This is to certify that the

thesis entitled

MICELLAR CHROMATOGRAPHY

OPTIMIZATION AND USE

presented by

NGANGA, PETER CEGE

has been accepted towards fulfillment
of the requirements for

_____ degree in _____

MSC Analytical Chemistry

William Reusch

Major professor

Date 6/14/85

MICELLAR CHROMATOGRAPHY, OPTIMIZATION AND USE

By

Nganga, Peter Cege

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirement
for the degree of

MASTER OF SCIENCE

Department of Chemistry

1985

ABSTRACT

MICELLAR CHROMATOGRAPHY, OPTIMIZATION AND USE.

By

Nganga, Peter Cege

Retention in reverse phase liquid chromatography is dominated by solvent-solute interations, with stationary phase-solute interactions making secondary contributions. Due to the many different ways in which a solute can interact with a micelle, aqueous micellar solutions might provide an altenative to the organic co-solvents presently used as mobile phases for reverse phase high pressure liquid chromatography.

The main limitation to micellar liquid chromatography is the low chromatographic efficiency associated with them, when compared to conventional hydroorganic mobile phases. It will be shown in this work that this loss of efficiency is due to poor mass transfer, of the solute between the stationary phase and the mobile phase. To optimize mass transfer, chromatographic conditions were varied for high pressure liquid chromatography, of a series of simple aromatic solutes. These variations included changes in temperature, stationary phase, surfactant concentration and flow rate.

The use of micellar liquid chromatography was demonstrated by the separation of several classes of pesticides. From this investigation it has been shown that, if operated at the optimum operating conditions, micellar chromatography will become a new and exciting chromatographic techinique.

To my wife, Emma Muthoni

and our son

Hezekiah Cege Nganga

ACKNOWLEDGEMENTS

I would like to say thank you to my major professor Dr Mathew Zabik, for giving me the opportunity to work in his lab and for his guidance in the course of the research work and thesis preparation. I have enjoyed and learned alot working under him and all those who work in his lab. Thank you Ines, for all the fights we have had, they kicked boredom out of the lab. Thank you Don for all the help you have given me with the computer. Dr Richard Leavitt for your help in the printing of the thesis. Sue, Nelson, Holly and Koffi, thank you for all that we have shared together.

I would also like to thank the Ministry of Agriculture (Republic of Kenya) together with USAID/USDA for their financial support.

Special thanks have to go to my mother Rose Wangari Cege and father Hezekiah Cege Nganga, for it is them who have made me what Iam, I will always be grateful to you two. I will always be grateful to my wife and son, for without their patience, love and sacrifice, I would not have done it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Reverse phase high pressure liquid chromatography is developing into a popular analytical technique. This is due to its uniqueness in its ability in being able to be manupulated into many different chromatographic conditions, that vary the retention and the separation of compounds of interest.

Retention in reverse phase liquid chromatography, is dominated by solvent-solute interactions, with the stationary phase solute interactions making secondary contributions. The key to separation then is, to be able to change the solvent solute interactions, in such a way as to shift the retention of overlapping compounds.

The expression reverse phase liquid chromatography, has been adapted to describe a system where, the mobile phase is more polar than the stationary phase. In the most commonly practiced reverse-phase chromatography, water is used as the principal or primary solvent (1). In order to change solvent-solute interactions, water is modified using solvents such as methanol, acetonitrile, isopropanol, tetrahydrofuran et cetra. Retention times are increased by increasing the water content of the mobile phase. Conversely an increase in the modifier concentration causes a decrease

in sample retention.

The main problem with solvents presently used, for high pressure liquid chromatography is their toxicity, cost and flammability characteristics. As the techinique continues to enjoy high popularity, research continues to try and find ways of minimizing the potential danger that exists in using these hydroorganic solvents.

The purpose of this investigation was to develop a liquid chromatographic method, that uses micellar mobile phases for use in the separation of various types of pesticides. In order to do this, it was necessary to find out whether it is possible, to use micellar aqueous solutions as mobile phases for high pressure liquid chromatography. If the micellar solutions can be used, the effects if any, of varying various chromatographic conditions, on the chromatograms' peak shapes, separations, and efficiency were investigated. In order to determine this, the investigation was divided into two parts. The effects of varying the chromatographic conditions were first studied. This was done using chromatograms obtained from high pressure liquid chromatography, of various aromatic hydrocarbons using micellar mobile phases. In this way, methods of optimizing the system were devised. The second part of the investigation was the actual use of micellar liquid chromatography for the separation of pesticides.

Compounds used in this investigation had to be detectable with a ultra violet-visable (U.V.-vis) detector, as the HPLC system employed a U.V.-vis detector. Four classes of pesticides were

experimented on in this investigation and three or four pesticides in each class were used as mixtures to be separated.

In this thesis, the theory behind micellar liquid chromatography will be looked at first, next, methods and materials used in the investigation will be presented. This will be followed by the results obtained from the investigation and a discussion of the results. The thesis will end with the conclusion.

In summary, the most commonly used mobile phases in reverse phase liquid chromatography are flammable, toxic and expensive. This investigation was to determine whether a micellar mobile phase had the same capabilities that hydroorganic mobile phases have, but without having their apparent drawbacks.

CHAPTER TWO

1. SURFACTANTS

A surface active agent, or a surfactant, is defined as a substance that, when present at low concentration, in a system, has the property of adsorbing onto the surface or interfaces of the system and of altering to a marked degree the surface or interfacial free energies of those surfaces or interfaces (2). An interface is a boundary between any two immiscible phases.

Surfactants have a characteristic molecular structure, consisting of a structural group that has strong attraction for the solvent, called the lyophilic group, together with a group that has very little attraction for the solvent, called the lyophobic group. This is the so called amphipathic structure.

Surfactants are classified according to the nature of their hydrophilic group. Anionic surfactants have a negative charge on the surface-active portion of the molecule, while cationic surfactants have a positive charge. Zwitterionic surfactants may have both positive and negative charges on the surface active portion of the molecule but non ionic surfactants have no apparent charge (table 1).

TABLE 1. Typical surfactants. Their critical micellar concentrations (CMC) and Aggregation numbers.

Surfactant	CMC(M)	Aggregation Number
ANIONIC		
Sodium dodecyl sulfate (SDS) $CH_3(CH_2)_{11}OSO_3^-Na^+$	0.0081	62
Sodium polyoxyethelene(12)--dodecylether (SDS 12EO) $CH_3(CH_2)_{11}(OCH_2CH_2)_{12}OSO_3^-Na^+$	0.0002	81
CATIONIC		
Cetylpyridinium chloride $C_{16}H_{33}N^+C_5H_5Cl^-$	0.00012	95
Cetyltrimethylammonium bromide (CTAB) $CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$	0.0013	78
NONIONIC		
Polyoxyethelene(6)dodecanol $CH_3(CH_2)_{11}(OCH_2CH_2)_6OH$	0.0000,	78
ZWITTERIONIC		
N-dodecyl-N,N-dimethylammonium--3-propane-1-sulfonic acid (SB-12) $CH_3(CH_2)_{11}N^+(CH_3)_2(CH_2)_3SO_3^-$	0.003	55

2. MICELLIZATION

Micellar formation or micellization, is the property that surface active solutes have, of forming colloidal like clusters in solution. It is an important phenomenon since a number of important phenomena such as detergency and solubilization depend on the existence of micelles in solution for them to occur.

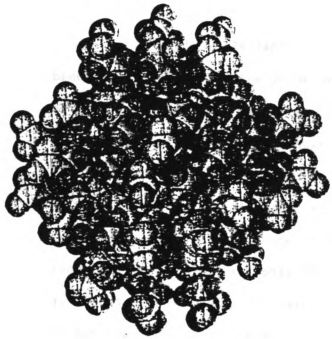
At low concentration in aqueous solutions, the surfactant is dispersed mostly as monomers, although dimers, trimers etc, can exist. At a critical surfactant concentration, the monomers assemble in covenient aggregates, to form micelles roughly spherical in shape and typically consisting of 60-100 monomers. The concentration at which this occurs is known as the critical micellar concentration (CMC). As the concentration of the surfactant is increased above the CMC, more micellar assemblies are formed with the ammount of free monomer, remaining approximately constant and equal to the CMC (3).

Micelle formation is believed to be the result of three primary forces, namely, hydrophobic repulsion between the hydrocarbon chains and the aqueous environment, charge repulsion of ionic head groups and Van der Waals attraction between the alkyl chains. The micelle structure, the aggregation number, the micelle size and the CMC are determined by the alkyl chain length, size of the head group structure and the interactions of the alkyl chains with one another and with the solvent (3).

In an aqueous solution the hydrocarbon moiety is directed inward, forming an oil like pool, and the more polar head groups are oriented outward into the bulk polar solvent. A micelle in a polar solvent can be considered to be roughly spherical, with the interior region containing the hydrophobic groups of the surfactant molecules, of radius approximately equal to the length of a fully extended hydrophobic group. This is surrounded by an outer region containing the hydrated hydrophilic groups and bound water (figure 1).

Micelles are generally 3-6nm in diameter, hence the macroscopic properties of the solution approximate those of a homogeneous solution but the solution is actually microscopically heterogeneous, comprised of at least two distinct media, the micelle aggregate itself and the surrounding bulk aqueous phase.

Micelles are not static, but are molecular assemblies that are in dynamic equilibria with their bulk solvent phase (4). Two basic processes occur, that is, the exchange with the solvent and other micelles, of monomers, dimers, trimers etc, occuring over a microsecond to a millisecond time scale and the actual replacement of a whole micelle by this process over a time frame from milliseconds to seconds.



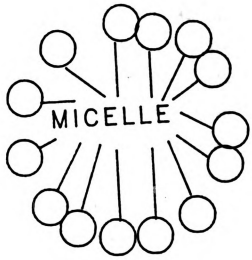


Figure 1. Micelle shape and structure

(a) A computer generated model of a micelle (12). (b) An illustration of a micelle shape and structure, showing the hydrophobic head groups and the hydrophilic tails.

3. MICELLE MOBILE PHASES

(a)Introduction

Charged surfactants of concentrations below the CMC have been widely used as mobile phase modifiers to augument the retention of charged eluites on nonpolar bonded phases. Early work by Farrula et al., (5) using paper chromatography, showed that, an increase in the Rf values is obtained, when a surfactant having an opposite charge to that of the ion chromatographed is present in the mobile phase. Knox and Laird (6) were the first to use surfactants in HPLC. In a technique they termed as "soap chromatography" they added a detergent at around the 1% level, to a water-propanol mobile phase and were able to get high resolution separations of a wide range of sulphonic acids and derived dye stuffs. The retention of ionized solutes on a nonpolar bonded stationary phase, is enhanced by the presence of a hydrophobic counter ion in the mobile phase (7). Retention of the solute molecules can be considered to occur, either by ion-pair formation in the mobile phase and the partitioning or adsorption of the complex into the stationary phase, or alternatively, by dynamic ion exchange which involves interactions between the ionized solute molecules and the counter ions adsorptively bound to the stationary phase (8).

Only recently have surfactants been the main organic modifiers at concentrations above CMC in reverse phase systems. Armstrong and Henry demonstrated that an aqueous solution of sodium dodecyl sulfate (SDS) micelles, can be a highly effective mobile phase in HPLC. They used it for the separation of phenols and polynuclear aromatic hydrocarbons (9).

(b)Selectivity

Column selectivity (α) is a measure of the separation of adjacent peaks in a chromatogram. It is the ratio of the capacity factors of two adjacent bands.

$$\alpha = \frac{k'_2}{k'_1}$$

Selectivity refers to the intrinsic capability of a given separation method to distinguish between two components. It is related to the fundamental physical chemical phenomena underlying a separation. It is, therefore, based on specific chemical interactions among sample molecules, the mobile phase and the stationary phase.

In reverse phase liquid chromatography using micellar mobile phases, retention of solutes generally decreases with increasing micelle concentration, but the rate of decrease varies considerably between different solutes, producing inversion in retention orders (10). This inversion is due to two competing equilibria, namely, solute-micelle association and solute-stationary phase interaction.

An increase in the micelle concentration may drive the solute into the moving micelle phase while having little or no effect on the stationary phase equilibria.

A solute can associate with a micelle through a combination of electrostatic, hydrophobic and steric interactions. Due to this complexity in the interactions of a solute and a micelle, some highly unusual chromatographic behaviour can be encountered (11). Compounds that associate or bind to micelles show decreased retention with an increase in micelle concentration. Compounds that do not associate with the micelles can show two types of elution behaviour, their retention can be unaltered by the micelle content of the mobile phase, or their retention can increase with increase in micelle concentration. Hence separations that involve combinations of binding, non-binding and anti-binding, solutes in micellar mobile phases show high degrees of selectivity.

(C)Detection

Sensitivity in liquid chromatography is of paramount importance. Use of micellar solutions have been shown to give dramatic enhancement of fluorescence, phosphorescence and chemiluminescence detection modes.

In both fluorescence and phosphorescence, one is concerned with measuring radiation emitted by a molecule after the molecule absorbs radiation. They differ in the process by which the molecule

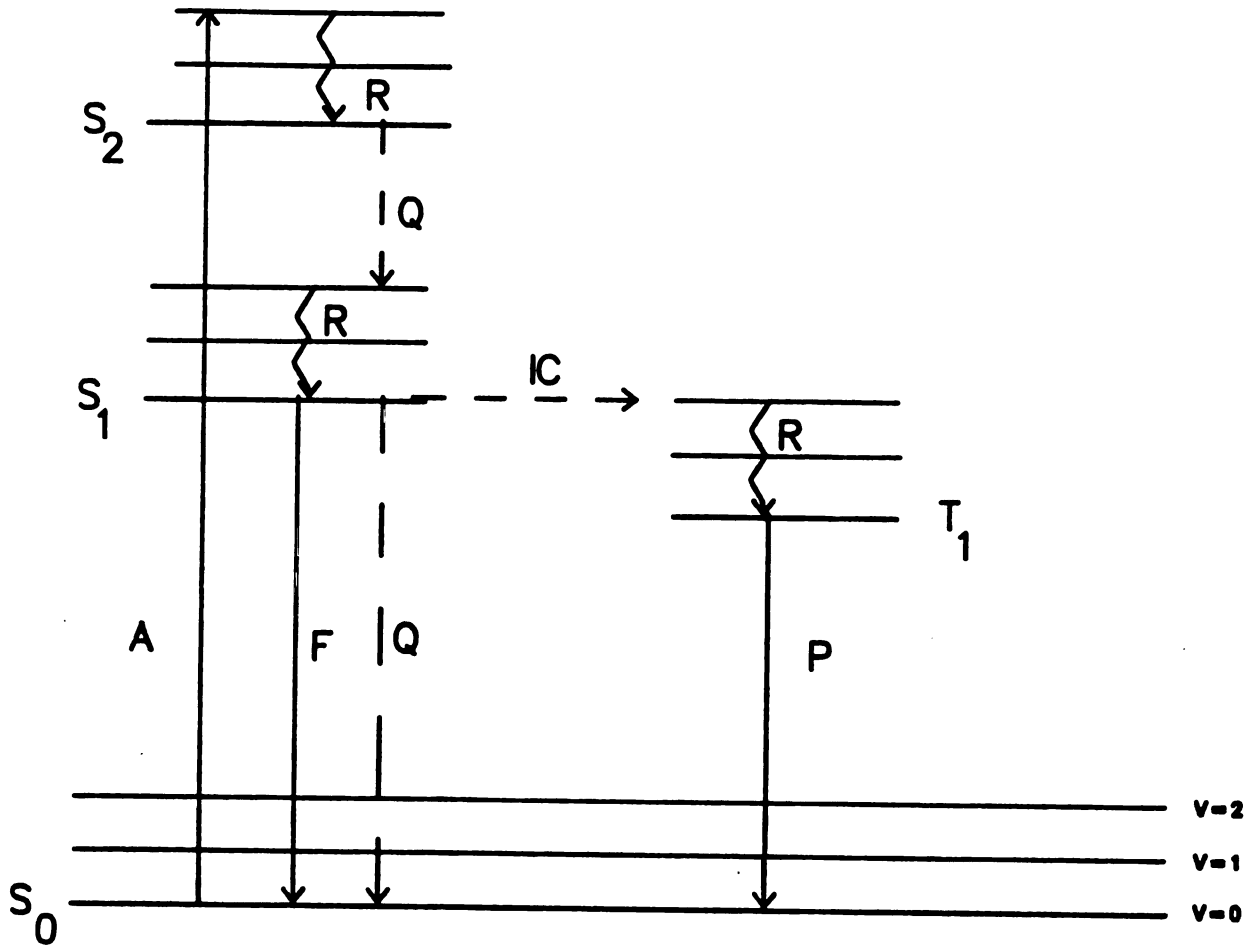


Figure 2. A simplified energy level diagram.

Singlet (S) and triplet (T) electronic levels are shown as heavy horizontal lines, lighter lines are vibrational levels. Continous vertical lines indicate transitions involving absorption or emission of radiation, others indicate non-radiative transitions. A is absorbance, F is fluorescence, P is phosphorescence, IC is intersystem crossing, R is vibrational relaxation and Q is quenching.

returns to the ground state ($T_1 \longrightarrow S_0$ rather than $S_1 \longrightarrow S_0$). See figure 2.

Figure 2 shows that the triplet states of a molecule are not normally accessible by direct excitation (a forbidden transition) but are populated by intersystem crossing, a radiation less transition between singlet and triplet electronic states. Transitions between states of different multiplicities are strictly forbidden quanta mechanically due to the selection rule requiring the conservation of spin angular momentum. However it is never really possible to have pure spin states because the spinning electron has a magnetic moment which can interact with the magnetic field associated with the orbital angular momentum. Therefore it is only possible to conserve total angular momentum rather than spin or orbital angular momentum independently. The mixing of states of different multiplicities is proportional to the spin-orbit interaction energy and inversely proportional to the energy difference between states being mixed (13). The spin-orbit interation energy for a hydrogen-like atom is proportional to Z^4. Where Z is the atomic number. This Z^4 dependence is the origin of the term heavy atom effect. Thus heavy ions such as iodide, silver and thallous are included in phosphorescence studies.

Phosphorescence 'lifetimes' (the time needed for the number of molecules in the exited state to fall to 1/e of its initial value) range from 1ms to 100ms. Due to these long lifetimes, the exited molecule can be deactivated by collisions with solvent molecules,

molecular oxygen (which has a triplet ground state) and other sample components.

For a number of years, the immobilization of the lumiphor in a rigid glass matrix at low temperatures was the only way of observing phosphorescence. However, Schulman and Walling (14), found that room temperature phosphorescence (RTP) could be observed from molecules adsorbed on filter paper and on surfaces of thin layer chromatography matrices. For solid surface RTP to occur, the phosphor has to be bound rigidly to the solid support in order to minimize collisional deactivation of the triplet state. It has been suggested that, this occurs through hydrogen bonding between the phosphor and the solid surfaces (15).

Although weak phosphorescence from the triplet state in fluid solutions has been observed, dramatic enhancement can be achived when the lumiphor is incorporated in micelles containing heavy atom counter ions in solution. The heavy atom-lumiphor interactions are no longer simply diffusion controlled as they are in conventional solutions (16,17). Micellar systems have been shown to (1) solubilize, concentrate, compartmentalize and organize solutes/reactants; (2) alter spectral parameters of solubilizates; (4) be chemically stable, optically transparent, and relatively non toxic (18). This then is the basis of the power pocessed by the micelle in enhancing phosphorescence. The ability of micelles to organize reactants on a molecular scale, increases the proximity of the lumiphore and the heavy metal counter ion so that the effective

concentration of the heavy metal is greatly increased and favorable orientation constraints facilitate more efficient spin-orbit coupling (17). This then leads to micellar stabilized room temperature phosphorescence (MSRTP). The ability of micellar solutions to mimic conventional liquid chromatography solvents, means that, it is possible to observe MSRTP with standard HPLC fluorescence detectors without further chemical manupulations (19). It is also possible to use conventional reverse phase HPLC and add reagents necessary for MSRTP post column.

Fluorescence and chemiluminescence are other detection modes improved by the presence of micelles in solution. The enhancement of fluorescence in micellar systems is said to be due to an increased quantum yield of the micelle-stabilized fluorescent solute. This is said to be brought about by altered micropolarity, restricted motion, effective shielding of the excited singlet state from quenchers present in solution and/or a combination of these factors (20). Armstrong et al (21) were able to use enhanced fluorescence as a detection mode, for HPLC separation of a variety of polynuclear aromatic hydrocarbons.

Use of micellar solutions have been shown to either enhance or reduce chemiluminescence intensities (22). This is a consequence of the micellar effects both on the rates of the chemiluminescence reactions as well as on the quantum yield. Cationic and zwitterionic micelles, cause an increase in the light output of chemiluminescence

reactions, while anionic or nonionic micelles diminish the intensity compared to that obtained in water.

4. THEORETICAL

As it has been noted before, aqueous solutions of surfactants at concentrations above critical micellar concentration (CMC) are microscopically heterogeneous, being composed of the amphiphilic micelle aggregate and the surrounding solvent. A solute in such a solution can be preferentially solubilized, not be affected by the presence of micelles, or be repelled from the micelle. This is a result of the solutes hydrophobicity, hydrophilicity or its ionization characteristics.

In order to understand and fully utilize micellar liquid chromatography, it is necessary to fomulate models that explain the elution behaviour of a solute in terms of chromatographic parameters, micelle characteristics and the equilibria involved between the solute, micelle, the stationary phase and the bulk solvent.

Armstrong and Nome (23) proposed a model that allowed the calculation of partition coefficients of solutes between water and micelles, between the stationary phase and water and between the stationary phase and micelles. This treatment works for non-ionizable solutes but could not be extended to solutes that are ionic or are ionizable with varying pH (11).

The model considered here was proposed by Arunyanart and Cline Love (24). This model, as described here, assumes minimal electrostatic interactions and is suitable for neutral solutes but can

be expanded to explain the elution behaviour of ionizable solutes with varying pH.

There are three equilibria involved in micellar liquid chromatography (figure 3). The first equilibria is the reversible equilibria that occurs when a solute in the bulk solvent E_m, interacts with the stationary phase sites L_s to form a complex EL_s. The second equilibria occurs when a solute in the bulk solvent, interacts with the surfactant in the micelle M_m, to form a complex EM_m. The third and final equilibria involves direct transfer of the solute in the micelle, EM_m, to the stationary phase. The following equations express these equilibria.

$$E_m + L_s \xleftarrow{\quad K_1 \quad} EL_s \qquad (1)$$

$$E_m + M_m \xleftarrow{\quad K_2 \quad} EM_m \qquad (2)$$

$$EM_m + L_s \xleftarrow{\quad K_3 \quad} EL_s + M_m \qquad (3)$$

From this you obtain,

$$K_1 = \frac{[EL_s]}{[E_m][L_s]} \qquad (4)$$

$$K_2 = \frac{[EM_m]}{[E_m][M_m]} \qquad (5)$$

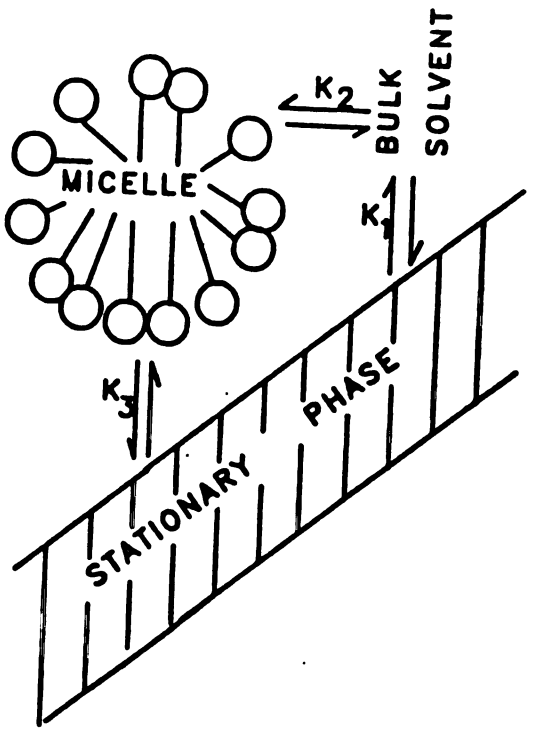


Figure 3. A schematic representation of a three phase model for micellar chromatography.

K_1 is the equilibrium constant for the solute between the bulk solvent and the stationary phase. K_2 is the equilibrium constant for the solute between the bulk solvent and the micelle. K_3 is the equilibrium constant for the solute between the micelle and the stationary phase.

$$K_3 = \frac{[EL_s][M_m]}{[EM_m][L_s]} \qquad (6)$$

Where $[M_m]$ = [surfactant] - CMC.

The concentrations are in moles/liter.

[Mm] is the concentration of surfactant in the micelle and [Surfactant] is the total concentration of the surfactant. From equations (4) and (5), we obtain,

$$[EL_s] = K_1 [E_m][L_s] \quad (7)$$

$$[EM_m] = K_2 [E_m][M_m] \quad (8)$$

Substituting in equation 6

$$K_3 = \frac{K_1[E_m][M_m][L_s]}{K_2[E_m][M_m][L_s]}$$

i.e.,

$$K_3 = \frac{K_1}{K_2}$$

hence, of the three equilibria described, only two are independent and it may be assumed that the solute binds independently to the stationary phase and to the micelle in the bulk solvent.

In liquid chromatography retention is usually measured in terms of the capacity factor k', which is the retention of a sample relative to that of a non retained sample, ie,

$$k' = \frac{V_r - V_0}{V_0}$$

where V_r = retention volume

V_0 = column void volume

It is also defined as,

$$k' = \frac{n_s}{n_m}$$

where n_s is the total number of moles of solute in stationary phase and n_m is the total number of moles of solute in mobile phase.

Hence in this case;

$$k' = \frac{[EL_s]p}{[E_m]+[EM_m]} \qquad (9)$$

where p is the phase ratio $\frac{V_s}{V_m}$.

V_s is the volume of the stationary phase,

V_m is the volume of tha mobile phase.

substituting,

$$k' = \frac{K_1[E_m][L_s]p}{[E_m]+K_2[E_m][M_m]}$$

ie, $$k' = \frac{K_1[L_s]p}{1+K_2[M_m]} \qquad (10)$$

Equation (10) predicts a parabolic curve dependence of k' on $[M_m]$, with an intercept value of $p[L_s]K_1$ at $[M_m] = 0$. If K_2 is known, it is

then possible to estimate the value of $p[L_s]K_1$ from only one measurement of k' at any surfactant concentration in the micelle. This intercept value can then be used to estimate k' at any surfactant concentration. Equation (10) can be linearized by taking recipricals

$$\frac{1}{k'} = \frac{[M_m]K_2}{p[L_s]K_1} + \frac{1}{p[L_s]K_1} \qquad (11)$$

$$\text{Slope} = \frac{K_2}{p[L_s]K_1}$$

$$\text{Intercept} = \frac{1}{p[L_s]K_1}$$

From this, the ratio slope/intercept = K_2

K_2 is the equlibrium constant for the solute between the bulk mobile phase and the micelle per monomer of surfactant. The equilibrium constant per micelle (K_{eq}) is obtained by multiplying K_2 with the aggregation number. K_{eq} obtained in this way should be the same as those obtained by other techniques such as luminescence or solubility.

Arunyanart et al., were able to test this model successfully (26) for a series of arenes. For ionizable solutes, one needs to consider all the principle equilibria taking place and this should result in an equation simillar to equation (10).

5. EFFICIENCY IN MICELLAR HPLC

A major drawback in micellar liquid chromatography is that it exhibits poor chromatographic efficiencies when compared to conventional mobile phases. Dorsey et al., (25) were the first to try to remedy this problem. They obtained efficiencies approaching those obtained using hydroorganic mobile phases by using mobile phases containing 3% propanol and temperatures of about 40 C.

In this discussion, a model proposed by Yarmchuck et al., (26) will be considered. The model treats restricted mass transfer in micellar liquid chromatography based on the kinetics of adsorption-desorption of the solute on the stationary phase and entrance-exit rates from micelles in the mobile phase. It provides an explanation why low efficiencies are observed for high pressure liquid chromatography using micellar mobile phases compared to hydrooganic mobile phases.

The distribution of the elution peak about its mean position (the retention volume of the peak) is normally measured in terms of the peak variance or standard deviation. Efficiency in chromatographic systems is measured in terms of the number of theoretical plates N, which is the ratio of the square of the retention volume (time) of the peak divided by its standard deviation.

$$N = \left(\frac{t_r}{\sigma_t}\right)^2$$

Where t_r is the retention time

σ_t is the band variance in time units.

Another measure of column efficiency is given by plate height H. also called height equivalent of a theoretical plate (HETP).

$$H = \frac{L}{N}$$

Where L is the length of the column.

In order to increase the column efficiency, the peak variance must be reduced. Van Deemter et al., (27), showed that the increasing variance of a band during migration arises from three main sources which occur independently within the column. These are axial molecular diffusion, multipath effect and slow mass transfer between the mobile phase and the stationary phase zones. Because these three dispersive processes are both independent and random, the variances produced by them are added to give the total variance.

$$HETP = H_{flow} + H_{diff} + H_{mass\ trasnsfer}$$

H_{flow}; The flow of a solute in a column is streamlined. In a packed bed the flow of the solute in a given streamline, is tortous. Although the flow is streamlined, the stream lines are of different lengths and the flow velocity varies in a random fashion along any given streamline, thus molecules of solute that are initially injected simultaneously at the top of a column into different streamlines would emerge with a spread of elution times. This is the so called eddy diffusion and is a result of irregular packing structure of the column

bed and particle size diameter of the packing material. H_{flow} is approximated by;

$$H_{flow} = Av^{1/3}$$

where A is a constant and v is linear velocity. H_{flow} should be the same for micellar mobile phases as well as for conventional ones in a given column.

$H_{diffusion}$; If a band of solute is allowed to remain static in a packed column, it will slowly spread along the axis of the column depending on the diffusion coefficient and the residence time. This type of diffusion also occurs when the band is moving. $H_{diffusion}$ is given by

$$H_{diff} = \frac{21D_m}{V}$$

Where 1 is the tortusity factor (normally 0.6) which allow for the restriction to diffusion by impermeable column packing particles. D_m is the diffusion coefficient of the solute in the mobile phase and v is the velocity. In liquid chromatography, diffusion rates for the solute are very low, hence H_{diff} can only be significant at very low flow rates. H_{diff} is usually given as

$$H_{diff} = \frac{B}{v}$$

Where B is a constant.

$H_{mass\ transfer}$; When a solute is retained by the stationary phase, they can only move within the packing particles by diffusion as there is no flow through the internal pores of the particles

themselves. The rest of the time they are in the moving phase and travel down the column at the average linear velocity of the eluent. When a particular molecule is in the stationary phase, it will lag behind the main band of the solute and when it is in the moving phase, it will run ahead. So the progress of a solute molecule down the column can be said to be occurring by a series of random stops, alternately behind and ahead of the main band. This type of progress is known as random walk. Theory shows that a group of molecules each independently perfoming a random walk becomes dispersed (28). The dispersion is proportional to the square of the mean step length and to the number of steps. The mean steplength will be proportional to the linear flow velocity of the moving phase and the time of residence of the molecules in the stationary phase, while the number of steps will be inversely propotional to the flow velocity. In micellar systems, the time of residence of the solute in the stationary phase is significantly affected by th presence of micelles in the mobile phase.

$$H_{\text{mass transfer}} = Cv$$

The total plate height equation then is

$$H = \frac{B}{v} + Av^{1/3} + Cv$$

From the above consideration, it can be seen that, in order to improve efficiency in micellar systems, the mass transfer term should be improved.

For good mass transfer and hence good chromatographic efficiency, the equilibration of solute between stationary phase and mobile phase should be rapid. This is the case with conventional mobile phases. In reverse phase liquid chromatography, in absence of side reactions, only one equilibrium occurs, that of the solute between the stationary phase and the mobile phase. However in micellar chromatography as has been noted previously, there are two solute equilibria (figure 3), one between the stationary phase and bulk solvent and a second between bulk solvent and the micelle. This two fold equilibria contributes to the uniqueness of micellar chromatographic systems but it is also responsible for the problems in efficiency of these systems since mass transfer across an additional barrier is required.

Figure 4 shows the four rate constants which are involved in a micellar system. K_i and K_e the entrance and exit rate constants of a solute with a micelle and K_d and K_a the adsorption and desorption rate constants of a solute with the stationary phase. If all these rate constants were large, mass transfer would not limit efficiency but as is shown below this is not the case.

Almgren et al., (29), calculated K_e values based on equilibrium data, assuming K_i is diffusion controlled and equal to $7 \times 10^9\ M^{-1}S^{-1}$ for all solutes. For a 0.1M SDS, with an aggregation number of 62, K_i is $1.0 \times 10^7\ S^{-1}$. Values for K_e ranged from $4.4 \times 10^6\ S^{-1}$ for benzene to $4.1 \times 10^3\ S^{-1}$ for pyrene, with small

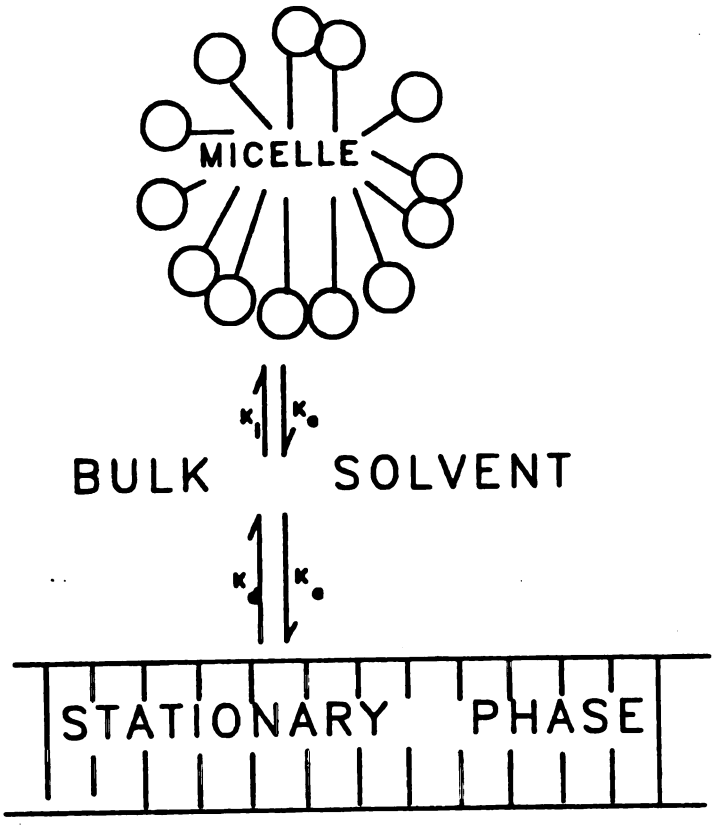


Figure 4. The two primary equilibria in micellar systems.

K_i is the entrance rate constant, K_e is the exit rate constant, K_d is the desorption rate constant and K_a is the adsorption rate constant.

and/or more polar molecules having large exit rates and large and/or more non-polar molecules having smaller exit rates.

K_d values can be calculated from chromatographic parameters substituted into equation (1) from random walk theory (28).

$$H = \frac{2k'}{1+k'} \times \frac{v}{K_d} \qquad (1)$$

Where H is the height equivalent of a theoretical plate, k', is the capacity factor and v is linear velocity. The adsorption rate constant K_a can be calculated from,

$$k' = \frac{K_a}{K_d}$$

Yarmchuck et al., calculated these values and obtained a K_a of about 300 S^{-1} for all solutes (a difussion controlled rate constant). Examples of K_d values that were obtained are 2.3/sec for 2-naphthol and 25.0/sec for phenol. Again K_d values were controlled by the size and polarity of the molecule.

Although Ki and Ka are both diffusion controlled, Ki is much larger than Ka. This is due to the distance which a solute molecule travels between stationary phase encounters as compared to the distance between micelle encounters. A 0.1M SDS solution contains 9.03×10^{17} micelles per cm^3. This results in the distance between

solute-micelle encounters being much less than between solute-stationary phase encounters. Therefore a solute molecule is more likely to enter a micelle than the stationary phase. While in the micelle the solute molecule becomes isolated and cannot partition to the stationary phase. However, for small or hydrophilic molecules, with large exit rate constants, they can readily move back to the mobile phase where they are available to the stationary phase. The overall result is that more hydrophobic molecules remain in the micelle much longer and spend less time in the bulk solvent as compared to more hydrophilic molecules. Hence their mass transfer between the micelles and stationary phase is inhibited. Small or hydrophilic molecules on the other hand do not have such a problem and for these solutes, efficiencies are not limited by micellar equilibria.

It should also be noted that those compounds with large micellar exit rate constants also have relatively large desorption rate constants compared to K_d's for more hydrophobic molecules. This further upsets the equilibrium resulting in a further loss in efficiencies for hydrophobic molecules

Mass transfer is also dependent on the diffusion constant of the solute. These are dependent on the viscocity of the mobile phase. In water the diffusion constants are about 10^{-5}cm /sec but in micelles it is only 10^{-6}cm /sec (30).

From this discussion, mass transfer seems to be the cause of the low efficiencies in micellar systems. This appears to be a consequence of slow micellar exit rates and slow desorption from the stationary phase, both of which are related to the hydrophobicity of the individual solute. Methods for overcoming some of these problems are shown latter in this report.

SUMMARY

Micellar formation is a property that surface active agents have of forming colloidal like clusters in solution. These clusters are called micelles and when present in aqueous solutions, micelles have the ability to preferentially solubilize solutes. This ability makes them capable of mimicking conventional hydro-organic mobile phases.

Due to the many different combinations with which a solute can interact with a micelle, a high degree of selectivity is often encountered in micellar chromatography. The ability of micelles to organize solutes on a mollecular scale leads to lower limits of detection in luminescence detection modes. There are two independent solute equilibria in micellar liquid chromatography, one between the stationary phase and bulk solvent and the other between the bulk solvent and the micelle. This two fold equilibria leads to problems with efficiency in micellar systems due to poor mass transfer.

Most of the work on micellar liquid chromatography, conducted so far has concentrated on developing the method. In this investigation, optimization of the technique will be done and use it for the separation of various types of pesticides. In this way we will ascertain whether this technique has practical value for the chromatographer.

CHAPTER THREE

EXPERIMENTAL

(a) Apparatus

The high pressure liquid chromatography system consisted of a model 110 solvent metering pump (Altex scientific inc., Berkeley, CA), and a model 7120 syringe loading sample injector with a 20-ul loop (Rheodyne inc., Berkeley CA). Due to the diversity of the separations conducted, columns packed with different stationary phases were required. All the columns were MPLC analytical cartridges (Brownlee labs Inc., Santa Clara, CA). The guard columns used were either 3cm x 4.6mm MPLC analytical cartridges or the new guard cartridge both from Brownlee labs. Use of elavated temperatures raises the solubility of the silica support used in the stationary phases of the analytical cartridges. This in turn lowers the column life as dissolution of the stationary phase occurs. To maximize the column life, a saturator column packed with silica, was placed between the pump and the injector. This precolumn was 15cm x 4.6mm, packed with silica gel (40-70um). The column temperature was controlled by inserting the precolumn, the injector and the analytical cartidge in a DuPOnt 860 column compartment (DuPont, Company, Scientific and Process Instruments Division Wilmington, DE). The column compartment was used to raise the solvent to a selected temperature and maintained the

sample injection valve and the column at that temperature during all chromatographic separations. A model 100-10 U.V.-Visable Spectrophotometer equipped with a 20 ul flow cell (HITACHI Scientific Instruments, Tokyo, Japan) was used for detection, and a strip chart recorder from Linear Instruments Corp (Irvine, CA) was used to record the chromatograms. A block diagram of the liquid chromatograph used is shown in figure 5.

To determine the optimum wavelength for U.V. absorption a U.V. scan was conducted for each solute using a Gilford system 2600 microprocessor controlled U.V. Visible spectrophotometer (Gilford instruments laboratories Inc., Oberin, Ohio)

(b) Reagents

The surfactant, sodium dodecyl sulfate (SDS) 99% was supplied by Sigma Chemical Company and used as received. The acetonitrile, 2-propanol and methanol used, were Baker analyzed HPLC reagent from J.T. Baker Chemical Company, Phillipsburg, NJ. The water used was lab distilled, then filtered and demineralized using a high capacity, standard Barnstead water purification cartridge (Barnstead Sybron Corp., Boston MA). It was then redistilled using a Corning Megapure three liter automatic still (Corning Waterware Corning, NY) before use. The solutes used were 99 % or more in

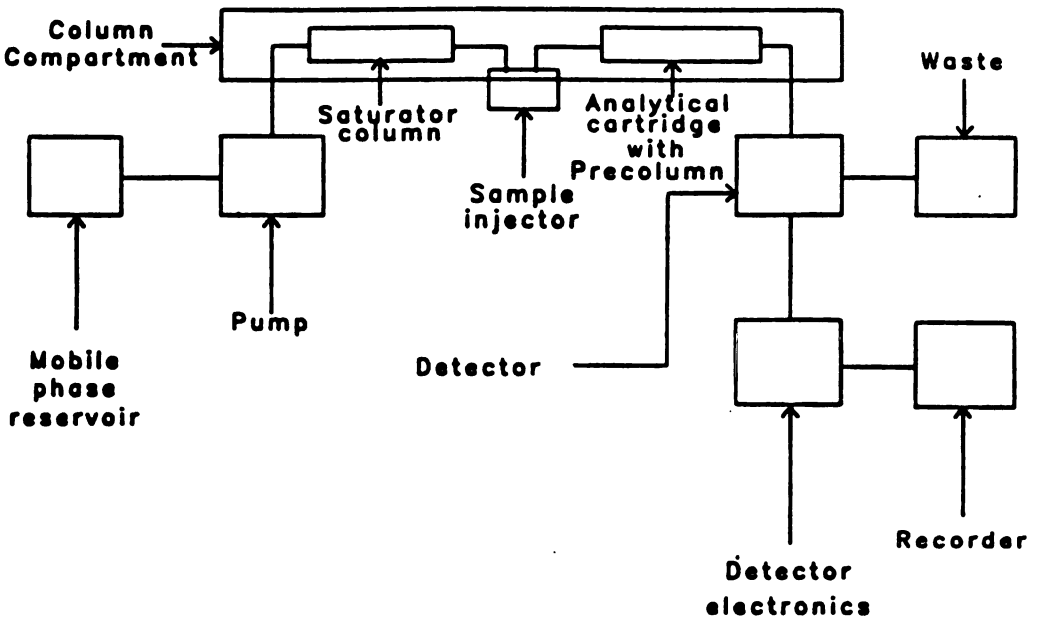


Figure 5. Block diagram of liquid chromatograph used

purity, they were obtained from various companies and were used as received.

(c) Procedure

The micellar mobile phases were prepared by disolving the appropriate amount of surfactant in modifier/water mixtures at the appropriate ratios. This was then filtered through a 0.47 um metricel membrane filter (Gelman instrument Co, Ann Arbor, Michigan). Stock solutions of the test solutes were prepared in methanol or acetonitrile and then diluted to the appropriate working conditions with the mobile phase. Retention times and peak widths were measured manually. To calibrate the pump flow rate, a flow rate of 2 ml/min was monitored by measuring the effluent with a 10 ml measuring cylinder for a sufficient length of time to measure 4 ml.

The void volume V_m of the system was measured by injecting 20 ul of 0.05M SDS with no modifier and measuring from the time of injection to the first deviation of the base line, multiplying this distance by the recorder chart speed gives t_0, the time needed for a non retained compound to elute. V_m is obtained by multiplying t_0 by the flow rate (F). The average volume obtained was 1.7 ml and was used for all k' calculations. 30 ul injections were made in order to make sure that the loop was completely filled. This ensured that a 20 ul injection was made for each and every injection.

Different Chromatographic conditions were used for each separation. These conditions are listed under the results, for each group of compounds. Before an injection was made, it was necessary to equilibrate the column at the set conditions, until a constant base line was obtained.

For U.V. scans, the solutes were prepared in methanol, diluted to the appropriate concentrations, and then scanned from 200nm to 350nm.

CHAPTER FOUR

RESULTS

1. Can micellar solutions be used as mobile phases for high pressure liquid chromatography ?.

Figure 6 shows a mixture of aromatic hydrocarbons obtained using 0.15M SDS mobile phase. The mixture injected had 0.1 ml/L phenol, 0.01 ml/L nitrobenzene, 0.2 ml/L benzene and 0.2 ml/L toluene. These solutes were used at these concentrations to obtain the results given in parts one to three of this chapter. There was no separation of benzene and nitrobenzene using these conditions. Toluene had a retention time of 25 min. while phenol had a retention time of 7.3 min.

Figure 6 gives a clear indication that it is possible to use micellar solutions as mobile phases for high pressure liquid chromatography. Micellar solutions are much weaker solvents as compared to ordinary hydroorganic mobile phases. This results in long retention times and broad peaks as shown in figure 6. Figure 7 is a chromatogram of the same mixture but this time using 50/50 water/acetonitrile, all the other chromatographic conditions being the same as before. In this case not only are the retention times much shorter but the peak widths are narrower.

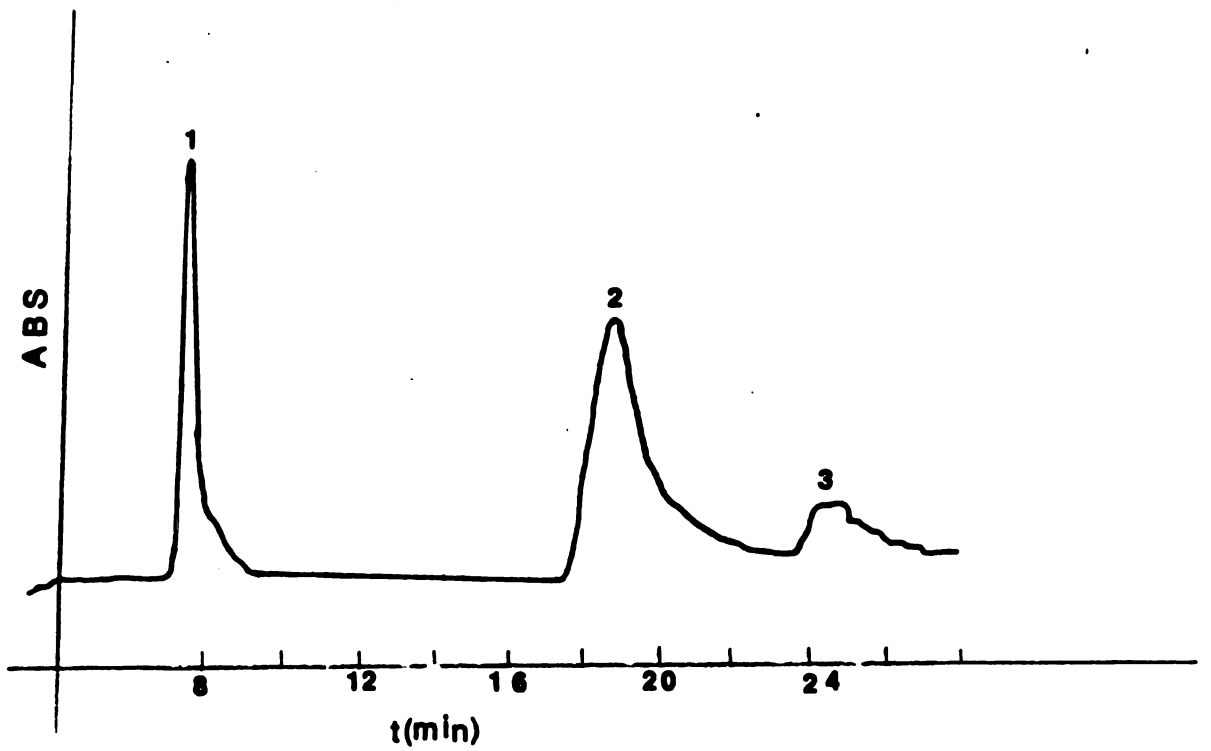


Figure 6. Micellar chromatograms of a mixture of aromatic hydrocarbons.

Chromatographic conditions: Detector, U.V., 255nm; Mobile phase, 0.15M SDS; Column, 25cm x 4.6mm C_{18} spheri-5 analytical cartridge; Temperature, 22 C; Flow rate, 1.5 ml/min; Chart speed, 5mm/min; Solutes: peak 1, phenol; peak 2, nitrobenzene/benzene; peak 3, toluene.

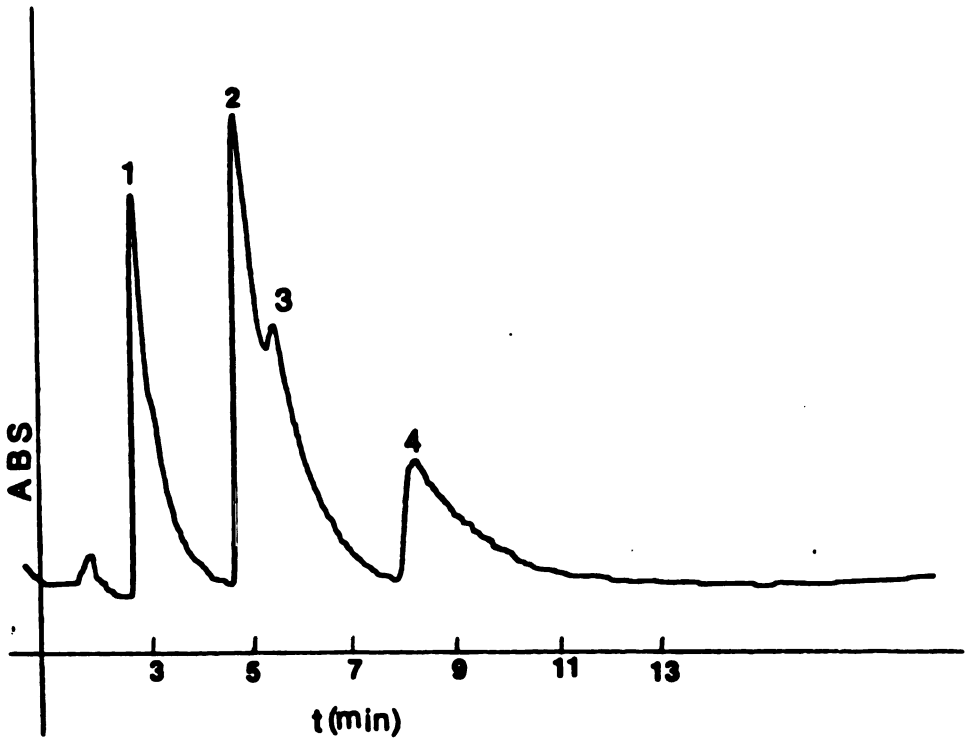


Figure 7. Conventional chromatograms of a mixture of aromatic hydrocarbons.

Chromatographic conditions; Mobile phase, 50/50, water/acetonitrile; Detector, U.V., 255nm; Column 250 x 4.6mm C_{18} spheri-5 analytical cartridge; Temperature 22 C; Flow rate, 1.5 ml/min; Chart speed 6.67 mm/min; Solutes: peak 1, phenol; peak 2, nitrobenzene; peak 3, benzene; peak 4, toluene.

Band width in liquid chromatography is commonly expressed in terms of the number of theoretical plates (N), which is a measure of chromatographic efficiency; the relative ability of given chromatographic conditions to provide narrow bands (small values of t_w) and improved separations.

$$N = 5.54\left(\frac{t_r}{W_{0.5}}\right)^2$$

Where t_r is retention time,

$W_{0.5}$ is peak width at half height.

Calculating N for nitrobenzene, From a band obtained using 0.15M SDS as mobile phase.

t_r = 18.8 min.

$W_{0.5}$ = 1.2 min.

N = 1360 plates

Normally the column used has more than 4000 plates.

Retention of a sample is expressed in terms of capacity factor k' which is the retention of a sample relative to the elution of a non-retained compound.

$$k' = \frac{V_r - V_0}{V_0}$$

Where V_r is sample retention time,

V_0 is elution time of a non retained peak.

For nitrobenzene, Using 0.15M SDS mobile phase

$$V_r = 28.35$$

$$V_0 = 1.7$$

$$k' = 15.7$$

The optimum capacity factor value range is roughly between one and ten. Separations that involve k' values greater than ten result in long retention times and excessive band broadening (31).

2. Optimization of micellar liquid chromatography

(a) The effect of hydroorganic modifiers on micellar chromatograms;

From figure 2, it is obvious that if micellar solutions are going to be used as mobile phases for high pressure liquid chromatography, then, some conditions have to be changed in order to obtain narrow peaks and shorter retention times. In reverse phase high pressure liquid chromatography, it is known that use of totally aqueous solutions usually results in broad peaks (32,25). This is presumably because of the poor wetting of the packing material or the slow equilibrium across the interface of the two highly dissimilar (hydrocarbon and aqueous) phases.

Table 2 shows the plate counts and capacity factors obtained after adding 10% of a hydroorganic solvent to act as modifier to a 0.15M SDS mobile phase. From the results, 2-propanol not only reduces the capacity factor k' by a factor of two but also nearly doubles the number of theoretical plates. It should be noted that all the modifiers tested increased the number of theoretical plates and also reduced the capacity factor.

Scot and Simpson studying the modification of C_{18} phases by organic modifiers have shown that, at a concentration of ca. 7% w/v propanol, 95% of the C_{18} surface is covered with the alcohol, however for 7% w/v ethanol only 86% of the surface is covered and only 67% for 7% w/v methanol (33). Hence the adhesion of the alcohol layer to the

Table 2. The effect of different organic modifiers on capacity factor (k') and the number of theoretical plates (N).

ORGANIC MODIFIER	k'	N
Methanol	11.1	1600
Acetonitrile	8.1	2300
Ethanol	9.1	1900
propanol	7.5	2500
none	15.7	1400

Table 3. Effect of varying the percent of organic modifier on capacity factor and the number of theoretical plates.

Percent of propanol in 0.15M SDS	k'	N
1	11.8	1700
2.5	10.3	1600
5.0	8.9	3000
7.5	8.4	3900
10	7.5	3600

Column,250mm by 4.6mm, C_{18} spheri-5 analytical cartridge; Mobile phase for table 2, 10/90 organic modifier/0.15 M SDS; Flow rate, 1.5mls/min; Detector, U.V., at 255nm; Temperature, 22 C; Sample, nitrobenzene.

hydrocarbon chain of the C_{18} column increases rapidly with the carbon chain length, thereby increasing the wetting of the column. Starting from butanol the higher alcohols have been shown to alter the shape and size of the micelle as they are incorporated in the apolar region of the micelle (34,35,36). This and problems with solubility prevented the use of these higher alcohols. It is important to use as little as possible of the hydoorganic modifier, this is in order to maintain the integrity of the micelle, and to retain the mechanism of separation as due to the presence of micelles rather than shift it to conventional reverse phase chromatography.

(b) The effect of varing the percent of organic modifier present in a micellar mobile phase.

Table 3 shows the effect of varying the percent of organic modifier on capacity factor and the number of theoretical plates. The sample used in this case was nitrobenzene. There was little change in the number of theoretical plates above 5.0% propanol. This is as expected since most of the column would be covered with propanol at this point. Capacity factor continued to decrease even above 10% propanol. Using more than 10% propanol would shift the separation mechanism from a micellar one, to a conventional mechanism, also, micelle structure destruction may occur if higher concentrations were used (35).

(c) Effect of SDS Concentration variation

Figure 8 shows a plot of log k' vs log of SDS concentration, for nitrobenzene and benzene, using a 10/90 propanol/[SDS] solvent. The capacity factor remains constant as the concentration of SDS is increased up to the critical micelle concentration (CMC). After the CMC, the capacity factor drops lineary with increase in SDS concentration. This is evidence that the micelle concentration controls the retention of these solutes. Below the critical micellar concentration, there are no micelles present in the mobile phase therefore increasing the concentration of SDS only increases the number of surfactant molecules in the mobile phase. This does not seem to have any effect on the retention of the solutes. Above the critical micellar concentration, the number of micelles present in the mobile phase, increases linearly with the increase of SDS concentration. This results in a linear decrease of the capacity factor just as would occur in coventional high pressure liquid chromatography, if the ammount of hydroorganic modifier is increased in a water/methanol or water/acetonitrile mobile phase.

The critical micellar concentration of SDS in pure water at 25 C is 0.0081M. Zana et.al, using tetradecyltrimethylammoniumbromide (TTAB) surfactant, showed that alcohols when present in aqueous micellar solutions, generally decrease the value of the CMC but for short chain alcohols (ethanol and propanol) the CMC first decreases and then increases with the alcohol concentration (36). The value CMC

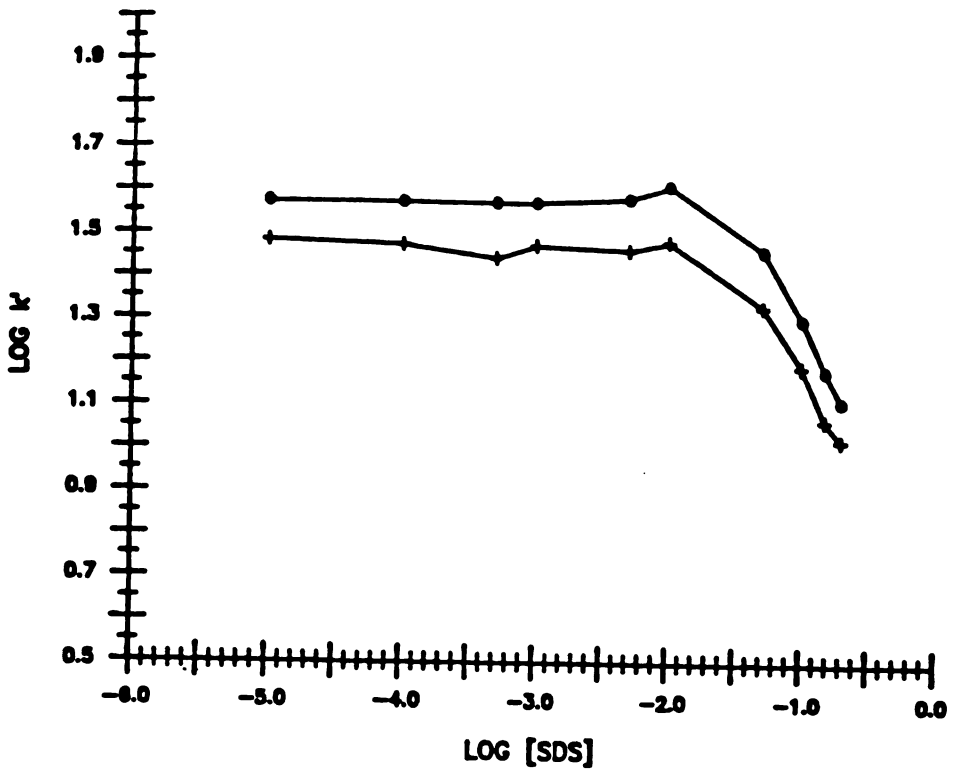


Figure 8. Log k' vs log [SDS]

Chromatographic conditions: Mobile phase, 10/90 2-propanol/[SDS]; Column, 250 x 4.6mm C_{18} spheri-5 analytical cartridge; Detector, U.V., 255nm; Sensitivity, 0.01 A.U.F.S, Flow rate 2.5 ml/min, Solutes; (●) benzene, (+) nitrobenzene.

calculated from figure 8 is 0.02M. 2-propanol is not expected to have a significant effect on either the shape or the characteristics of the micelle at this concentration, due to its size and its shape. The controlling factor for the micelle size, is the surface area available per charged head group, or the surface charge density (35). Small alcohols reduce this charge density as they tend to stay at the polar part of the micelle. As a result more surfactant molecules are needed to form the micelle, hence the increase in CMC. All the same it is prudent to keep the concentration of the hydroorganic modifier to a minimum.

To explain the theory behind micellar liquid chromatography, Arunyanart and Cline Love proposed a three phase model relating capacity factor to micellar mobile phase concentration (24). The model discussed elsewhere in this thesis proposes that there are two independent solute equilibria in micellar liquid chromatography. One between the stationary phase and the bulk solvent and the other between the micelle and the bulk solvent. From equilibria and capacity factor equations, the equation shown below was obtained (see chapter 2).

$$\frac{1}{k'} = \frac{[M_m]K_2}{p[L_s]K_1} + \frac{1}{p[L_s]K_1}$$

Where $[M_m]$ is the concentration of the surfactant in the micelles, K_2 is the solute equilibrium between the micelle and the bulk solvent per monomer of surfactant. p is the phase ratio V_s/V_m, $[L_s]$ is the

concentration of the stationary phase sites and K_1 is the solute equilibrium between the stationary phase and the bulk solvent. If the model is correct then a plot of 1/k' vs $[M_m]$ should be linear.

The experimental values of capacity factors obtained with variying SDS concentration are tabulated in table 4, and the graphs obtained by plotting the reciprical capacity factors versus SDS concentration are shown in figure 9. It is obvious from an inspection of this graph that the relationship between the reciprical capacity factors and the concentration of the surfactant is linear. This shows that the behavior predicted by equation (11) is followed. The slope/intercept ratio is equal to K_2. In order to obtain K_{eq}, the equilibrium constant per micelle, it is then necessary to multiply K_2 by the aggregation number. Using linear regression the values for benzene are;

$$\text{Slope} = 0.262$$

$$\text{Intercept} = 0.029$$

$$K_2 = \frac{\text{Slope}}{\text{Intercept}} = 8.91$$

$$K_{eq} = 8.91 \times 62 = 550 \text{ L/Mol}$$

The K_{eq} value obtained from literature (29) for benzene is 1.6×10^3. This literature value, was calculated from data obtained from aqueous micellar solutions with no hydroorganic modifier added. The value calculated above was obtained with a mobile phase of 10/90 2-propanol /0.15M SDS. The modifier as noted before is expected to change the aggregation number of the surfactant and it should also affect K_2 for

Table 4. Variation of capacity factor with SDS concentration in mobile phase

	Total SDS in mobile phase Mol/L			
	0.05	0.1	0.15	0.2
Compound	SDS in micelles; [Mm]#, Mol/L			
	0.03	0.08	0.13	0.18
Phenol	6.35	5.62	4.29	4.0
Nitrobenzene	21.35	15.32	11.65	10.47
Benzene	28.71	19.88	15.18	12.97
Toluene		30.32	21.64	17.53

SDS in micelles, [Mm] = [SDS] - CMC
Where CMC is the critical micelle concentration. The value used in this case is 0.02 M, obtained from figure 8. [SDS] is the concentration of sodium dodeocyl sulfate in the mobile phase.

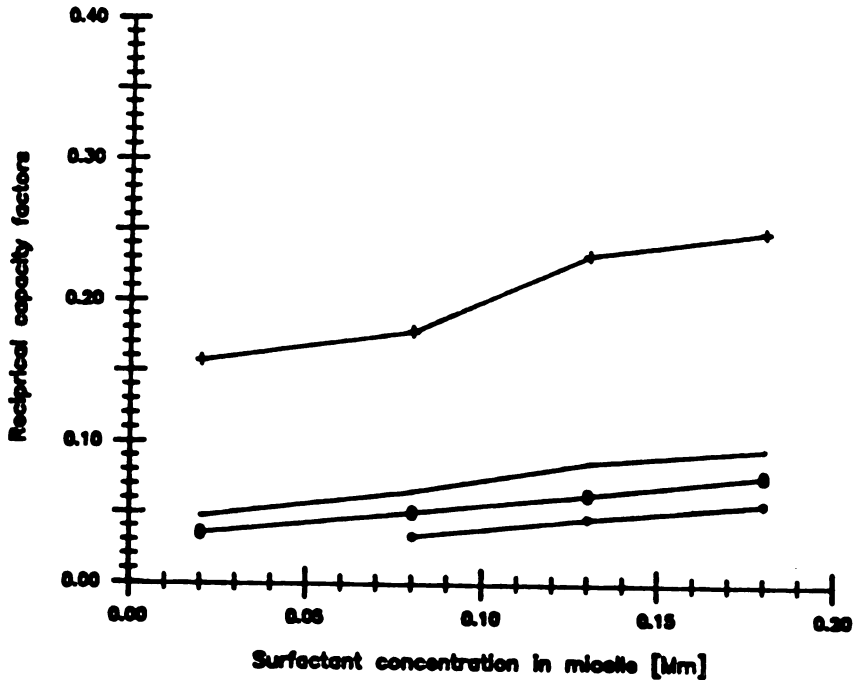


Figure 9. Reciprical capacity factors vs surfactant concentration.

Chromatographic conditions; Column 250 x 4.6mm C_{18} spheri-5 analytical cartridge; Detector, U.V., 255nm, Sentivity 0.01 A.U.F.S., Solutes: (+) Phenol, (.)Nitrobenzene, (0) Benzene, (*) Toluene.

benzene. If experimental conditions were the same, then K_{eq} obtained using spectroscopic or solubility techniques should be the same as the one obtained using micellar liquid chromatography.

3. Mass transfer and efficiency.

The problem encountered when using micellar solutions as mobile phases for liquid chromatography are supposed to originate from poor mass transfer of solute between the mobile phase and the stationary phase (chapter two). In conventional chromatography, only one equilibrium occurs in the mass transfer of the solute. This is the equilibration of the solute between the stationary phase and the mobile phase. In micellar chromatography there are two solute equilibria, one between stationary phase and the bulk solvent and the other between the micelle and the bulk solvent. Figure 4 shows the four rate constants that are involved in a micelle system. Changing these rate constants will greatly affect the mass transfer of the solute.

(a) Effect of temperature on efficiency.

In order to improve mass transfer the overall rate constants should be increased, notably exit rate constant (from the micelle) and the desorption rate constant (from the stationary phase). The easiest way to acomplish this is by raising the temperature. Raising the temperature not only increases the kinetics of the system but also lowers the viscosity of both the mobile and stationary phases, thereby increasing the rate constants.

Table 5. Effect of temperature on chromatographic efficiency.

Compound	Temperature (C)	Capacity factor (k')	Efficiency
Phenol		3.5	2300
Nitrobenzene	22	8.7	3300
Benzene		10.3	3600
Toluene		14.6	3500
Phenol		3.1	3200
Nitrobenzene	35	7.8	3200
Benzene		9.9	4100
Toluene		14.0	3800
Phenol		3.1	2900
Nitrobenzene	40	7.5	4100
Benzene		9.6	5200
Toluene		13.8	4300
Phenol		3.0	2800
Nitrobenzene	45	7.1	3800
Benzene		9.3	4700
Toluene		13.3	4800
Phenol		2.9	2700
Nitrobenzene	50	6.8	4800
Benzene		9.2	4600
Toluene		13.1	4700

Chromatographic conditions; Detector U.V., 255nm; mobile phase 10/90 2-propanol/0.15M SDS; Column 250 x 4.6mm, C_{18} spheri-5 analytical catridge; Flow rate 1.5 ml/min

A series of solutes were chromatographed at temperatures between 22 C and 50 C and the results obtained are tabulated in table 5. Overall, efficiency (N) increases as the temperature increases. Efficiency first increases as the temperature increases and then it levels off and starts to decrease. For small and polar solutes like phenol, the temperature increase that is needed to reach the maximum efficiency is small (13 C). Toluene which is more non-polar needs a higher temperature increase (23 C) to get to its maximum efficiency. More non-polar compounds would be expected to need higher temperature rises in order to reach the optimum efficiency. It should be noted that there is only a slight decrease in capacity factor values as the temperature is increased. This indicates that mass transfer and not the partition coefficient is primarily affected by the temperature increase.

(b) Effect of flow rate.

Table 6 shows the efficiencies obtained for a group of solutes with varing flow rates. For polar solutes like phenol, there is little change in efficiency between 0.5ml/min and 1.5ml/min. Past 1.5ml/min the efficiency decreases as it would be expected to in conventional chromatography. For non-polar solutes the number of theoretical plates drops continously as flow rate is increased. It would be expected that larger and more non-polar molecules would have even greater decreases due to increased flow rates.

Table 6. Effect of flow rate on efficiency for micellar chromatography.

Compound	Flow rate (ml/min)	Efficiency (N)
Phenol		3200
Nitrobenzene	0.5	6000
Benzene		6500
Toluene		6100
Phenol		3000
Nitrobenzene	1.0	4100
Benzene		4800
Toluene		5400
Phenol		3500
Nitrobenzene	1.5	4200
Benzene		4300
Toluene		4200
Phenol		2400
Nitrobenzene	2.0	2900
Benzene		3000
Toluene		4000
Phenol		1800
Nitrobenzene	2.5	2600
Benzene		2700
Toluene		3700

Detector; U.V., 255nm, Mobile phase; 10/90 2-propanol/0.15M SDS Column 250 x 4.6mm C_{18} speri-5 analytical cartridge; Temperature 22 C.

The slow equilibrium kinetics in micellar solutions is emphasized at high flow rates. For small molecules the exit and desorption rate constants are relatively large and hence mass transfer is fairly rapid. For large hydrophobic molecules, the rates are much slower and hence mass transfer is inhibited. At high flow rates a micelle containing a hydophobic solute molecule will be moved for a longer distance before it can exit from the micelle and partition into the stationary phase.

(c) Effect of surfactant concentration.

Table 7 shows how efficiency varies with the concentration of SDS. The column efficiency decreases with an increase in SDS concentration, again the highest decrease being for the more non-polar compounds. This can also be explained in terms of reduced mass transfer between the mobile phase and the stationary phase. The entrance rate into the micelle is dependent upon the concentration of the micelle in the mobile phase, hence an increase in the micelle concentration increases the probability of a solute encountering a micelle, thereby increasing the entrance rate constant. Increasing SDS concentration also increases the viscosity of the mobile phase and this results in an overall reduction in mass transfer.

Table 7. Variation of efficiency with SDS concentration in mobile phase.

Compound	[SDS] (M)	Efficiency (N)
Phenol		3500
Nitrobenzene	$5x10^{-2}$	5200
Benzene		6300
Phenol		2900
Nitrobenzene	$1x10^{-1}$	4300
Benzene		4600
Toluene		5000
Phenol		1800
Nitrobenzene	$1.5x10^{-1}$	4600
Benzene		4200
Toluene		3700
Phenol		1600
Nitrobenzene	$2x10^{-1}$	3900
Benzene		3200
Toluene		3500

Detector U.V., 255nm, Mobile phase 10/90 2-propanol/[SDS], Column 250 x 4.6mm C_{18} speri-5 analytical cartridge, Temperature; 22 C, Flow rate 2.5ml/min

4. Separation of pesticides using micellar chromatography.

The main objective of this investigation was to determine whether it is possible to use micellar solutions as mobile phases for high pressure liquid chromatography of various groups of pesticides.

It has been shown that the main problem with micellar chromatography is the loss of chromatographic effeciency. This loss has been shown to be due to poor mass transfer of solute between the mobile phase and the stationary phase. In order to improve mass transfer, and hence chromatographic effeciency, it is necessary to use elevated temperatures, low flow rates and dilute micellar solutions. This is especially necessarily so, for non-polar solutes which tend to stay in the micelle or in the stationary phase. The use of dilute micellar solutions and low flow rates results in long retention times, to overcome this, it is necessary to use columns that are more polar than would normally be used for a given class of pesticides.

(a) Separation of s-Triazine herbicides

s-Triazines are normally used as herbicides. After application it is usually necessary to find out whether residues of this pesticides remain as pollutants in the environment. Figure 10 shows the s-triazines that were used in this investigation. The chromatogram obtained after HPLC of the triazines is shown in figure 11. A C_8 column was used due to the long retention times that

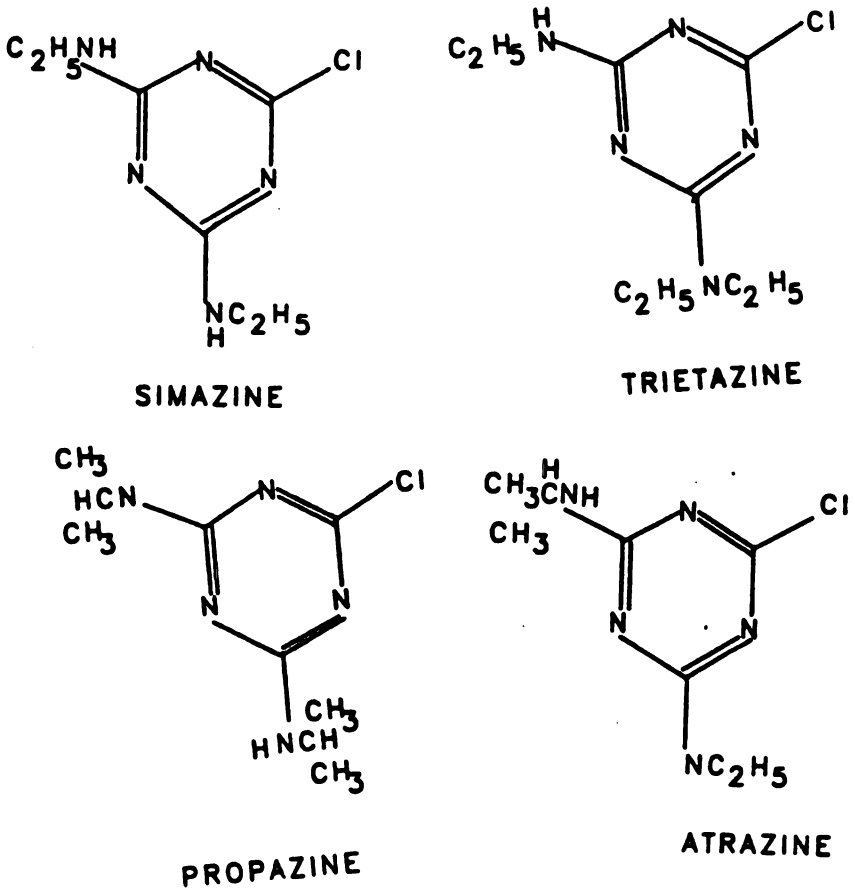


Figure 10. Structures of s-triazines used.

The working concentrations were; Simazine, 5ppm; Atrazine, 10ppm; Propazine, 5ppm; Trietazine 5ppm.

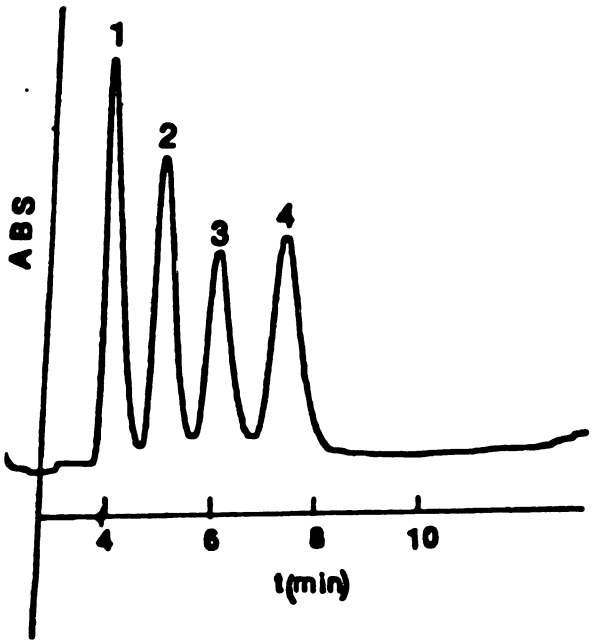


Figure 11. Micellar chromatogram of s-triazines.

The chromatographic conditions used were; Mobile phase 10/90 2-propanol/0.1M SDS, Column 25cm x 4.6mm C_8 spheri-10 analytical cartridge, Temperature 60 C, Flow rate 1ml/min, Chart speed 6.67mm/min Detector U.V., 235nm, Solutes: peak 1, simazine; peak 2, atrazine; peak 3, propazine; peak 4, trietazine.

resulted when the more hydrophobic C_{18} column was used. Higher temperatures than those previously used were necessary since these are more hydrophobic compounds than those used before. To determine the optimum wavelength for the detection of these compounds, it was necessary to run a U.V. scan for each compound. It was found that, the most appropriate wavelength was 235nm.

The separation mechanism is as in conventional chromatography, where the polarity of the compound determines its retention time. The less hydrophobic compound simazine, elutes out of the column first and the more hydrophobic compound trietazine is retained the longest.

(b) Organochlorines

Organochlorines are a group of broad action and persistent insecticides. They are stable under most environmental conditions and are resistant to complete breakdown by enzymes present in soils, microorganisms and by higher organisms. The persistence of these pesticides in the environment is mainly due to the fact that they are soluble in the organic fractions of soil, tissues et cetra and are virtually insoluble in water.

Although their use is now limited in most of the developed countries, their cost and availability continue to make them the main method of insect control in the developing countries.

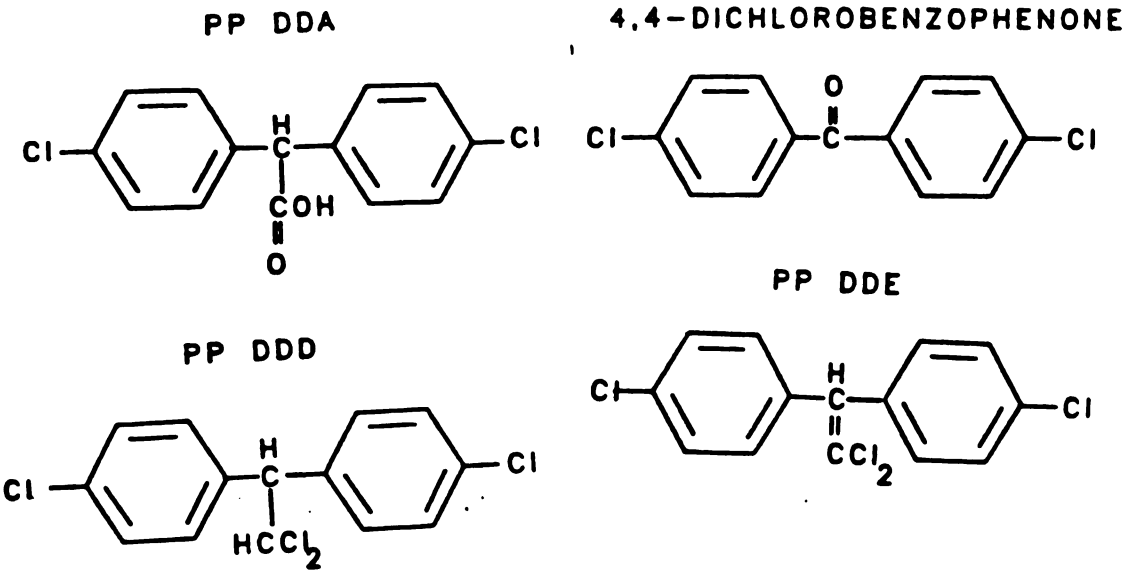


Figure 12. Molecular structures of the organochlorines used.

The working concentration was 2ppm for each solute.

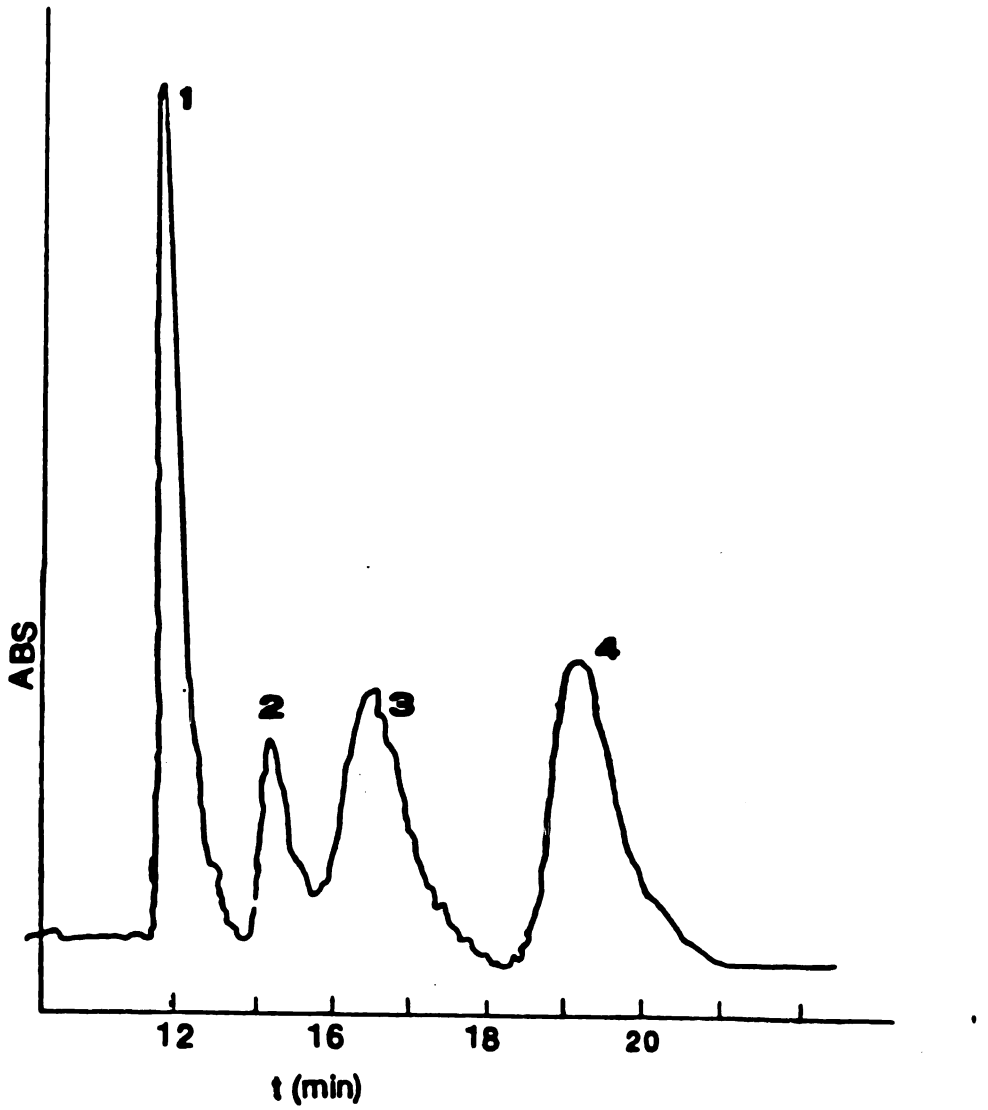


Figure 13. Micellar chromatogram of organochlorines

The chromatographic conditions used were; Mobile phase; 10/90 2-propanol/0.05M SDS, Column; 10cm x 4.6mm cyano spheri-10 analytical cartridge, Temperature; 65 C, Flow rate; 0.3ml/min, Chart speed; 5mm/min, Detector; U.V., 235nm, sensitivity; 0.01 a.u.f.s., Solutes: peak 1, DDA; peak 2, 4,4-dichlobenzophenone; peak 3, DDE; peak 4, DDD.

The structures of the four chlorinated pesticides used are shown in figure 13. The chromatogram obtained from their HPLC is shown in figure 12. By obtaining the U.V. spectra of the four compounds, it was determined that their most convenient wavelength for detection was 235nm. Again the polarity of the compound determines the retention time of the solute.

A Cyano column was used for the seperation of these compounds. Surfactant molecules are expected to coat the stationary phase surface and the retention mechanism would be due to the interaction of the pesticides with the surfactant molecules (acting as the stationary phase) and the mobile phase. From the chromatogram, it can be seen that the most polar compound had the least retention time while the least polar compound had the longest retention time.

(c) Polycyclic aromatic hydrocarbons (PAH).

Recently polycyclic aromatic hydrocarbons have received a lot of attention due to their carcinogenic properties. Modern analytical chemistry has assumed a very important role in answering the highly complex questions arising from PAH carcinogenesis. This role concerns not only the detection and quantition of PAH in complex environment mixtures but also the products formed during metabolism (37).

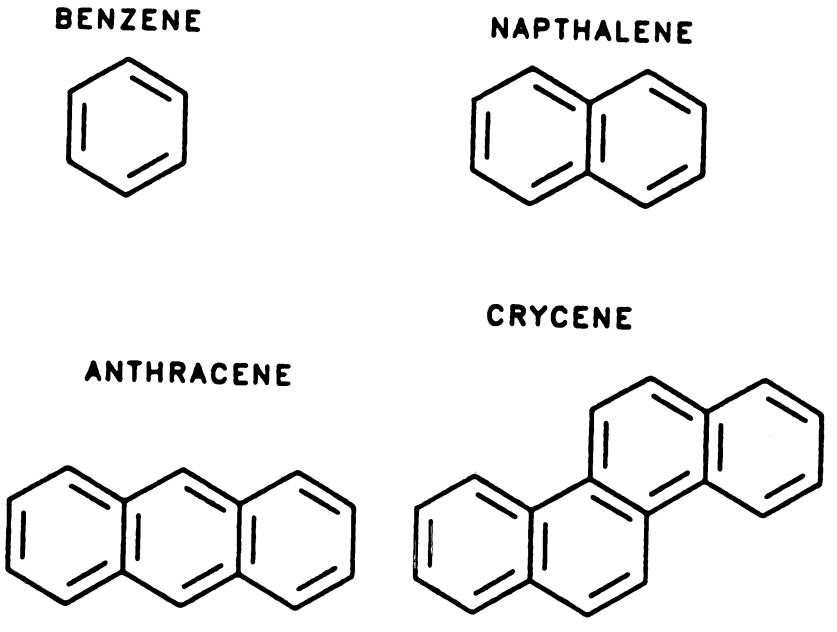


Figure 14. Molecular structures of polycyclic aromatic hydrocabons.

The working conditions were; Benzene; 200ppm, Napthalene; 10ppm Anthracene; 1ppm, Crysene 5ppm.

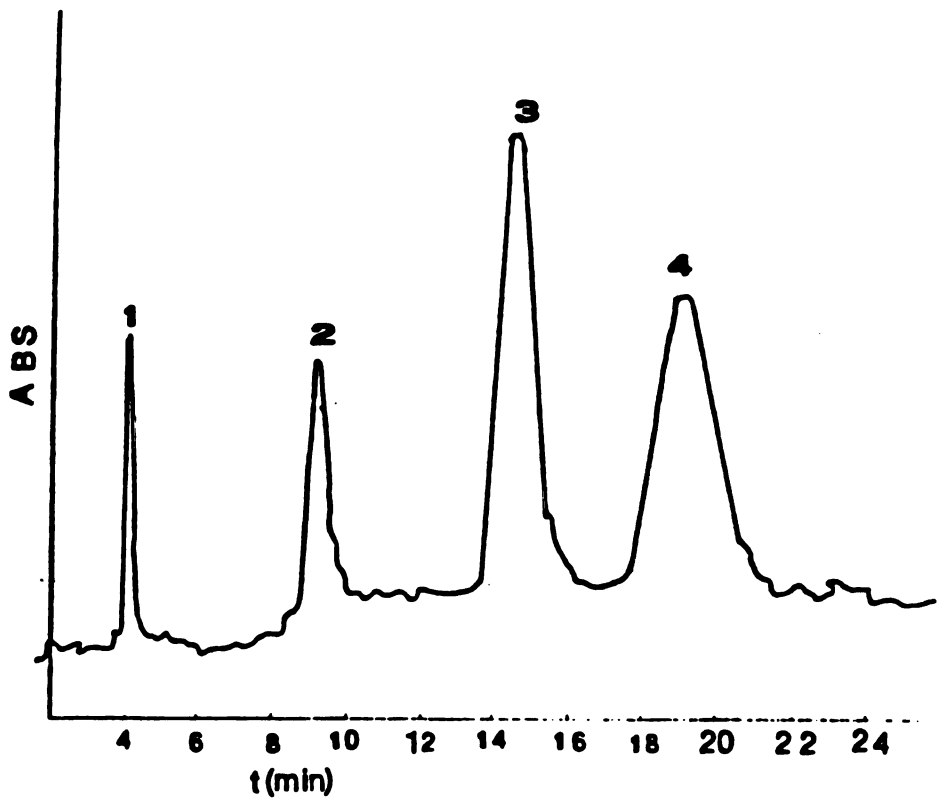


Figure 15. Micellar chromatogram of Polycyclic aromatic hydrocarbons.

The chromatographic conditions used were; Mobile phase; 10/90 2-propanol/0.05M SDS, Column; 10cm x 4.6mm C_2 spheri-10 analytical cartridge, Temperature; 65 C, Flow rate; 2.5ml/min, Chart speed; 5mm/min Detector; U.V., 255nm, Solutes: peak 1, benzene; peak 2, napthalene; peak 3, anthracene; peak 4, crysene.

The structures of the group of PAHs that were separated are shown in figure 14 and the chromatogram obtained is shown in figure 15. The use of a C_2 column means again that the surface of the stationary phase will be coated with the surfactant molecules and the separation mechanism will again be due to the interaction of the solute with the surfactant molecules (acting as the stationary phase) and the mobile phase. The polarity of the solutes again determines their retention times. It is important to use high temperatures in order to increase the rate constants discussed earlier as these compounds are very hydrophobic. Since the concentration of the SDS used in this separation is low, high flow rates are necessary in order to reduce the retention times.

(d) Chlorinated phenols.

Chlorophenols are used for the manufacture of many pesticides and hence are usually present as contaminants in pesticides. Their levels in the environment and in the pesticides they are used to make is important to the pesticide chemist.

The structures of the chlorinated phenols used, are shown in figure 16 and the chromatogram obtained shown in figure 17. From pure polarity considerations, the order of elution would be, m-chlorophenol, dichlorophenol, trichlorophenol, tetrachlorophenol and pentachlorophenol. From the chromatogram it can be seen that this is not the order followed. m-Chlorophenol has the shortest retention

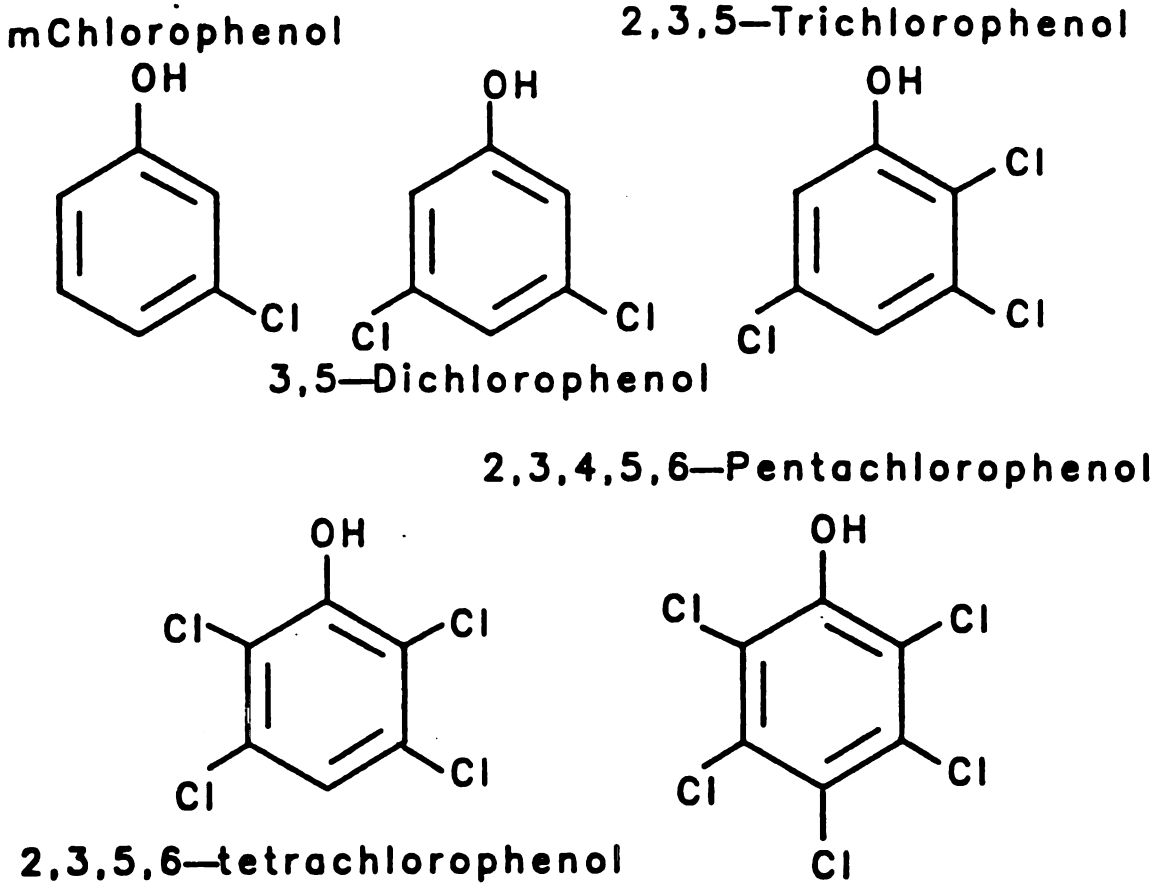


Figure 16. Molecular structures of chlorinated phenols used.

· The working concentration was 10ppm for each solute.

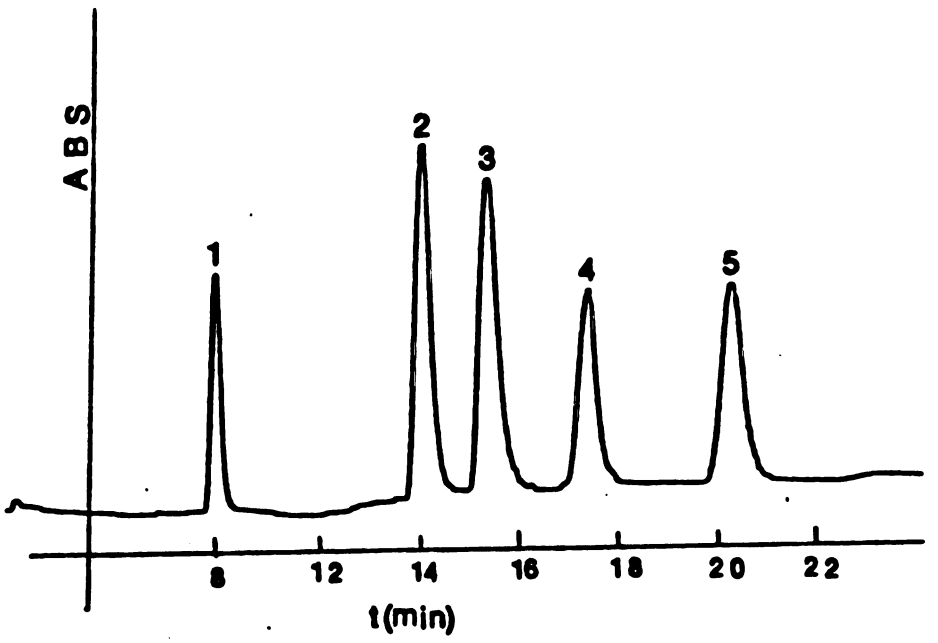


Figure 17. Micellar chromatogram of chlorinated phenols.

The chlomatographic conditions used were; Mobile phase; 10/90 2-propanol/0.05M SDS, Column; 22cm x 4.6mm C_8 spheri-5 analytical cartridge, Temperature; 65 C, Flow rate; 2ml/min, chart speed; 6.67mm/min, Detector; U.V., 230nm, Sensitivity; 0.01 a.u.f.s., Solutes: peak 1, m-chlorophenol; peak 2, tetraclorophenol; peak 3, pentachlophenol; peak 4, dichlorophenol; peak 5, trichlorophenol.

time followed by tetrachlorophenol, next is pentachlorophenol, then dichlorophenol and lastly trichlorophenol. Yarmchuk et al., have shown that a plot of log of capacity factor (for a series of solutes) versus log of surfactant concentration results in linear plots as would be expected, (retention time decreases with increase in surfactant concentration) but the plots are not parallel but intersect one another. Thus not only is the capacity factor but also the separation factor is changing, resulting in an inversion of retention orders (10). This is a result of the two competing equlibria, namely solute-micelle association and solute-stationary phase equilibria. This same type of separation is occuring with the chlorophenols with the exit rate constants from the micelle for tetrachlorophenol and pentachlorophenol being so low that their retention times are much lower than would be expected from purely polarity considerations alone.

Conclusion

Aqueous micelle solutions offer an alternative to hydroorganic solvents as mobile phases for reverse phase high pressure liquid chromatography. The main limitation of micellar chromatography is the low chromatographic efficiency associated with them. This results in band broadening and eventually poor separations. The main reason for low chromatographic efficiency is poor mass transfer of the solute between the stationary phase and the mobile phase. To improve mass transfer and hence chromatographic efficiency, it is necessary to use elevated temperatures, low flow rates and dilute micellar solutions.

The elution behavior of a solute in micellar chromatography can be explained in terms of the chromatographic conditions being employed and the two principle equilibria that occur in micellar chromatography. The conditions that are easily varied in micellar chromatography include the surfactant concentration, the stationary phase (column), the temperature and the flow rate.

The surfactant concentration variation only varies the concentration of micelles present in the mobile phase. This is the main way of varying the solvent strength in micellar chromatography. This is equivalent to varying the concentration of the organic modifier e.g., methanol in conventional reverse phase high pressure liquid chromatography. When chromatographing large and non-polar

solutes, it is necessary to use a surfactant concentration close to but above the CMC.

Micellar solutions are not as strong solvents as hydroorganic solvents are. The need to use dilute solutions leads to long retention times for non polar solutes when using hydrophobic stationary phases. Therefore when chromatographing non polar solutes, short chain stationary phases should be used, this may eliminate the need to add hydroorganic modifiers in the mobile phase as there is no need to wet the stationary phase.

Micellar chromatography should be carried out at elevated temperatures. For small and polar solutes, only slight increases are needed but for bigger and non polar solutes, higher temperatures are needed. Operating commercial silica based columns above 60 C leads to rapid deterioration of the column as the silica support is solubilized. To prevent this, it is necessary to use a saturator column in micellar chromatography. The use of polymer based columns would eliminate the need for a saturator column.

The main problem encountered in this investigation was the lack of sensitivity for the solutes by the U.V., detector used. This made it necessary to use high concentrations of the solutes which in turn lead to band broadening and poor separations. Micellar solutions have been found to enhance fluorescence and room temperature phosphorescence of certain solutes (chapter 2). Use of these detection modes should have better sensitivities for aromatic

compounds and especially better resolution would be obtained for the polycyclic aromatic hydrocarbons and the organoclorines.

The columns used for the separation of PAH and organochlorines were 100 x 4.6mm C_2 and cyano, spheri-10 analytical cartridges respectively. Better resolution for these compounds would be expected if columns with 5um packing particle size were used. Longer columns would also have improved the resolution.

The use of reversed micelles to do normal high pressure liquid chromatography was not investigated at this time. Since water is the strongest solvent in normal HPLC, small variations in water content in the mobile phase couses dramatic differences in retention times. Use of micelles can solubilize this water and therefore make normal HPLC less dependent on the water content.

From this investigation, it can be concluded that micellar solutions can be used as mobile phases for high pressure liquid chromatography. If the correct chromatographic conditions are used, micellar chromatography can offer high sensitivity and selectivity for the separation and detection of compounds of interest.

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