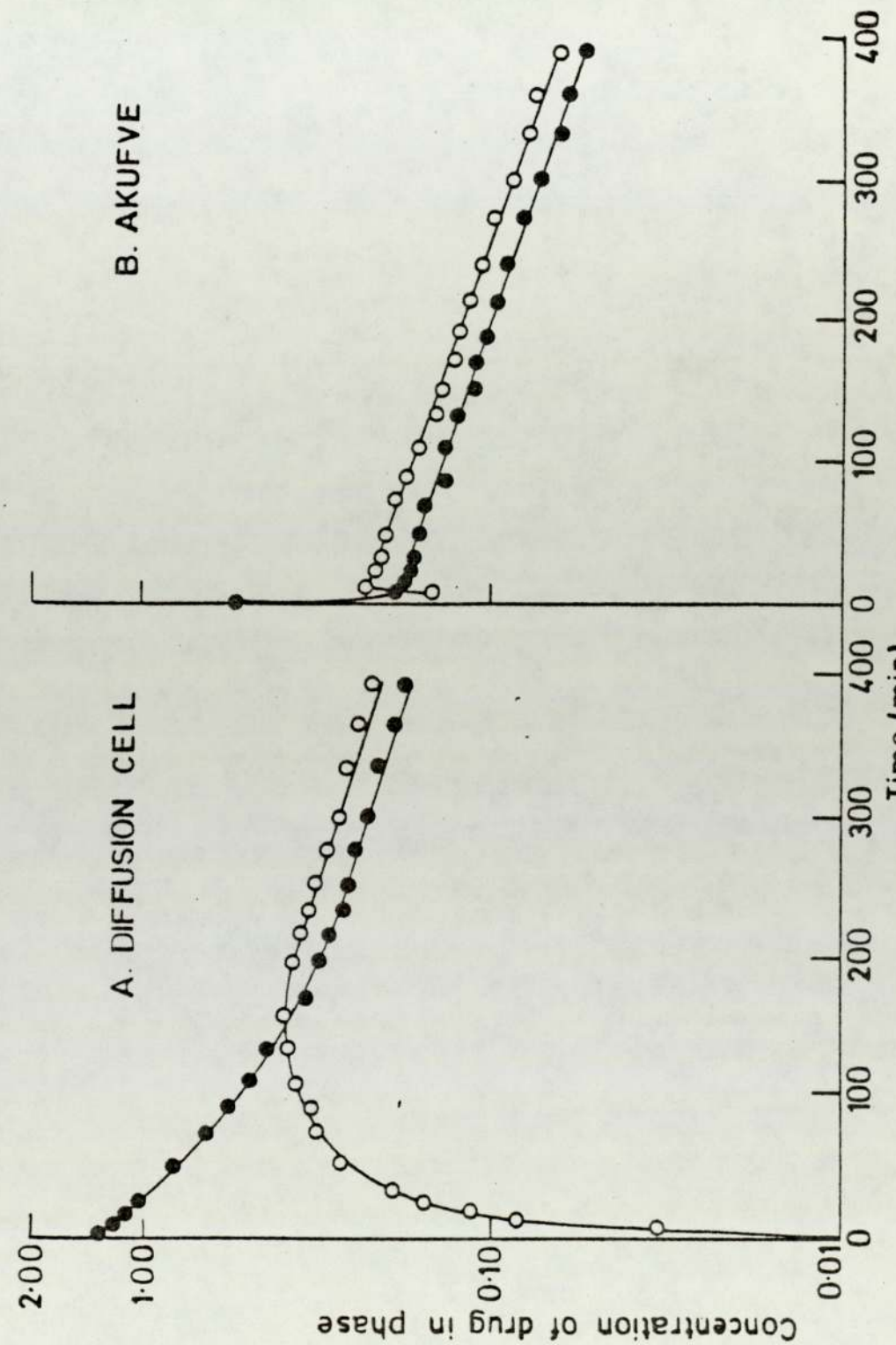


Fig. 45. The distribution of an unstable species (amoxycillin) in an organic (isobutanol) and aqueous (0.5N.HCL) phase with time at 37°C.

CHAPTER 4: DISCUSSION

4.1.1. a) EFFECT OF CONCENTRATION

For the system phenol distributed between either cyclohexane and water or octanol and water phases there appeared to be no change in partition coefficient with respect to initial concentration of the distributed species.

In both methods used the results (p.106) at the very lowest concentrations are somewhat anomalous. The nature of these anomalies suggests that equilibrium effects tend to play a part probably due to the different mixing techniques used in each of the systems.

Long term shake flask techniques (quoted in the literature) give values closely corresponding to those obtained on both the AKUFVE and the semi-continuous apparatus.

Since spectroscopic methods were used exclusively for the assay of all the compounds used in our studies and owing to the nature of these compounds solute concentrations were too low to give rise to the interactions which could produce variation of partition coefficient arising from any concentration effects.

4.1.2. b) EFFECT OF IONIC STRENGTH.

The results (pp 84-89) obtained for all three experimental methods although differing in absolute value, all tend to exhibit the same trends (slopes) albeit in some cases with slight discrepancies at the lower ionic strengths.

In all four systems studied there is a consistent increase in partition coefficient as the ionic strength of the aqueous phase is increased. This phenomenon is attributable to the fact that the added ionic species interacts with the solvent molecules and decreases their free energy thus giving rise to an increase in free energy and hence activity of the solute (drug) species (c.f. Gibbs-Duhem equation). In order to maintain the thermodynamic partition coefficient constant, solute is "salted" out of the aqueous phase into the organic phase i.e.

Gibbs-Duhem

$$X_1 d\mu_1 + X_2 \, d\mu_2 = 0$$

decreasing $X_1 d\mu_1$ causes a corresponding increase in $X_2 d\mu_2$. Now the thermodynamic partition coefficient is given by:-

$$K_D^X = \frac{X_o}{X_w}$$

Thus for any increase in X_w which is directly related to the aqueous phase activity (concentration), we get a compensating increase in X_o which is directly related to the organic phase activity (concentration).

4.1.3. c) EFFECT OF pH.

For the systems phenol, p-cresol in aqueous-octanol or aqueous-cyclohexane phases the partition coefficient (apparent) decreased rapidly above pH 9. At lower pH the partition coefficient remained constant, neutral molecules being preferentially partitioned into the organic phase, (pp 91-94).

It has already been shown (c.f. introduction) that for an ionisable species the true partition coefficient is given by the expression:-

$$K_D(\text{true}) = \frac{K_D(\text{apparent})}{(1 - \alpha)}$$

$$\text{where } \alpha = \frac{1}{1 + \text{antilog}(\text{pH}-\text{pKa})}$$

for a base

Hence the partition coefficient will only appear constant when the undissociated form predominates in the partition process.

In the cyclohexane system the apparent partition coefficient falls quite rapidly over a small range of pH and since K_D (apparent) falls when most molecules are ionised one can deduce from the graphs that for both phenol and cresol pKa values lie between 9 and 11. In the case of octanol, however, the change in partition coefficient with pH is not so sharply defined as for cyclohexane e.g. using cresol, K_D (apparent) seems to decrease from pH 6 to 11 gradually. The differencein the two systems suggests that other factors, for example, the partitioning of other than neutral species may also be affecting the partitioning of the phenols in octanol.

This is even more apparent in the results (pp 131-132) attained on the AKUFVE for the pH-partition profile for the weak base propranolol hydrochloride. This drug was selected for study because its molar absorptivity in the aqueous phase is, unlike other systems studied e.g. quinoline, independent of pH.

At low pH, where all the drug is ionised, it is contained totally in the aqueous phase. As the pH is increased the proportion of unionised form increases and the drug is contained more and more in the organic phase.

As can be seen almost total extraction of the compound into the organic phase occurs, anomalously, well below the pK_a of the free base (50 per cent of ionised and unionised species). This will be due to the fact that the hydrochloride was used and the primary dissociation of this species into free base and hydrochloride ionic species would tend to mask the true partitioning process:-

$$|Pr^- + H^+| \quad |H^+ + Cl^-| \rightleftharpoons |PrH| \quad HCl \rightleftharpoons |PrH|$$

totally ionised species. pH6-7 base hydrochloride pH9-10 free base.

However, since all the available results for comparison were determined classically for the hydrochloride the AKUFVE data were used directly as a comparison and were accordingly found to be remarkably consistent.

Results obtained for quinoline distributed between water-octanol and water-cyclohexane have not been recorded since the complex variation of molar absorptivity with pH made interpretation of the data extremely difficult and therefore somewhat tenuous.

4.1.4. d) EFFECT OF TEMPERATURE.

As can be seen from the results obtained (pp.97-130) the derived partition coefficient values are not "approximately independent of temperature" (c.f. also Crugman, 1971) as has sometimes been claimed. For phenol distributed between water and octanol a 20°C increase in temperature gives a 14 per cent decrease in partition coefficient. Similarly for cresol in the same solvent system there is a 20 per cent decrease in partition coefficient for a temperature rise of 20°C.

In contrast for all those drugs distributed in the aqueous - cyclohexane system the partition coefficient increases with temperature.

Such changes in partition coefficient reflect the thermodynamic properties of the various species and the nature of solute - solvent interactions.

Cyclohexane is a simple non-polar solvent where these interactions will be relatively simple as well as minimal in effect. Octanol however, is a much more complex polar solvent that is extensively hydrogen bonded. Many different solute-solvent and solvent-solvent interactions are possible that can change markedly with temperature e.g. monomeric octanol has been reported to be in equilibrium with tetrameric octanol (Hansch 1971) and this equilibrium will change with temperature. Octanol in equilibrium with an aqueous phase also contains a large quantity of water (27 percent water on a mole fraction basis) and can be considered more properly as a ternary system.

The results given in Table 29 for the Gibbs free energy, enthalpy and entropy of transfer from water to cyclohexane were calculated using the methods outlined in the introduction to this thesis (theory section). The thermodynamic partition coefficients (related to mole fractions) were naturally used for these calculations.

It is interesting to see how the high enthalpy of transition, due presumably to strong hydrogen bonding interactions in the aqueous phase particularly for the least sterically hindered structures of a given series, can be offset by the change in entropy which accompanies the transition from a highly structured solvent-solute structure, arising from the hydrogen bonding in the aqueous phase, to a non-structural arrangement in the cyclohexane phase. These two "opposing" processes determine the overall free energy and hence magnitude of the partitioning process.

In the series phenol, o-cresol, m-cresol the oxygen atom in the phenol molecule will have the highest electron density in this series (from basic "resonance" theory) since neither o-cresol nor m-cresol can donate electronically to the hydroxyl group, the former due to its lack of co-planarity and the latter owing to the electronically unfavourable arrangement.

Additionally the hydroxyl groups on o-cresol will undergo steric hindrance to hydrogen bond formation and this explains its relatively low enthalpy of interaction in this series. Phenol will have the highest interaction energy owing to its non-hindered high electron density hydroxyl grouping. The m-cresol has a lower, non-hindered energy of interaction which is intermediate between that of phenol and o-cresol.

Further examination of these phenomena shows that the degree of solvent molecule structure around the solute molecules in the aqueous phase decreases in the sequence o-cresol > phenol > m-cresol, a fact which is reflected in the results for the entropy of partitioning for these three compounds, (Gould, 1959).

A similar argument can be applied to the series 2,3; 2,4; 3,4 - xylenols although owing to the increased steric effects of the extra functional groups the enthalpy of partitioning will tend to be lower and the entropies of partitioning higher for this series than for the mono-substituted phenols, and this is also born out in the results.

4.1.5. e) EFFECT OF CHEMICAL STRUCTURE AND SOLVENT SYSTEM.

Considering first the data given (pp. 80-83) in tables 2 and 3 (plotted in Figs. 8 and 9) for the alkylated phenols it can be seen that all three methods (in those cases where experiments have been performed) and the literature values show the same trend i.e. the partition coefficients were found to be higher in the octanol-water system than in that of the cyclohexane - water, however, the change in partition coefficient with each addition of a methylene group to the homologous series was greater for cyclohexane than for octanol.

In the cyclohexane - water system, the organic phase has a minimal hydrogen bonding ability. Octanol however, has a terminal hydroxyl group which can hydrogen bond with the hydroxyl of the phenol compounds. Octanol can act as both donor and acceptor of hydrogen bonds and in so doing with the solute decreases the energy required to transfer the solute molecules from the aqueous to the organic phase.

Octanol dissolves 2.3 moles of water per litre at saturation due to its polar nature. In contrast, cyclohexane dissolves only 2.5×10^{-3} moles of water per litre The transfer of a phenol molecule from water to cyclohexane may involve complete dehydration of the polar hydroxyl function of the phenol. In the case of octanol, however, it is likely that the polar function would be in some way solvated by the water or hydroxyl function of the alcohol.

The slope of log K_D vs. number of methylene groups added was about 0.55 for the cyclohexane system and 0.4 for the octanol system i.e. as is generally found the more non-polar solute molecules have higher partition coefficients with non-polar than with polar solvents as well as being more sensitive to changes in the non-polar functions within the solute molecule when distributed between aqueous and the more lipophilic phases e.g. cyclohexane.

The series of xylenols studied also show this tendency for the partition coefficient between cyclohexane and water to increase with increasing number of non-polar groups within the solute molecule (c.f. Table 4).

It is a well known observation that compounds tend to be most soluble in solvents with a similar composition to their own, although the applicable theories tend to be complex and are usually grouped under the heading of Van der Waals or London forces. These arise from induced electronic interaction between the polarisable regions of adjacent molecules thereby producing a relatively weak bond.

The three isomers o-cresol, m-cresol, p-cresol exhibit a decreasing trend in their partition coefficients due to the increasing aqueous phase-solute interactions arising through hydrogen bond formation in going from left to right in the above sequence. This is so because the electron density on the phenolic oxygen is lowest for o-cresol owing to the molecules' lack of coplanarity and hence inability to form a fully delocalised electron system coupled with the steric hindrance arising from the two large ortho-groups. Hydrogen bond formation is thus somewhat inhibited by this structure.

The meta-isomer is electronically unfavourable for a high electron density on its phenolic oxygen whereas in p-cresol maximum delocalisation of electron charge can occur without any steric effects also coming into play.

These phenomena can also be observed in a similar fashion throughout the isomeric analogues of the di-alkyl substituted phenols also studied.

4.1.6. f) EFFECT OF MIXED SOLVENTS.

With p-cresol as solute in the aqueous-cyclohexane/octanol system, a linear relationship between partition coefficient and percentage octanol in cyclohexane was observed. The graph given as Fig 19 (p.96) implies that the increased degree of hydrogen bonding commensurate with the higher octanol concentrations is responsible for the enhanced values of the partition coefficient.

The results obtained for p-n-propyl-phenol (Fig. 18) (p.95) are however, not quite so regular. The partition coefficient was in fact unexpectedly high, possibly due to there being both polar (octanol) and non-polar (cyclohexane) groups in the organic phase which could interact synergistically with the polar phenolic and large non-polar extranuclear, alkyl chain of the solute molecule. This would explain the reversed trend in partition coefficient values above an optimum solvent mixture of 80 per cent octanol/20 per cent cyclohexane as organic phase, owing to the reduced non-polar solute-solvent interactions.

4.1.7. g) ION-PAIR EXTRACTION.

The increasing ionic strength in the aqueous phase leads to a decrease in the partition coefficient for the alkylbenzyldimethylammonium ion-pairs. This is probably due to increased solute (ionic species) - solvent (sodium chloride solution) interactions which will delocalise the electronic charges on the ions and thereby enhance their stability in the aqueous phase. Although the overall thermodynamic Gibbs free energy is implied to be negative in this situation it must be borne in mind that this parameter is a composite one as shown in the relationship:-

$$\Delta G = \Delta H - T\Delta S$$

During partition of a solute between two phases, any changes in the orientation of the solute-solvent interactions will be related to the entropy term in the Gibbs equation. Similarly there will also be a change in enthalpy associated with the energy of these interactions.

It would appear that extensive studies would have to be carried out in order that each contributing variable could be isolated for accurate assessment. However, overall trends were clearly indicated by our experiments (p.135).

4.1.8. h) EXTRACTION OF UNSTABLE SPECIES.

A typical drug concentration versus time plot is given in Fig. 45. p.138 The concentrations in the two phases change with time, though an equilibrium situation is not reached since the compound is undergoing simultaneous loss from the aqueous phase. After an initial distribution period a first order decrease in the concentration of solute in both phases is obtained. From the results obtained on the AKUFVE it can be observed that no distribution effect takes place since equilibration is reached rapidly (Fig. 45). For the system and the conditions represented in Fig. 45 it can be seen that the gradients obtained with both methods are very similar. The ratio of the concentrations in the two phases found when using the AKUFVE does not necessarily provide the partition coefficient ratio, but this can be checked using data from the diffusion cell. If correlation is established then the AKUFVE may be used for examining variables such as ionic strength, pH. etc. on the partition coefficient of the unstable drug.

4,1.9. GENERAL CONCLUSIONS

Our detailed assessment of the AKUFVE has shown that it can provide a rapid and accurate method for the determination of partition coefficient values of drug substances in instances where a range of experimental conditions are investigated, for example solute concentration, pH, ionic strength and temperature. It can also be used to examine the distribution behaviour of unstable substances.

However, as is to be expected with any experimental procedure, there are a few disadvantages which may preclude its use in some instances i.e.

(a) Cleaning

After each partition study the apparatus has to be stripped down and cleaned. This applies in particular to the centrifuge. An experienced person would take from half to one hour to perform this consequently the AKUFVE is not suitable for the routine determination of a large number of 'one off' partition values.

(b) Quantities of solvent and solute

The volumes of organic and aqueous phases might be considered excessive in some instances. In normal operation 500 ml of each is employed. Not only is there the cost of the organic phase but also the quantity of available solute. In the studies carried out we employed model drug compounds that were available in large quantities. However, in reality with new drugs the researcher may have at his disposal only a few milligrammes, insufficient to provide suitable detectable concentrations in the aqueous and/or organic phases.

(c) Solvent loss

Solvents with high vapour pressures, such as cyclohexane and chloroform are able to escape from the AKUFVE unless precautions are taken to provide a closed system. For example in some of our detailed temperature studies, with cyclohexane as organic solvent, up to ten per cent of the solvent could be lost. As a result, in these investigations a cyclic

procedure was adopted (low temperature to high temperature back to low temperature) and any hysteresis effects were noted and a correction made. No doubt a totally closed system could be made without undue difficulty.

4.1.10. THE 'MINI'-AKUFVE

Throughout our studies it was realised that the latter two disadvantages outlined above could be largely alleviated if a smaller version of the AKUFVE had been available.

Current literature from the manufacturer of the AKUFVE system (MEAB Metallextraktion AB; Sweden) informs us that they have just begun production of the H-10 apparatus which utilises a smaller centrifuge than in the standard AKUFVE.

Salient features of the H-10 centrifuge are:-

Flow capacity	100 l.p.h.
Bowl volume	0.015 l.
Maximum rotational speed	24,000 r.p.m.
Hold-up time	0.5s.

This smaller apparatus would no doubt be an invaluable asset to any researcher engaged in the area of study undergone in the preparation of this thesis.

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