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Enantioselective Enrichment of Selected Pesticides by Adsorptive Bubble Separation

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List of Abbreviations

ABS	Adsorptive Bubble Separation
DCPP	Dichlorprop
DME	Dichlorprop methyl ester
MCPP	Mecoprop
α-HCH	α-Hexachlorocyclohexane
FME	Fenoprop methyl ester
HCB	Hexachlorobenzene
CD	Cyclodextrin
HPβCD	2-Hydroxypropyl-β-cyclodextrin
TMβCD	Heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin
MβCD	Methylated-β-cyclodextrin
MeO	Methanol
THF	Tetrahydrofurane
HPLC	High Performance Liquid Chromatography
CE	Capillary Electrophoresis
CSP	Chiral Stationary Phase
CMPA	Chiral Mobile Phase Additive
GC	Gas Chromatography
ECD	Electron Capture Detector
FID	Flame Ionization Detector

DST	Dynamic Surface Tension
ee	Enantiomeric excess
ER	Enantiomeric ratio
E_R	Enrichment ratio
ppm	Parts per million
mM	Milimolar
c1, c2	Concentrations of first and second eluted (in GC) enantiomer
A_1, A_2	Peak areas of first and second eluted (in GC) enantiomer
A_{IS}	Peak areas of internal standard (in GC)

INTRODUCTION

In 1848, when examining tiny crystals of tartaric acid, Louis Pasteur noticed that the crystals came in two asymmetric forms that were mirror images of each other. He sorted the two forms with a pair of tweezers into two piles and discovered that a solution of one form would rotate polarized light clockwise, while a solution of the other form would rotate the light anticlockwise, and an equal mix of the two would not rotate the light at all. This was the first chemical experiment in which chirality was demonstrated.

In the conclusion of his observations, Pasteur wrote: “Most natural organic products, the essential products of life, are asymmetric and possess such asymmetry that they are not superimposable on their images…..”

In 1874, two scientists J. A. Le Bel and Dr. J.H van’t Hoff independently argued that the spatial arrangement of four groups around a central carbon atom is tetrahedral. As often, when unconventional scientists depart from traditional ways of thinking, they were harshly criticized. Dr. van’t Hoff’s work was dismissed as “**...**childish fantasy...” and he was criticized for having “...no taste for accurate chemical research...”. Nevertheless, abundant evidence supporting their hypothesis accumulated, and in 1901, van’t Hoff was the first recipient of the Nobel Prize for Chemistry. The asymmetric carbon proposed by Van’t Hoff had the correct tetrahedral shape, whereas Le Bel, proposed a square pyramid.[27]

The works of Van’t Hoff and Le Bel marked the beginning in the field of stereo-chemistry.

The separation of chiral compounds has been of great interest because the majority of bioorganic molecules are chiral. Amazingly, chirality is more the rule than the exception in the living world; and the important building blocks of life, such us DNA, RNA and proteins, are all composed of chiral molecules. Moreover, they are

homochiral – proteins consist of L-amino acids and DNA and RNA consist of the D-enantiomers of deoxyribose and ribose, respectively. Because of chirality, living organisms show different biological responses to one of a pair of enantiomers in drugs, pesticides, or taste compounds, for example.

Chirality is a major concern in the modern pharmaceutical industry. This interest can be attributed largely to a heightened awareness that enantiomers of a racemic drug may have different pharmacological activities, as well as different pharmacokinetic and pharmacodynamic effects. The body being amazingly chiral selective, will interact with each racemic drug differently and metabolize each enantiomer by a separate pathway to produce different pharmacological activity. Thus, one isomer may produce the desired therapeutic activities, while the other may be inactive or, in worst cases, produce unwanted effects.

Interest in environmentally stable chiral compounds is relatively recent, being know that many of agrochemicals are used as racemic. König et al. separated racemic a-hexachlorocyclohexane (a-HCH) by chiral gas chromatography (GC) using cyclodextrin (CD) as a chiral stationary phase (CSP). The cited authors suggested that this technique could be used for enantioselective analysis of chiral pollutants. Shortly thereafter, chiral environmental analyses of several persistent organic compounds (POPs) were reported, such as chlordanes, *o,p'*-DDT, toxaphenes and atropisomeric PCBs. By then it was well known that organochlorine (OC) pesticides and other POPs were ubiquitously distributed throughout the world. In spite of restrictions and bans, these lipophilic compounds are found in all environmental compartments, even in such remote areas as the Arctic and Antarctic.[27]

Since:

1. a significant number of environmental chemicals are chiral,
2. these chemicals are released into the environment as racemates,
3. enantiomers frequently exhibit different toxicological and other biological activities, and
4. most biochemical processes in nature are stereospecific,

there is a growing concern about the ecotoxicological effects of chiral pesticides and pharmaceutical drugs in current use[27].

Worldwide, the market for chiral chemicals sold as single isomers was §6,63 billion in 2000 and will grow at 13.2% annually to §16.0 billion in 2007, according to a recent study made by the market research firm Frost & Sullivan, London.[58] The drug industry is the engine that is driving this strong growth, accounting for 81.2% of the total, the remaining being divided among agrochemical, food, flavors, and fragrances industries.

As a result of this, nowadays the preparation and control of enantiomerically pure compounds is of immense interest. Thus, the interest in finding new cheap, environmentally friendly techniques for the separation of isomers (enantiomers) is increasing.

Foam fractionation can represent an alternative to the classical methods used for obtaining the pure enantiomers. Foam fractionation is one member of a group of processes known as Adsorptive Bubble Separation (ABS) methods.

Chromatographic separation methods are based on the differential interactions between a stationary phase and the target molecules in a mobile phase. The targets are absorbed at the inter- or extra-surface of the stationary phase and then eluted sequentially. The basis of separation by foam fractionation is the difference in

surface activity of molecules in a mixture coupled with the very high surface-to-volume ratio of the foam. The separation material (stationary phase, mobile phase, elution solutions) used in chromatographic methods is not used in foam fractionation. Thus, a distinct advantage of foam fractionation over the chromatographic separation is its efficiency of processing large amounts of material at relatively low equipment, operation and labor costs.

Foaming has long been employed in the purification and concentration of conventional surface-active substances. The surface activity of proteins is well recognized and foam-formation of proteins has been studied [28, 73, 74, 116] in the food and pharmaceutical industry. Foams are also being considered for use in isolating active principles from medical plants for pharmaceutical use, removing of detergents from sewage, enhanced oil recovery, insulating and reducing the impact of explosions.[119]

REASONS AND SCOPE OF THE THESIS

The growing use of pure enantiomers in the pharmaceutical, agrochemical, food, flavors, and fragrances industries calls for search for new enantioselective methods applicable on large industrial scale.

As previously was emphasized, foam fractionation methods have various field of applicability. Theoretically, with these methods, any dissolved substances can be concentrated and/or separated if an appropriate collector with adequate surface activity can be found. Foam fractionation shows particular promise in the concentration and/or separation of substances, present in low concentrations, from large volumes of liquids.

Cyclodextrins (CDs) are the most widely used molecules that form host-guest type inclusion complexes. Although as recently as the 1970's these long time known

molecules were merely scientific curiosities, available only as expensive fine chemicals, by the end of 20th century they were produced and used industrially in thousand tons amounts[160].

The spectacular development of CD's technology relies on a series of reasons like:

i. they are semi-natural products; produced from a renewable natural material, starch, by a relatively simple enzymatic conversion,
ii. they are produced in thousand ton/year amounts by environment-friendly technologies,
iii. their initially high prices have dropped to levels where they become acceptable for most industrial purposes,
iv. through their inclusion complex forming ability, important properties of the complexed substances can be modified significantly. This unprecedented "molecular encapsulation" is already widely utilized in many industrial products, technologies, and analytical methods,
v. any of their toxic effect is of secondary character, and can be eliminated by selecting the appropriate CD-type or derivative, or mode of application,
vi. consequently, CDs can be consumed by humans as ingredients of drugs, foods, or cosmetics.

Cyclodextrins represent also one of the most widely used groups as chiral selectors. CDs are extensively used as stationary bonded phase (CPS) in high-performance liquid chromatography (HPLC) and gas chromatography (GC), or as mobile additives in HPLC and capillary electrophoresis (CE).

Because natural CDs are typically surface-inactive organic compounds, they have not attracted a surface chemical interest. Instead, some modified CDs tend to be surface active. As they present a distinct hydrophobic and hydrophilic part, they possess surfactant proprieties and behave as conventional surfactants.[4, 19, 55]

This raised the idea of using modified CDs as *surface-active chiral collectors* in conjunction with a foam separation technique.

The present work is intended to illustrate the potential of foam fractionation as a cheap, clean, and easy to operate method for concentration and/or enantiomeric separation using the surface activity of some derivatizated CDs.

There are other chiral selectors with surface-active proprieties. The following reasons have determinated the choice of CDs for this work:

- CDs are practically nontoxic for humans and for environment,
- CDs are available at very low purchasing price,
- CDs present very broad chiral recognition spectra,
- CDs are able to form host-guest complexes with a large variety of compounds.

As to yet, the surface-active properties of modified CDs have not been well studied.
In spite of the great advantages and promises of foam fractionation, only one attempt of enantiomeric enrichment using *surface-active chiral collectors* was briefly reported with no further studies or developments.[145]

There were several interrelated objectives in this work.
The first one was a trial to find out if the enrichment of different compounds can be achieved using modified CDs as surface-active collectors and to develop a method for optimizing the process. In parallel, attempts to obtain the maximum enantiomeric excess (“enatiomeric enrichment”) for these compounds, which are also known to be able to enantioselective associate with CDs, were done. The influence of parameters and conditions of the process were investigated in accordance with the theory available in the field of foam fractionation and enantiomeric separation.

Gas chromatography was used as an efficient and sensitive method in determination of the enantiomeric excess (“enantiomeric enrichment”).

Compromises between the CD’s chiral selectivity toward certain molecules, the solubility of these molecules and of the CDs, and the possibility to use foam fractionation method with these specific pairs CDs – selectands had to be done. Moreover, the choice of the compounds was done taking also into account that these should be volatile enough in order to be analysed with chiral GC methods.

1. FOAM SEPARATION

1.1 Overview

Foam separation is a new separation technology developed in recent decades. Although at the beginning of the 20th century foam flotation had been widely used in the mine metallurgy industry, only in the last three decades has foam separation been used to separate ions, molecules, colloids, and deposits in many fields such as environmental protection, biotechnology, and pharmaceutical industry.

Foam separation is one member of a group of processes known as Adsorptive Bubble Separation (ABS) techniques that concentrate and/or separate species depending on their surface activity. ABS techniques are divided into two main groups: *foam separation* and *non-foaming adsorptive bubble separation*.

As their names suggest, first technique requires the generation of foam or froth to carry off the objective species while in *non-foaming adsorptive bubble separation* these are levitated on bubbles surface to the top of the liquid pool where they re-deposit at the surface of the liquid pool or encounter a solvent layer to which they are transferred as the bubble moves trough the solvent layer. A schematic representation generally accepted as the nomenclature for ABS techniques is presented in Figure 1.1.

Foam separation includes *foam flotation* and *foam fractionation*.
Foam flotation is the removal of particulate material by foaming.
Foam fractionation, the second subdivision of foam separation, is further described in detail, being the method utilized in this work.

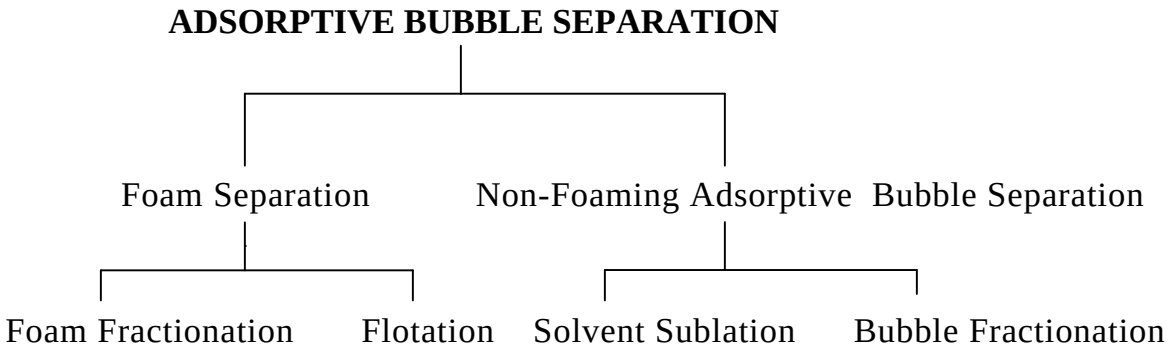


Figure 1.1. Schematic Representation of Adsorptive Bubble Separation Methods.

1.2 Foam Fractionation

In foam fractionation solute species adsorb at the gas-liquid interface between a dispersed phase (gas bubble) and a continuous phase (bulk liquid). Foam fractionation processes have been used to concentrate (enrich) and/or to remove surface-active agents (surfactants) from aqueous solutions. Non-surface active materials can be also removed by interaction with surfactant and carried along into the foam. In this case, the surfactant is called *collector*. Moreover, foam fractionation is used for separation of species from complex mixture depending on their surface activity.

Thus, the main principles of the foam fractionation are:

1. in bulk solution the surface-active substances preferentially adsorb, function on their surface activity, onto the gas-liquid interface (bubbles surface);
2. the bubbles rise through the solution and accumulate above the bulk liquid surface to form a foam phase with a small amount of liquid entrained in the spaces between the bubbles;
3. because of gravity, this liquid tends to drain down and return to the bulk solution thus drying the foam phase.

When the foam is removed and collapses, a concentrated solution (the foamate) is obtained. After foam removal, a residual diluted solution remains.

Consequently, the factors governing the efficiency of foam fractionation are:

(i) physicochemical properties of surface-active substance (surfactants),

(ii) foam properties,

(iii) operating variables.

1.2.1 Adsorption in the bulk solution

1.2.1.1 Surfactants

A surfactant is an organic compound, which reduces the surface tension of a liquid (aqueous or non-aqueous). Surfactants are commonly referred to as surface-active agents, emulsifiers, wetting agents, or simply detergents. Surfactant molecules consist of both hydrophilic head group (water-attracting) and hydrophobic tail group (water-repelling) moieties in their structure and are thus referred to as amphiphilic / amphipathic molecules (Figure 1.2).

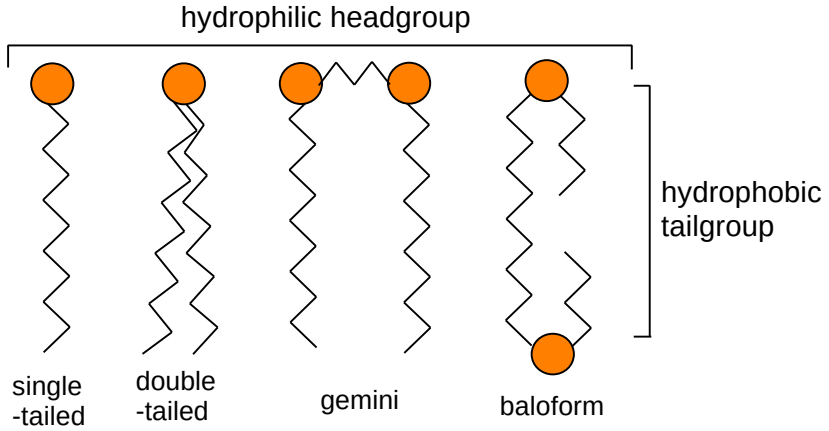


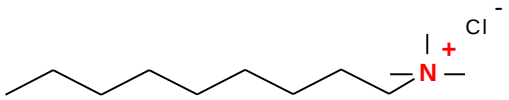
Figure 1.2. Structure of different types of surfactant monomers.

The hydrophobic tail group can be a long, short linear or branched hydrocarbon chain that interacts weakly with the water molecules. The hydrophilic head group,

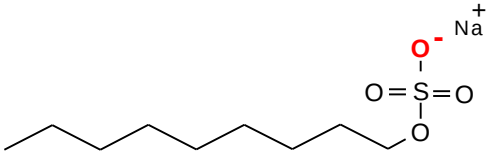
which can either be ionic or non-ionic, usually interacts strongly with an aqueous environment through a solvation process involving dipole-dipole or ion-dipole interactions.

Surfactants are classified into four groups: anionic, cationic, zwitterionic, and non-ionic depending on the charge of the hydrophilic head group in aqueous solution (Figure 1.3).[16]

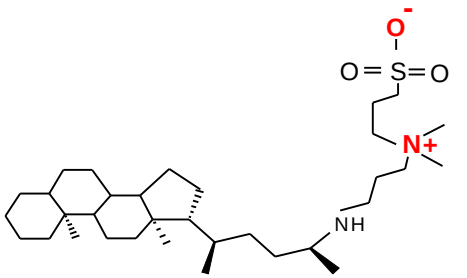
Cationic
(cetyltrimethylammoniumchloride)



Anionic
(Sodium dodecyl sulfate)



Zwitterionic
(CHPS)



Nonionic
(N-Docyl-β-D-Glucopyranoside)

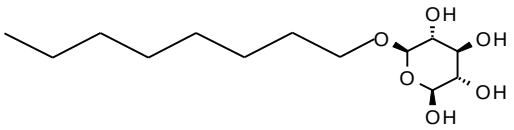


Figure 1.3. Classification of surfactants.

In solution, surfactant molecules self assemble to form clusters, known as micelles, trough the micellization process. The self-association process is concentration dependent. For example, in dilute solutions, surfactant molecules exist as individual species (monomers). As the concentration increases, the monomers begin to aggregate to form micelles depending on temperature, pressure, and the presence of additives. Micelles are non-covalently bonded macromolecular aggregates that continually associate and dissociate with the monomeric forms of the surfactant on a

time scale of 10^{-6} to 10^{-3} seconds. Micelles typically form at a well-defined concentration of surfactant known as the critical micelle concentration (cmc).

Micelles in aqueous solution provide a transient hydrophobic environment different from bulk water, in which discriminative interactions with analytes may occur. When an additive is incorporated into the micelle, three types of interactions are possible (Figure 1.4). First, the additive molecule may be adsorbed onto the surface of the micelle by electrostatic or dipole interaction (A); second, it may behave as a co-surfactant by participating in the formation of the micelle (B); and third, it may be incorporated into the core of the micelle (C).

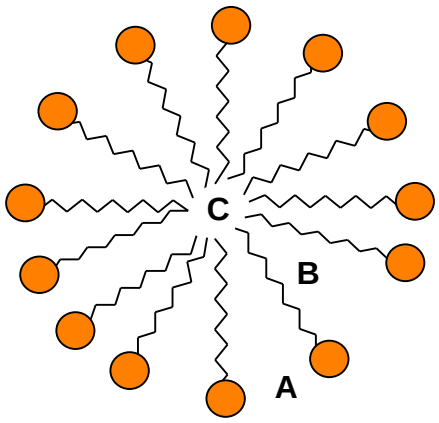


Figure 1.4. Schematic of interaction between three types of analytes (A, B, and C) and an ionic micelle.

For example, highly polar molecules will be mainly adsorbed onto the surface of the micelle unless they are substantially hydrophobic as a whole. Polar molecules having both hydrophilic and hydrophobic groups in the molecule may behave as co-surfactants. In principle, micellar size and shape are sensitively dependent on the molecular structure of the surfactant, nature of the solvent (temperature, pressure, ionic strength, presence of additives etc), and concentration of the surfactant solution. As a result, various shapes of micellar structures may be formed.

1.2.1.2 Dynamic surface tension and adsorption

Surface tension is an intermolecular, attractive force between adjacent molecules (Figure 1.5 A), expressed in force per unit width, as dynes/centimeter (dynes/cm) or milliNewtons/meter (mN/m).[16] At 20°C, water has a high surface tension in the range of 72.8 dynes/cm, while alcohols are in a low range of 20 to 22 dynes/cm.

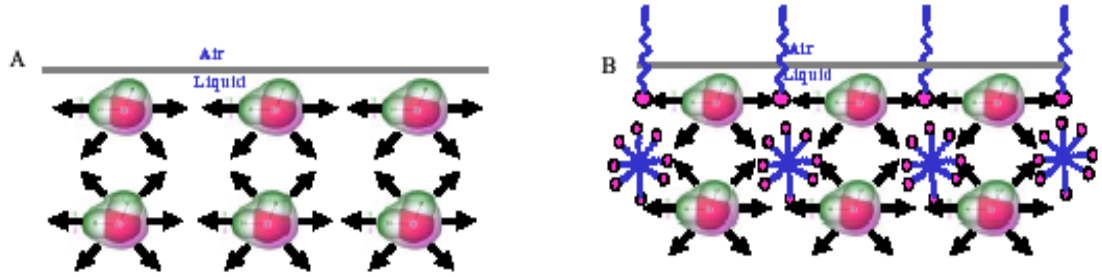


Figure 1.5. Effect of surfactant molecules on the surface tension of water.

The surface tension of water is affected by temperature, pressure, presence of additives, and surfactants. Surfactant molecules arrange at the surface of the water such that the hydrophilic part interacts with the water and the hydrophobic part is held above the surface of water. The presence of surfactant molecules on the surface disrupts the cohesive energy of the water molecules hence, lowering the surface tension (Figure 1.5 B).

A freshly formed interface of a surfactant solution has a surface tension, γ, very close to that of the solvent, γ_0. Over a period of time, γ will decay to the equilibrium value, γ_{eq}, and this period of time can range from milliseconds to days depending on the surfactant type and concentration. This dynamic surface tension (DST or $\gamma(t)$) is an important property as it governs many important industrial and biological processes.[75]

For a surfactant solution at equilibrium, the interfacial (or surface excess) concentration of surfactant is given by G_{eq}. However, adsorption is a dynamic phenomenon, and at equilibrium the adsorbing flux of monomers to the surface, j_{ads}, is equal to the desorbing flux, j_{des}. This is shown in Figure 1.6. If the surface is stretched, by creating a liquid drop or by forming an air bubble in a liquid, the surface excess concentration, G, immediately after the perturbation will be now less than G_{eq}. Therefore, to re-establish equilibrium the adsorbing flux j_{ads} will now be greater than the desorbing flux, j_{des}, and in order to obtain equilibrium, there will be an overall transport of monomer from the bulk to the interface. If the equilibrium surface is contracted, then $G > G_{eq}$ and therefore $j_{des} > j_{ads}$ in order to re-establish equilibrium, and there will be an overall transport of adsorbed monomer from the surface into the bulk back diffusion (Figure 1.6).

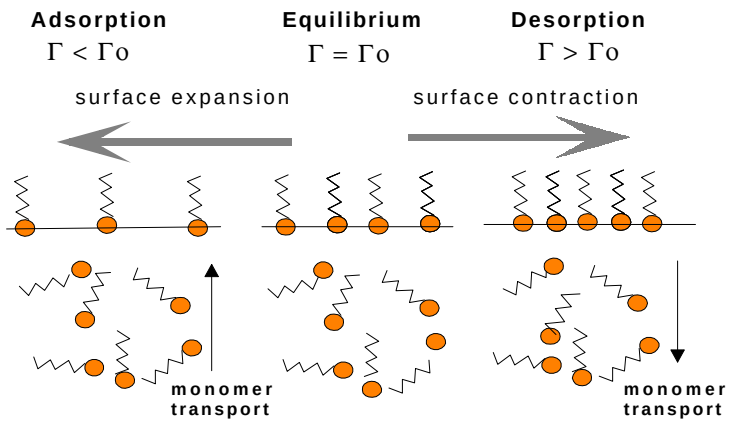


Figure 1.6. Surface expansion and contraction may drive the flux of monomer to the interface.

A simple relationship describing this kinetic mechanism is described in Eq. 1.1.

$$\frac{dG}{dt} = j_{ads} - j_{des} \qquad (1.1)$$

As stated, when a fresh surface is created, initially the surface excess of monomer is less than the equilibrium value, and since $G < G_{eq}$ there will be a flux of monomer

from the bulk to the interface. This flux will cause the surface tension to decay from γ_0 to γ_{eq}, where the interfacial concentration has reached its equilibrium value, i.e. G_{eq}.

Broadly speaking there are two main models for monomer transport and adsorption, and these are depicted in Figure 1.7. Once the monomer has diffused to the subsurface it will either instantaneously adsorb at the interface in accordance with a diffusion-controlled model (1) or will have to pass through a potential barrier to adsorb (2).
The subsurface may be taken as an imaginary plane, a few molecular diameters below the interface.

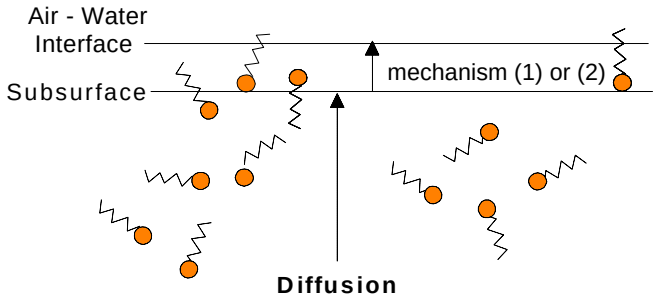


Figure 1.7. Transport and adsorption of monomer to the interface.

1.2.1.3 Mechanisms of surfactant adsorption

Diffusion-only mechanism

The most theoretical work regarding surfactants absorption onto gas/liquid interface was published by Ward and Tordai in 1946.[75]

The Ward and Tordai theory accounts for the diffusion of monomers from the bulk to the interface, and also the back diffusion into the bulk as the interface becomes more crowded. At the start of the process monomers from the subsurface adsorb directly, the assumption being that every molecule arriving at the interface is likely

to arrive at an empty site, a reasonable postulate for the start of adsorption. However, as the surface becomes fuller, there is an increased probability that a monomer will arrive at an already occupied site. Back diffusion from the subsurface to the bulk must then also be considered.

Mixed diffusion-kinetic controlled adsorption:
presence of an adsorption barrier

In this activated-diffusion mechanism, the monomers undergo diffusion from the bulk to the subsurface, obeying the same diffusion equations as for the diffusion only mechanism. However, once in the subsurface the monomer is not instantaneously adsorbed at the interface. It may have to do any of the following:

Overcome any potential energy barrier

In order to adsorb, a monomer has to do work against the increasing surface pressure π. When π is high it is unlikely that every molecule reaching the subsurface will have enough energy to adsorb at the interface. Only those molecules possessing an energy greater than a specified activation energy will be able to adsorb.

Be in the correct orientation for adsorption

For the monomer to penetrate the surface film, it may have to adopt a certain configuration. This is particularly the case for very long chain surfactants, polymers and proteins where the monomer may not adsorb if the chain is closely entangled within itself. In preference to rearranging itself in order to reach the adsorbed state, it may back diffuse into the bulk.

Strike an ‘empty site’ in the interface

When present in the subsurface there has to be an ‘empty site’ in the interface above the monomer. Unlike the two factors above which were thermodynamically based, this is a statistical parameter.

The presence of micelles, and the time-scale for break-up, may hinder adsorption
Once the surfactant solution is above its cmc, the micelles present in the solution have a certain lifetime for break-up. If the micelles are stable entities with long lifetimes, the molecules in the micelles may not be available for adsorption, and hence the surface tension will decay more slowly. In effect, the concentration of molecules diffusing to the interface will be equal to the cmc.

The term “adsorption barrier” can be used as a catch-all to incorporate any or all of the above factors that affect surfactant adsorption. This barrier will decrease the adsorption rate, and hence the transfer of monomer from the subsurface to the interface is the rate-determining step.

1.2.1.4 Thermodynamics of adsorption

The Gibbs equation
Several theoretical models are available for describing the adsorption of materials at the gas-liquid interface. The most popular, developed by Lemlich is based on the Gibbs Adsorption Theorem.[75]
Assuming that activities may be given by concentrations (dilute solutions), the surface excess may be obtained from the Gibbs Equation:

$$\Gamma = -\frac{1}{nRT} \cdot \frac{d\gamma}{d\ln(c)} \qquad (1.2)$$

where:

n = l for non-ionic surfactants, neutral molecules or ionic surfactants in the presence of excess electrolyte,

n = 2 for ionic surfactants, assuming electrical neutrality of the interface

Γ - is the equilibrium surface excess,

R - the gas constant,

T - the Kelvin temperature, and

c - the bulk surfactant concentration.

The adsorption isotherm, Γ vs. *c*, can therefore be obtained by measuring the surface tension γ at various bulk surfactant concentrations.

Empirical and theoretical adsorption isotherms

The main problem for interpreting DST is the application of an appropriate isotherm. The purpose of an adsorption isotherm is to relate the surfactant concentration in the bulk and the adsorbed amount at the interface. It is assumed that adsorption is monomolecular.
Apart from the Gibbs isotherm a number of other equations are also used .

Henry isotherm
The simplest isotherm is

$$\Gamma = K_H c \qquad (1.3)$$

where K_H is *the equilibrium adsorption constant*, which is an empirical measure of the surface activity of the surfactant. This isotherm is only valid at low surface concentrations due to the assumption that there is no interaction between the adsorbed monomers and also there being no defined maximum value of Γ.

Langmuir isotherm
This is the most commonly used non-linear isotherm. It is based on the assumption of equivalent and independent adsorption sites on the surface.

The rate of change of surface coverage due to adsorption is proportional to both the concentration of surfactant in solution, and the number of vacant sites available. The maximum number of sites available is (or surface excess at saturation) Γ_{max} .

$$\frac{d\Gamma}{dt} = K_{max}\, c\, G_{abs}\left(1 - \frac{G}{G_{max}}\right) \qquad (1.4)$$

The rate of change of Γ due to desorption is proportional to the number of adsorbed species:

$$\frac{d\Gamma}{dt} = K_{des}\, G \qquad (1.5)$$

At equilibrium, these two rates are equal, and introducing the Langmuir equilibrium adsorption constant $K_L = k_{ads}/k_{des}$, results in the Langmuir isotherm:

$$\Gamma = \Gamma_{max}\left(\frac{K_L c}{1 + K_L c}\right) \qquad (1.6)$$

Deviations from the Langmuir isotherm may be attributed to the failure of the assumption of equivalent and independent sites. For example, intermolecular Deviations from the Langmuir isotherm may be attributed to the failure of the assumption of equivalent and independent sites. For example, intermolecular forces act between the molecules at the interface, and these can be relatively small van der Waals or London dispersion forces, or larger forces due to electrostatic effects or hydrogen bonding. The enthalpy of adsorption often becomes less negative as Γ increases, suggesting the most energetically favorable sites are occupied first.

Frumkin isotherm

This approach builds on the Langmuir equation and it also accounts for solute/solvent interactions at a non-ideal surface. It has been used in the study of many systems, and is most appropriate for non-ionic surfactants. Eq. 1.7 gives its usually quoted form:

$$c = \frac{1}{K_F} \cdot \frac{G}{G_{max} - G} \exp\left[-A\left(\frac{G}{G_{max}} \right) \right] \qquad (1.7)$$

The three variables are: the maximum adsorption Γ_{max} , the Frumkin adsorption constant K_F, the constant A which depends on the non-ideality of the layer. If *A*=0, then this equation reduces to the Langmuir isotherm (Eq. 1.7)

Freundlich isotherm

This originated as an empirical equation, but can be theoretically derived by a model which considers the enthalpy of adsorption varying exponentially with surface coverage. It can be thought of as a summation of a distribution of Langmuir equations and its usual form for surfactant adsorption is given in Eq. 1.8 below:

$$G = kc^{1/n} \qquad (1.8)$$

where *k* and *n* are both constants.

Volmer isotherm

This model accounts for non-ideal non-localised adsorption and also for the finite size of the molecules, with their interactions being calculated from statistical mechanics. Its usual form is quoted below:

$$c = K_v \left(\frac{G}{G_{max} - G} \right) \exp\left(\frac{G}{G_{max} - G} \right) \qquad (1.9)$$

where K_v (units of concentration) is a constant. This isotherm is not currently applied in the study of adsorption dynamics but is included in this discussion for completeness.

The above is a list of traditional isotherms used in solution chemistry, although there have been recent advancements to account for other physical properties of surfactants.

1.2.2 Foam phase

In foam fractionation, gas is sparged to produce bubbles which rise to the top of a liquid column, producing foam. As the bubbles travel through the continuous phase, surfactants adsorb at the gas-liquid interface. When the gas bubbles emerge from the liquid they form cells with a honeycomb structure (Figure 1.8). These cells accumulate above the bulk liquid surface to form a foam phase with a small amount of liquid entrained in the spaces between the bubbles.

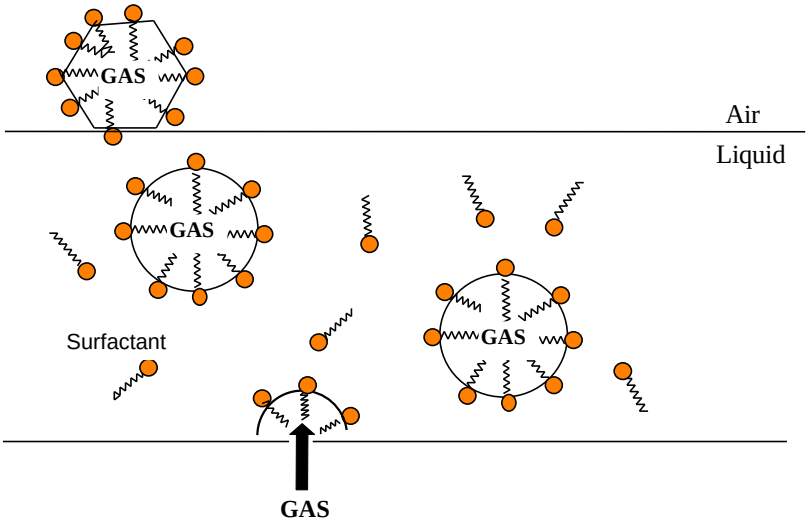


Figure 1.8. Schematic representation of foam formation

Foams are highly concentrated dispersions of gas (dispersed phase) in a liquid (continuous phase) containing surface-active macromolecules, such as surfactants. These preferentially adsorb at the gas-liquid interface and are responsible both for the tendency of the liquid to foam and the stability of the resulting foam. Foams can persist for a few minutes or several days depending on the conditions.[119]

Liquid-based foams exhibit striking mechanical properties. If pushed gently, they resist deformation elastically like solids. If pushed hard, they can flow and deform arbitrarily like liquids. Thus, they are neither solid, liquid nor vapour, yet they exhibit the feature of all three basic state of matter. Such behaviour characterizes *soft matter*.

1.2.2.1 Foam structure and geometry

The physical properties of bulk foam ultimately arise from the physical chemistry of bubble interface and the collective structure formed by the random packing of gas bubbles. Polyhedral bubbles filled with gas and separated by liquid films or lamellae compose foams. The bubbles differ markedly in their shape and size. Even among bubbles having the same volume, there is a variation in the number of faces and the number of edges per face. There is some order, however, amidst this chaos. Almost without exception, foams always obey a few simple rules, referred to as Plateau's laws in honor of the Belgian physicist Plateau who first formulated them.

Plateau's laws state:

1. Three and only three films (or lamellae) meet at an angle of 120 degrees to form a *Plateau border*.
2. Four and only four edges (Plateau border channels) meet at a point at an angle of 109.6 degrees to form a *vertex* (Figure 1.9).

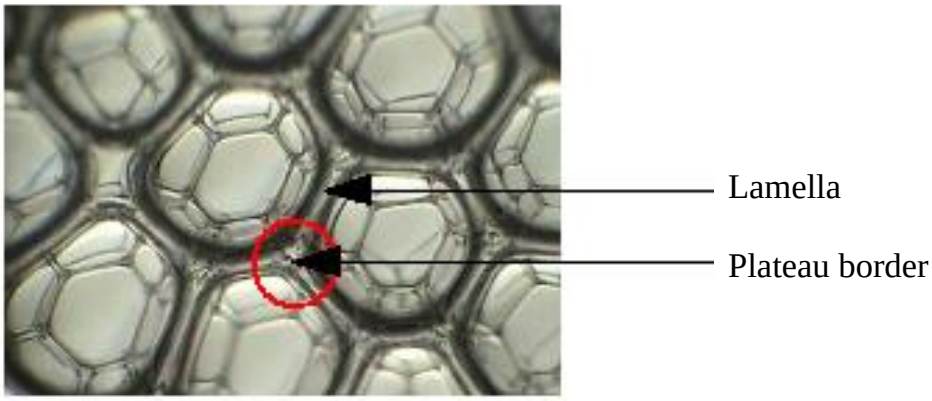


Figure 1.9 Typical picture of foam. Plateau borders and lamellae can be observed.

Depending on surface activity of surfactants, the resulting foams are unstable (wet, large bubbles) or meta-stable (dry, small bubbles) foams. In unstable foams, the bubbles are not drained and have a spherical form that is only slightly distorted by their neighbours whereas, meta-stable foams persist long enough for drainage to proceed extensively so that the bubbles press against each other and will distort in shape rather than coalesce. The region of contact will then flatten out into a film with a thickness determined by the combination of applied van der Waals force (attractive, originating from dielectric mismatch between gas and liquid) and electric double-layer force (repulsive, originating from the adsorbed surfactant).

1.2.2.2 Foam stability

The development and stability of the foam is governed by three main processes:

- *Drainage*
- *Coarsening*
- *Film rupture*

Drainage

Plateau border channels form a complex interconnected network through which liquid flows out of the foam under the action of gravity. At the same time, the liquid in the films is sucked into the plateau border channels due to the capillary pressure (Figure 1.10).

The fluid flow is complex because involves many parameters like:

i. bubble size and shape,
ii. type of surfactants,
iii. initial concentration of surfactants in the bulk solution,

iv. concentration of unabsorbed surfactants in the interstitial liquid (high surfactant concentration can lead to the formation of micelles),
v. packing of surfactants molecules along the gas-liquid interface of bubbles,
vi. fluid characteristics (viscosity, density, etc.),
vii. presence of additives, etc.

Drainage of interstitial liquid in foams with larger bubbles is more rapid than in foams with smaller bubbles. This occurs due to the larger cross-sectional area of the Plateau borders in larger bubble foams.

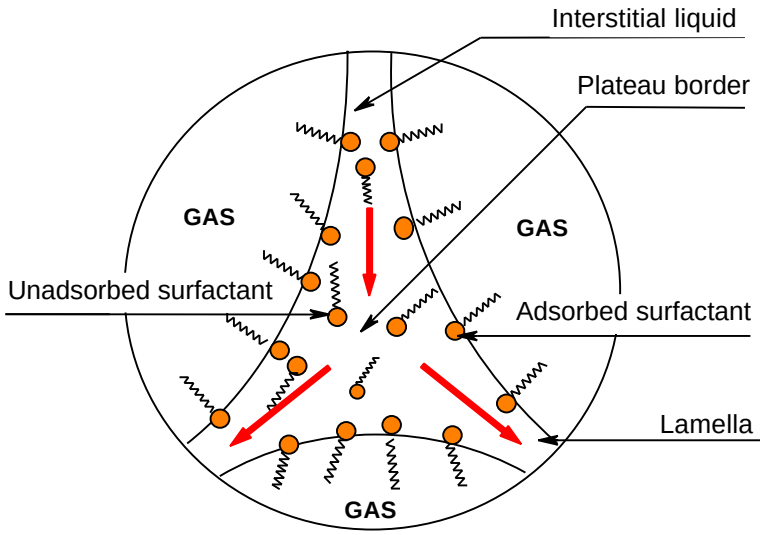


Figure 1.10. Drainage in Plateau borders and in lamellae

During the drainage, unabsorbed surfactant and/or less surface-active molecules will flow back to the bulk solution. A part of them will be further adsorbed onto the bubbles (if there are free attaching sites) or will reach the bulk solution modifying continuously its concentration.

Two mechanisms are responsible for the fluid flow in foam. Flow in the Plateau border channels occurs due to the gravity, while the flow in the films is driven by the capillary pressure.[119]

Figure 1.11 shows a cross-section of a draining film. The surface is curved at the edges where neighboring films come together to form a Plateau border channel. Because of this curvature, the pressure is smaller at the edges then at the center of the film and a radial flow is induced leading to a reduction of the film thickness in time.

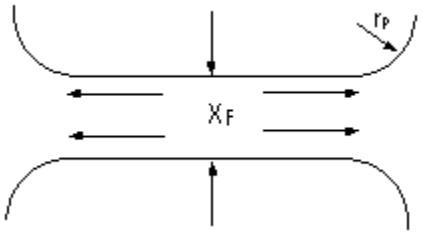


Figure 1.11 A draining film.

x_F = film thickness

r_p = curvature radius

The driving force (ΔP) causing the flow is a net result of the suction pressure in the adjacent Plateau border channels and the disjoining pressure (Π) in the films:

$$\Delta P = \frac{g}{r_p} - \Pi \qquad (1.10)$$

where γ is the surface tension and r_p is the radius of the curvature of the Plateau border channels.

The disjoining pressure (Π) refers to the repulsive force that arises when the film surfaces are close enough (x_F < 1000 Å) to interact with each other. When Π is positive (repulsive), it opposes film thinning, while when it is negative (attractive), it increase the driving force (ΔP) and accelerates film thinning. In most general case, Π is computed as a sum of an attractive force van der Waals force (Π_{WW}), a repulsive force due to the interaction of the electrical double layers (Π_{DL}) on the two surfaces and a short range repulsive force (Π_{SR}) which result from steric interactions when long chained molecules are adsorbed on the surfaces or due to the hydration forces that are set up due to the ordering of water molecules near charged surfaces:

$$\Pi = \Pi_{VW} + \Pi_{DL} + \Pi_{SR} \qquad (1.11)$$

The plot of Π versus x_F is referred to as the disjoining pressure isotherm and plays a crucial role in determining the rate of film thinning and its stability to rupture.

Film rupture

The drainage of interstitial liquid results in the thinning of the film by suction. When the films get too thin (x_F < 1000 Å) and week (which is characteristic for large bubbles), they will rupture leading to the direct coalescence of neighbouring bubbles, and, eventually, the foam will collapse and vanish. Coalescence results in a decrease in number of the bubbles and in an increase in the mean bubble volume.

Film rupture is promoted, among other parameters, by the presence of a weakly surface-active compound, which determines the prevalence of the attractive van der Waals forces over the repulsive electric double-layer forces. Also, a low viscosity solution will lead to a very fast drainage of interstitial liquid from the lamellae.

When two bubbles coalesce into a single larger bubble, the total gas-liquid interface is reduced. With a loss of surface area, greater amounts of surface-active molecule will be forced into the interstitial liquid, which ultimately will influence the drainage.

Foam Coarsening

As a result of gas diffusion from smaller bubbles to larger bubbles, as a result of surface tension that makes the pressure in smaller bubbles greater than the pressure in larger ones, some bubbles will grow while other shrink and disappear. The net result of this process is that the average bubble size grows in time, process known as foam coarsening.

Foam coalescence and coarsening lead to a bubble size distribution in the foam, with the bubbles being larger in the region where these two phenomena occur. Direct visual measurements of bubble diameters in a foam column indicates that average bubbles size grows as the foam is forced up the column.

1.2.3 Foam Fractionation experimental system

Devices used in foam fractionation consist essentially of a column, which contains the materials to be separated, a gas delivery system to introduce the gas in a dispersed form, and a foam collector. The foam fractionation column is the device were concentration and/or separation of the desired materials takes place. The column is usually made of glass and the height as well as the area of the cross section depends chiefly on the foam generating method and on the final desired purpose.[158]

There are two modes of foam fractionation: simple mode (batch or continuous) and higher mode (with enriching and/or stripping) (Figure 1.11).

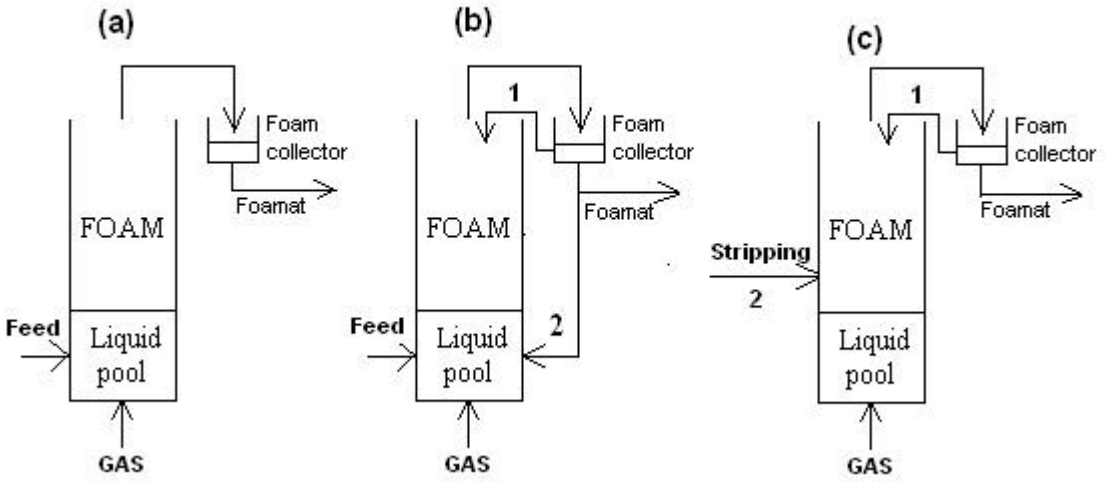


Figure 1.12 Foam Fractionation:
(A) Batch mode, (B) Continuous mode, (C) Higher mode

The main features of a foam fractionation column operating in batch mode (Fig.1.11 A) entail the continuous withdrawal of foam. This is accomplished by first, running the column for a while at gas rates sufficient to drive the foam up in the column and then, all the produced the foam (or part of it) is collected.
In the continuous mode part of the foamate is introduced back on the top of the column or in the liquid pool (Fig. 1.11 B, 1 or 2)

In the enriching mode, part of the foamate is fed back on the top of the top of the column and flows in counter-current to the rising foam. Since the reflux is richer than the interstitial liquid, the mass transfer resulting from this counter-current may considerably increase the enrichment (Fig. 1.11 C, 1).

In the stripping mode, the “stripper” feed enters the column at some distance above the liquid pool in counter-current to the rising foam (Fig. 1.11 C, 2) and tends to replace interstitial liquid. The overall result is an improvement in the degree of stripper. The stripping process can be successful in removing excess of surfactant acting as a collector.

The combined mode is a combination of the enriching and stripping modes.

The foam fractionation columns can be used as single-stage or multi-stage systems. In a multi-stage system, column cascades, in which the residual solution from one column is re-foamed in the second, third, and so on column, are used. In many cases, a high degree of foam concentration is achieved by re-foaming the foamat from the first column in secondary columns. (Figure 1.12)

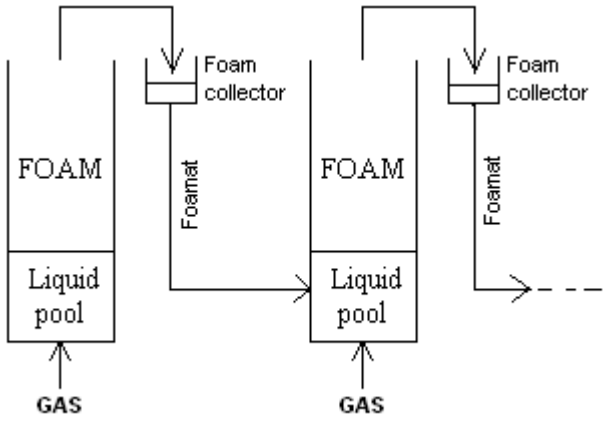


Figure 1.12 Multi-stage Foam Fractionation

1.2.4 Operating variables

Successful implementation of foam fractionation depends upon optimization of the complex interactions between solution condition and foam fractionation operating parameters.

The parameters that influence foam fractionation include basic variables such as concentration of solutes and surfactants, auxiliary materials, temperature, pH value, ionic strength and operating variables as gas flow rate, height of liquid pool, foam height and internal reflux ratio.

Concentration of Solute and Surfactant

The efficiency of foam fractionation is largely dependent on the initial concentration of materials to be concentrate and/or separated present in a diluted solution. There is an optimal concentration range, which is more suitable to achieve the more effective results.

At higher concentrations of surfactant, micelles are formed. This might has a negative or positive effect on the foam fractionation efficiency.

Micelles are non-covalently bonded macromolecular aggregates that continually associate and dissociate with the monomeric forms of the surfactant on a time scale of 10^{-6} to 10^{-3} seconds. Each micellar aggregate may fluctuate in size around the vicinity of its mean value by picking up or releasing some monomers at a time, reflected as a fast relaxation process. If the micelles are stable, above the critical micelle concentration, any increase in the surfactant concentration results in the incorporation of surfactant monomers in the micelles.

If the micelles, which are in the subsurface, are meta-stable, they can ‘leak’, thereby releasing one or two molecules, and then leave this region to be replaced by another micelle that can similarly supply more monomer. Since this characteristic monomer lifetime is typically less than 10^{-6} to 10^{-3} seconds, the micelles can be treated as a sink of monomers, which are readily available for adsorption over the timescale of the DST, and therefore the micelles will not limit adsorption. It can be concluded that the shorter relaxation time is important for supplying fresh monomer from the micelle to the interface, rather than assuming that the monomer is tightly bound and only released over the longer relaxation time.[75]

Addition of Organic Solvents

The solvent traditionally used in foam fractionation is water. Therefore, the foam fractionation of compounds that are not soluble in water is problematic. Addition of small quantities of organic solvent, in which these compounds are soluble, might enhance the foam fractionation efficiency. On the other hand, these can be detrimental on the foam stability.

Solution viscosity

An important parameter in foam separation is the viscosity. An increase in the solution viscosity will lead to a slow drainage between bubbles allowing thus, to the un-adsorbed molecules to re-adsorb onto the bubbles surface.

Solution pH

In general, the pH value of a solution will determine the charge sign and magnitude for a large variety of molecules. Therefore, adsorption of these molecules at the gas-liquid interface of dilute aqueous solutions and the extent of their removal by foam fractionation can be positively influenced by the solution's pH value.

It is well established that the surface activity of molecules possessing different functional groups is maximal at a pH equal to their isoelectric point (pI), where the net charge of the molecule is zero. This phenomenon is relating to the maximal packing of the molecule at the interface as a result of minimized electrostatic repulsion. Thus, a working solution at pH corresponding to their pI will enhance the performances of foam fractionation

Gas Flow Rate

The gas flow rate has a marked effect in foam fractionation process. It determines the speed of gas bubbles in liquid pool and, in consequence, the time available for surfactant adsorption onto the bubble surface. It determines also the speed of foam rising up in the column, as well as its height.

Flow rate should be correlated with the surface activity of surfactant, its concentration, and the solution viscosity. There must be sufficient gas flow to obtain foam and to maintain it at a certain height.

Temperature

Temperature is an important operating variable for the cases where the foam stability of surface-active components is different at different temperatures. The effect of temperature is complex because of its influence on various other factors such as adsorption, surface elasticity, and viscosity.

Height of Liquid Pool Height and of Foam column

In foam fractionation, the height of liquid pool above the sparger can affect the process efficiency. The variation of height of liquid pool implies a change of contact time between the solution and the rising bubbles before they reach the top of the liquid pool. To a certain extent, increase in pool height results in an increase of bubbles residence time in the pool and thus, more time for the bubbles to approach adsorption equilibrium.

Foam phase is important to obtain a separation during foam fractionation. An increase in foam height to a certain extent leads to significant changes in the mass transfer process due to the increase in the interfacial transfer area and the overall increase of drainage and internal reflux.

There are several attempts to mathematically model the foam and foam fractionation process [30,43,102,117,118,119,126] with the intended goal of formulating a generalized approach to the choice of optimal column operation parameters and the liquid pool conditions. Ideally, the choice of conditions would be based strictly upon the physical dimensions and characteristics of the foaming column and the physicochemical characteristics of the molecular components of the feed solution. In practice, models of foam fractionation have considerable empirical character.

Success in constructing truly predicative models of the foaming process devoid of a number of adjustable empirical adjustable parameters has been very limited. The number and complexity of the mass transport processes involved in foam fractionation makes rational model construction very difficult. Often, a great number of simplifying assumptions about the various phenomena occurring foaming are required, leading to questions as the physical relevancy of the resultant model.

2. CHIRALITY

2.1. Overview

The constituent atoms of stereoisomers are attached in the same order and differ only in the arrangement of their atoms in space.

There are two general categories of stereoisomers: enantiomers and diastereomers. Enantiomers are stereoisomers whose molecules *are* mirror reflections of each other, while diastereomers are stereoisomers whose molecules *are not* mirror reflections of each other. Compounds that exist as enantiomers are chiral. In extremely simple terms, chirality is “handedness”, that is the existence of left/right opposition. The word chiral comes from the Greek word *kheir*, meaning “hand” and it was stated by Lord Kelvin in 1904, in his *Baltimore Lectures on Molecular Dynamics and the Waves Theory of Light*: *…”I call any geometrical figure, or groups of points, chiral, and say it has chirality, if its image in a plane mirror, ideally realized, cannot be brought to coincide with itself”*. This statement is universally accepted as the definition of chirality.

The chirality of a molecule can be attributed to any of the following (Figure 2.1):[104]

- The carbon atom in the two molecules on either side of the mirror plane is surrounded in a tetrahedral spatial arrangement by four different substituents, which make it an asymmetrically substituted carbon atom. In such an arrangement, the carbon atom is called a stereogenic center or a *center of chirality* (A).
- *Axial chirality* can occur. Examples for such a stereochemistry are allenes and cumulenes. In the former class, the substituents have not necessarily to be different since the second double bond causes the loss of the C3 rotational symmetry element (B). In the latter class, only the members with an odd number of cumulated carbon atoms are potentially chiral, whereas an even number of carbon atoms results in E-/Z-isomerism (D) (geometric isomerism).

- Another type of axial chirality is represented by *atropisomers*, which possess conformational chirality (C): As long as the *ortho*-substituents in tetrasubstituted biaryls are large enough, the rotation around a C-C single bond will be hindered and prevent the two forms from interconverting.
- Finally, there exists *planar chirality,* which arises from the arrangement of atoms or groups of atoms relative to a stereogenic plane (not illustrated). However, this form of chirality is rather rare.
- *Helicity* is a special form of chirality and often occurs in macromolecules such as biopolymers, proteins and polysaccharides. A helix is always chiral due to its right-handed (clockwise) or left-handed (counter-clockwise) rearrangement.

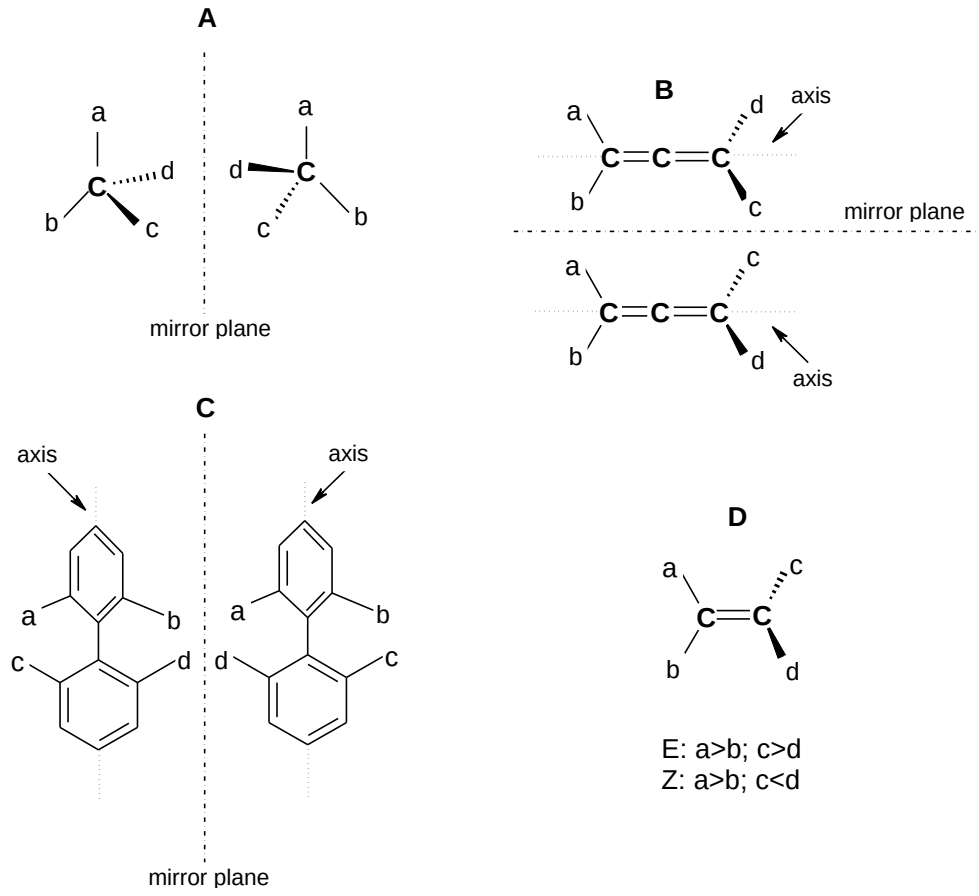


Figure 2.1. Mirror images of pair of enantiomers: (A) Central chirality, (B) Axial chirality, (C) Antropisomerism, (D) E-/Z-isomerism=geometric isomerism

In a non-chiral environment, the optical antipodes of a racemate possess the same physical and chemical properties. The name optical antipode was derived from their ability to rotate under equal (non-chiral) conditions the plane of plane-polarized light in opposite directions (optical activity). In a racemic mixture or racemate, the ratio of the two enantiomers is one (50:50 appearance of each optical isomer) and the sum of the optical rotations should be zero. Therefore, the net rotation may be used as an indication for the enantiomeric composition.

In the case when a stereoisomer has more than one stereogenic center, *e.g. n* chirality centers, the number of theoretically possible diastereo(iso)mers can be derived from 2^n. This phenomenon is also exploited when optical isomers are brought into a chiral environment. There, the enantiomers of a racemate behave differently due to the formation of diastereo(iso)mers. In the presence of two chiral compounds, in the simplest case, there are two stereogenic centers, one in each molecule, so that 2^2 diastereo(iso)mers will be formed:

$$(\pm)A + (\pm)B \longrightarrow (+)A(+)B + (+)A(-)B + (-)A(+)B + (-)A(-)B$$

of which (+)*A*(+)*B* and (-)*A*(-)*B* behave like optical antipodes (a pair of enantiomers), as do (+)*A*(-)*B* and (-)*A*(+)*B*. All other combinations possess different physical and chemical properties (diastereo(iso)mers) on the basis of which they can be discriminated in achiral media.

Another point connected to chirality is the nomenclature of enantiomers. In the beginning, the optical isomers were distinguished with (+)/(–) signs or d/l, indicating the direction in which the enantiomers rotate the plane of polarized light: (+) or d (*dextro*) stand for a rotation to the right (clockwise), whereas (–) or l (*levo*) indicate a rotation to the left (counter-clockwise). The main drawback of such an assignment is that one cannot derive the number of chirality centra from it. This is possible when applying the *R*/*S* notation, which describes the absolute configuration (the spatial arrangement of the substituents) around the asymmetric carbon atom.

This assignment is based on the Cahn-Ingold- Prelog (CIP) convention. It has mostly replaced the older D/L notation, which correlates the configuration of a molecule to the configuration of D/L-glyceraldehyde according to the Fischer convention. Today, the latter nomination is predominantly restricted to amino acids and carbohydrates.

The assignment of *R* or *S* according to CIP follows the sequence rule, *i.e*. the order of priority of the substituents on the center of chirality. It can be determined on the basis of the decline in the atomic number of the atoms directly bonded to the center of chirality. In the case that two or more of these atoms are identical, the next bonded atoms have to be considered, eventually the third bonded atoms etc. In doing so, the branch containing the atoms with the highest atomic numbers has the highest priority. If the atoms are connected by double or triple bonds, their weight is higher than that of two or three singly bonded atoms. When isotopes of atoms are involved, the order of priority can be determined by putting in order the decline in their mass number. Note, that in closely related structures the nomination of the absolute configuration may change, whereas the spatial arrangement of the substituents is maintained.

2.2 Chirality and its consequences

Although pure enantiomers of chiral compounds have identical physico-chemical properties, their behavior in biochemical processes might be strikingly different.[1]

The four valences of carbon imply that, if four different groups are attached to one carbon, the spatial arrangement results in a center of asymmetry and occurrence of stereoisomers, enantiomers by their mirror image relationship. In the early 1930’s, Easson and Stedman introduced a model which laid the basis for the initial

understanding of stereochemical differences in pharmacological activity, the so-called “three-point attachment model” (Figure 2.2).

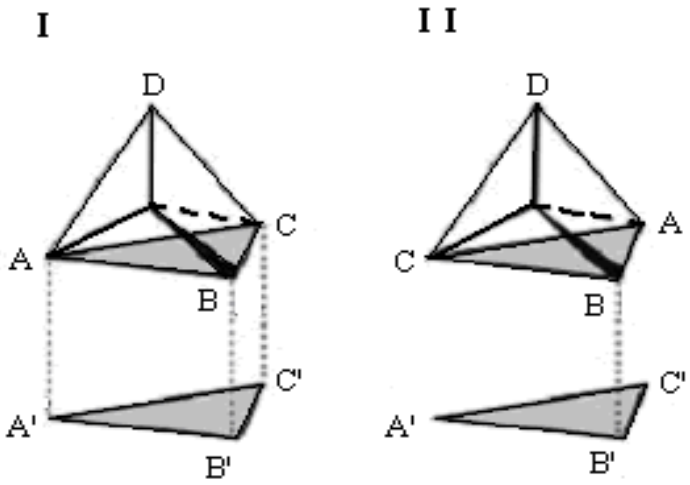


Figure2.2. Three-point attachment model modified from Easson and Stedman (1933); Ogston (1948). A’, B’ and C’ are the binding sites in the active site of the enzyme. For one enantiomer of the chiral substrate (*diagram I*), the three ligands (A, B, C) are oriented counterclockwise and coincide with the binding sites of the enzyme. It can be seen than the ligands of the other enantiomer (*diagram II*) bind ineffectively to the enzyme.[1]

They described the differences in bioaffininity to the differential binding of enantiomers to a common site on an enzyme or receptor surface, with the receptor or enzyme needing to possess three non-equivalent binding sites to discriminate between the enantiomers. The one enantiomer that simultaneously interacts with all of the sites is called eutomer (“active” enantiomer), whereas the other, which binds to less than three sites at the same time, is called distomer (“inactive” enantiomer, enantiomorph).

Since amino acids and proteins, the main building blocks of living systems, are chiral, the action of enantiomers in such an environment is expected to be different.

Some fifty years after Pasteurs remarkable discovery, the first pharmacological observations on the differential pressor effects of the (-)- and (+)-isomers of adrenaline were made.

Hence, it is obvious that today the main interest in the development of new drugs is directed towards finding the eutomer. This is because the inactive enantiomer may not only be ‘ballast’, but also antagonistic to the action of the eutomer, or even toxic. The potency ratio between the active enantiomer and the less active one is called eudismic ratio.

Among the bioactive synthetic compounds, most of the chiral drugs are administered as racemates, despite the fact that the optical isomers of a racemic drug can exhibit different pharmacological profiles in living systems. These differences can be expressed in *e.g.* the affinity of the enantiomers for certain receptor subtypes or enzymes, distribution rates, their metabolism and excretion, in antagonistic actions relative to each other, or their toxicological properties. Obviously, the more chiral centres present in a (drug) molecule, the more complex the situation becomes.[104]

The effects of differently acting stereoisomeric drugs can be categorized basically as follows:[1]

A. The stereoisomers of a chiral drug may have similar modes of action, but may differ in their affinity to a receptor or an enzyme, resulting in different reaction rates.
B. The inactive stereoisomer may act as a competitive antagonist.
C. Enantiomers may have opposite or different effects, as in the case for barbiturates, where the (-)-enantiomer is a sedative and the (+)-enantiomer has convulsive effects.

D. Even applied as a single enantiomer, the formation of harmful metabolites, as well as chiral inversion or racemisation, can occur *in vivo*. (A negative example is thalidomide, which was introduced to the market in the late 1960's as a sedative, in the racemic form. Even when applied in the therapeutic and harmless (+)-form, the, *in vivo*, interconvertion into the harmful (–)-isomer was shown to be responsible for the disastrous malformations of embryos when thalidomide was applied to women during pregnancy.)
E. In contrast to this, the inactive enantiomer may antagonize the side effects of the active isomer. (In the case of the diuretic idacrinon the presence of distomer is useful promoting the efficacy of the therapeutic eutomer by antagonizing one of its side effects.)

As this list shows, the relationship between the effects of active and inactive stereoisomers in pharmacological context is quite complicate and cannot be easily predicted.

The U.S. Food and Drug Administration, in 1992, issued a guideline that for chiral drugs only its therapeutically active isomer will be brought to market, and that each enantiomer of the drug should be studied separately for its pharmacological and metabolic pathways. In addition, a rigorous justification is required for market approval of a racemate of chiral drugs. Presently, a majority of commercially available drugs are both synthetic and chiral. However, a large number of chiral drugs are still marketed as racemic mixtures. Nevertheless, to avoid the possible undesirable effects of a chiral drug, it is imperative that only the pure, therapeutically active form be prepared and marketed. Hence there is a great need to develop the technology for analysis and separation of racemic drugs.[103]

Chiral compounds are also important in the agrochemical industries. Due the different behavior of enantiomers in a chiral environment, the relationship between

chirality and biological properties plays an important role in pesticide chemistry.[115] In 1995, chiral compounds accounted for 25% of all agrochemicals as compared to 19% in 1980; the compounds sold as single isomers accounted for only 7% of the total market value.[114] After their field application, pesticides generally undergo a series of biological mediated reaction in which the differences in activity of the isomers may have important consequences: in some cases only one of the isomers has a pesticidal activity while the other may have toxic effects against non-target organisms. The use of racemats contributes to useless environmental loading; furthermore, additional costs are involved in both producing and removal processes of the non –active isomers.

The agrochemical industry and government regulators are beginning to take enantioselectivity into account. For example, the (R)-(+)- enantiomer of the herbicide dichlorprop (as well as the (R)-(+)- enantiomer of all the phenoxypropionic acid herbicides) is the active enantiomer, killing the weeds, while the (S)-(-)-enantiomer is inactive. In order to reduce the amount of herbicide used and to avoid the possibility of the unnecessary enantiomer causing some adverse impact, several European countries have decreed that only the (R)-enantiomers will be used.

2.3 Mechanism for Chiral Recognition

Separation of enantiomers from a racemic mixture is an important and challenging field of molecular recognition. The problem with enantiomeric separation is that in an achiral environment, enantiomers display identical physical and chemical properties. In order to distinguish between two enantiomers, a chiral selector must be introduced into the separation media. In addition, the chiral selector must be compatible in size and structure to the racemate for the separation to occur. The interaction of the chiral selector with the enantiomers of the solute results in the

formation of two transient or long-lived diastereomers, which differ in their thermodynamic stability, solvation in the mobile phase, or binding of the complex to the solid support. These differences in thermodynamic stability, solvation, and binding will occur provided that at least three active points of the chiral selector participate in the interaction with the corresponding sites of the solute molecule.

The mechanism of chiral recognition is inadequately understood because of the multiplicity and complexity of the interactions of the enantiomers to be separated with the chiral selector. However, the idea of three-point interaction described by Easson and Stedman[16] is compulsory for chiral recognition to occur. The rule proposes that chiral recognition depends on the degree of the interaction exhibited between each enantiomer and the chiral selector. One enantiomer (eutomer) should interact simultaneously with all three sites and at least one of the three interactions should be stereochemically dependent. Conversely, the other enantiomer (distomer) should only achieve two of these interactions due to spatial restrictions.

In Figure 2.2, chiral recognition depends on the absence of C-C’ and A-A’ fit conjunction interactions. The interactions at the three points can be both attractive and repulsive. Multiple modes of interactions that could be possible include dipole-dipole bonds e.g. hydrogen bonding, which involves secondary amine and carbonyl groups of the chiral selector with the hydroxyl, acidic, and amino groups of the analytes. Steric interactions arising from the bulky non-polar groups attached near the chiral center of the chiral selector provide conformational control, which is necessary for chiral separation. Interactions between electron pair p-donor and p-acceptor of aromatic rings of racemic analytes and the chiral selectors as well as ion-dipole bonds, and Van der Waals forces play a remarkable role in chiral recognition. It should be noted that not all interactions between the enantiomer and the chiral selector would meet the three-point criterion. The total enantioselectivity can depend strongly on composition, temperature, and pH of the mobile phase.

Therefore, optimization of the separation media should be accomplished in order to maximize the three-point interactions for chiral separations.

In some cases, the three-point attachment model needs to be expanded to so called four-location model Mesecar and Koshland Jr., 2000) (Figure 2.3).[1] When isocitrate dehydrogenase is provided with the substrate racemate, L- isocitrate is exclusively bound to the protein crystals in the absence of Mg^+, but in the presence of Mg^+ the D-isomer binds. The crystal structure revealed that the three of four groups od C2-atom of isocitrate bind in to the same three residues, but not the fourth group. In other words, the protein needs not three but four locations in the active side to differentiate between the two enantiomers. In general, the three-point model works as long as it is assumed that the binding site can be approached only from one direction. But if the active site is in a cleft or on protruding residue, only binding of the fourth group enable the protein to distinguish between the enantiomers.

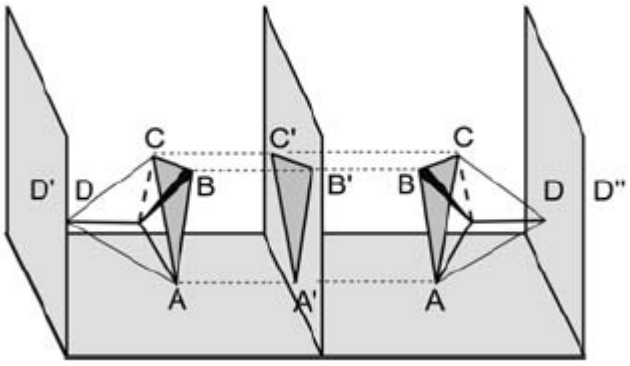


Figure 2.3. The four-location model. A', B', C' are the binding sites in the active site of the enzyme. If the active site can be approached from both sides, both enantiomers can bind. In such a case, an additional binding site - a fourth location (D', respectively) –is necessary for the selective recognition of an enantiomer.[1]

2.4 Resolution of Optical Isomers

The field of chiral recognition and separation of chiral compounds has received considerable attention in the past few decades. This is due to its pharmaceutical importance and to its relevance to the more general aspects of molecular recognition.

There are various methods for enantiomeric analysis, which do not require the separation of enantiomers. These include polarimetry, nuclear magnetic resonance, isotopic dilution, calorimetry, and enzyme techniques. The disadvantages in all these techniques, however, are the need for pure chiral samples and their relative slowness. A typical analytical problem requires separation and quantitation of enantiomers and sometimes identification of the levorotatory or dextrorotatory enantiomer.

To date, the most applicable tool for enantioseparation remains column chromatography on chiral stationary phases (CSPs). Here, enantiomers (selectands) are separated based on their differential recognition by an immobilized asymmetric molecule (selector), in either the gas or liquid phase. The detailed mechanism by which such recognition takes place has been characterized for a number of model systems.[86, 99,122]

In the following, the chromatographic resolution methods are described.

2.4.1 Chromatographic Methods

Both gas chromatography (GC) and high performance liquid chromatography (HPLC) provide fast and accurate methods for enantiomeric separation and allow quantitation of both mass and optical rotation (for HPLC) if appropriate detection devices are used.
Chromatographic chiral separation dates back in 1939 when Henderson and Rule demonstrated the separation of *d*,*l*-*p*-phenylenediiminocamphor on *d*-lactose. Both Kotake et al. in 1951, and Dalgliesh in 1952 utilized paper chromatography, a cellulose support, to separate chiral amino acids. Although Dalgliesh was not the first to observe such separations, he correctly attributed the enantioselectivity to adsorption by cellulose and proposed the Three Point Rule for asymmetric recognition. This rule was later restated by Pirkle as “Chiral recognition requires a minimum of three simultaneous interactions, with at least one of these interactions to be stereochemically dependent.” In 1960, Klem and Reed first reported the use of a silica gel support for chiral HPLC chromatographic separation. In 1966, Gil-Av and Feibush reported the first successful GC direct enantiomeric separation.[103]

Chromatographic methods are considered the most useful for chiral separation. There are two approaches: *indirect*, which utilizes derivatizing agents, and *direct*, which uses chiral stationary phases or chiral mobile phase additives.

The *indirect* chromatographic separation of racemic mixtures into their enantiomers can be achieved by derivatization with a chiral derivatizing agent (CDA) prior to analysis. The introduction of a second chiral center leads to the formation of a pair of diastereomers. These differ in their physical and chemical properties and thus can be separated from each other in an achiral environment. A condition for a succesful derivatization is the presence of suitable functional groups in the analyte. Also, to increase the ‘physicochemical differentiation’, the derivatization should occur close

to the chiral atom. Although the indirect chromatographic approach has the advantage to predetermine the elution order, which can be important for the determination of optical purities, there are some limitations to this technique. First of all, the derivatization procedure can be time-consuming. This can be due to different reaction rates of the individual enantiomers, and for a proper interpretation of the analytical result it is necessary to have the reaction completed before analysis. Also, the formation of unwanted products should be avoided and the diastereomeric mixture must be chemically and stereochemically stable. This also counts for the CDA, which must be of high optical purity and stable in solution (reaction conditions) or solid state (long-term storage). For preparative purposes, the indirect chromatographic approach includes an additional synthesis step because the derivatizing agent has to be cleaved off the separated diastereomers after their resolution in a non-chiral environment. Hereby, impurities can be introduced or even the racemization of the just resolved enantiomers could be caused. Still, once a suitable CDA and derivatizing procedure have been found, the indirect approach offers some advantages for preparative separations over direct preparative separations, mainly due to the fact that in large scale resolutions non-chiral media are better handled than, *e.g.*, chiral stationary phases. The conditions can be adjusted more easily to obtain the desired resolution.

To avoid the extended sample preparation for indirect (analytical) separations, one can chose the *direct* chromatographic approach, where the phenomenon that enantiomers behave like different compounds in a chiral environment is exploited. There are two ways of achieving direct chromatographic enantioseparations: either by chiral mobile phase additives (CMPAs) in normal- or reversed- phase chromatography, or by the utilization of chiral stationary phases (CSPs). In the latter, the chiral selector is chemically bound, coated or otherwise attached to the surface of the support material. In either case, the formation of transient

diastereomeric complexes between the analyte and the chiral discriminating agent is being pursued.

Various interactions, such as charge transfer (π-π) complexes, hydrogen bonding interactions, dipole stacking, as well as differences in the stability of these transient complexes may contribute to the enantiodiscrimination.
In the following, the main chromatographic techniques and their use in chiral separations are discussed in more detail, without attempting to be exhaustive.

2.4.1.1 Direct Chromatographic Methods (Chiral Mobile Phase Additives)

Direct separation of enantiomers on an achiral column using a chiral mobile phase additive (CMPA) is applied only in HPLC and CE. In GC the mobile phase is an inert carrier gas, where the possibility of selective interactions with the analyte or the stationary phase is minimal. However, in HPLC, the mobile phase is a dynamic part of the system that influences both analyte and stationary phase interactions. In this method, enantiomeric separation is accomplished by the formation of a pair of transient diastereomeric complexes between racemic analyte and the chiral mobile phase additive. Chiral discrimination is due to differences in the stabilities of the diastereomeric complexes, solvation in the mobile phase, and/or binding of the complexes to the solid support. The three major approaches in the formation of diastereomeric complexes are:

(1) transition metal ion complexes (ligand exchange),
(2) ion pairs, and
(3) inclusion complexes.

Many racemic mixtures can be separated on conventional achiral LC columns by using an appropriate chiral mobile phase additive (CMPA). Additives such as α-, β-, γ- Cds have been successful used. Advantages of this technique are as follows:

(1) less expensive conventional LC columns can be used; (2) a wide variety of possible additives are available; and (3) different selectivity's from the chiral phases can be obtained. However, the problems with this technique include: many chiral additives are costly, and, sometimes, have to be synthesized, the mode of operation is complex and inconvenient for preparative applications because the chiral additive must be removed from the enantiomeric solutes.

2.4.1.2 Direct Chromatographic methods (Chiral Stationary Phases)

Enantiomeric separation by using chiral stationary phases (CSPs) is based on the formation of transient diastereomeric analyte-CSP complexes between the enantiomers and the chiral molecule that is an integral part of the stationary phase.
At present, there are over a hundred CSPs that are commercially available. There are five major classes of CSPs based on the type of analyte-CSP complexes formed:
Type 1 or "Pirkle" phase forms analyte-CSP complexes by attractive-repulsive interactions, mainly by π electron donor-acceptor mechanisms.
Type 2, exemplified by derivatized cellulose, involves attractive interactions followed by inclusion into chiral cavities.
Type 3 CSPs, such as cyclodextrins (CDs) and crown ethers, form inclusion complexes. In the Type 4 CSP, the analyte is a part of a diastereomeric metal complex (chiral ligand-exchange chromatography).
Type 5 CSP is a protein, e.g., bovine serum albumin, and the analyte-CSP complexes are based on the combination of hydrophobic and polar interactions.

2.5 Thermodynamics of enantioseparation

2.5.1 Enatioseparations in Chromatographic Techniques

The separation of the R and S enantiomers involves the reversible formation of a pair of reversible diastereomeric analyte-chiral selector C complexes, [R·C] and [S·C]:[103]

$$R_M + CSP = [R \cdot C] \qquad (2.1)$$

$$S_M + CSP = [S \cdot C] \qquad (2.2)$$

where R_M and S_M represents the R and S analytes in the mobile phase, before interacting with the CSP. The differences in the stability between these diastereomeric complexes lead to a difference of retention time. The enantiomer that forms the less stable complex will be eluted first. These diastereomeric complexes must therefore differ adequately in free energy for an enantiomer separation to be observed.

In chromatography, the magnitude of solute retention is expressed by the *retention factor*, *k*, which is a measure of the stoichiometric mass distribution of analyte between the stationary and mobile phases.

$$k = \frac{\text{analyte mass in stationary phase}}{\text{analyte mass in mobile phase}} \qquad (2.3)$$

For the enantiomers R and S, their respective retention factors k_R and k_S expressions are:

$$k_R = (R{\cdot}C)_S / R_M \quad (2.4)$$

$$k_S = (S{\cdot}C)_S / S_M \quad (2.5)$$

where $(R{\cdot}C)_S$, $(S{\cdot}C)_S$, R_M, and S_M are the masses of R and S in the stationary phase and mobile phase, respectively.
Expressing k_R and k_S in terms of molar concentrations

$$k_R = [R\text{-}C]_S \cdot V_S / [R]_M \cdot V_M \quad (2.6)$$

$$k_S = [S\text{-}C]_S \cdot V_S / [S]_M \cdot V_M \quad (2.7)$$

where $[R\text{-}C]_S$, $[S\text{-}C]_S$, $[R]_M$, and $[S]_M$ are the molar concentrations of R and S in the stationary phase and mobile phase, respectively, and V_S and V_M are the volumes of the stationary phase and mobile phase. In a given column the volume ratio of the stationary and mobile phases, φ, is fixed so that the mass distribution ratio is simply given by

$$k_R = \varphi([R\text{-}C]_S / [R]_M) \quad (2.8)$$

$$k_S = \varphi([S\text{-}C]_S / [S]_M) \quad (2.9)$$

Considering the equilibrium distribution of the R and S enantiomers between the stationary and mobile phases, the respective equilibrium constants denoted by K_R and K_S are:

$$K_R = [R\text{-}C]_S / [R]_M \quad (2.10)$$

$$K_S = [S\text{-}C]_S / [S]_M \quad (2.11)$$

Eqn. 2.10 and 2.11 can be rewritten as

$$k_R = \varphi K_R \qquad (2.12)$$

$$k_S = \varphi K_S \qquad (2.13)$$

The enantioselectivity (or chiral separation factor), α_{RS}, which describes the selective interaction of the enantiomers with the chiral selector is

$$\alpha_{RS} = k_R / k_S \qquad (2.14)$$

when $k_R > k_S$.

Because retention factor, k, is directly related to its equilibrium distribution constant, and from the relationship of the Gibbs free energy change and the distribution equilibrium constant, K,

$$\Delta G = - RT \ln K = - RT \ln (k / j) \qquad (2.15)$$

Eqn. 2.14 becomes

$$\alpha_{RS} = \exp[\Sigma (-\Delta G_R)/ RT] / \exp[\Sigma (-\Delta G_S)/ RT] \qquad (2.16)$$

where the $\Sigma (-\Delta G_R)$ and $\Sigma (-\Delta G_S)$ are the total molecular free energies of adsorption of the R and S enantiomers, respectively.
In the case of enantiomers, all analyte-stationary phase interactions except chiral interactions, $\Sigma (\Delta G)$, are identical and cancel.

$$\alpha_{RS} = \exp[\Sigma (-\Delta G_R - \Delta G_S)/ RT] \qquad (2.17)$$

Rearrangement of Eqn. 2.17 gives the relationship between α_{RS} and $\Delta(\Delta G)$, the difference in the molar free energy of the interaction for the two enantiomers with a chiral stationary phase.

$$\alpha_{RS} = \exp\Sigma [(-\Delta(\Delta G)/ RT] \qquad (2.18)$$

The difference $\Delta(\Delta G)$ is solely responsible for enantioselectivity. It must be remembered that the enantiomers have equal internal energies, their solvation enthalpies are equal in a given mobile phase. The larger the value of $\Delta(\Delta G)$ the better is the enantiomeric separation. Since $\Delta(\Delta G)$ determines enantioselectivity, the designer of a CSP should maximize $\Delta(\Delta G)$ while minimizing the adsorption energies, ΔG_R and ΔG_S. Large ΔG_R and ΔG_S lead to long retention times and broad chromatographic peaks.
Applying the Gibbs-Helmholtz , G = H - TS, Eqn. 2.18 becomes

$$R \ln \alpha_{RS} = - \Delta(\Delta H) / T + \Delta(\Delta S) \qquad (2.19)$$

where *R* is the gas constant, $\Delta(\Delta H)$ and $\Delta(\Delta S)$ are the differences in the enthalpy and entropy of the interactions of R and S enantiomers with the stationary phase, respectively.

The $\Delta(\Delta H)$ and $\Delta(\Delta S)$ values can be obtained by measuring α_{RS} of an enantiomeric pair at different temperatures and plotting $R\ln\alpha_{RS}$ versus $1/T$ (Figure 2.4). If $\Delta(\Delta H)$ is a constant within the temperature range, a straight line should be obtained. In chromatography, this means that the retention mechanism is invariant under the temperature range. The slope is $\Delta(\Delta H)$ and the intercept is $\Delta(\Delta S)$.

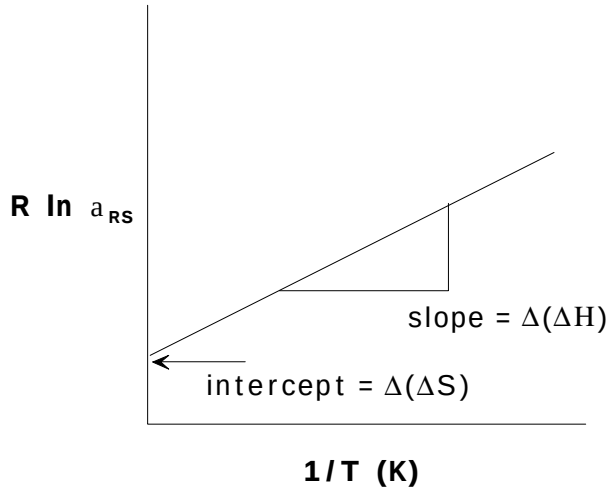


Figure 2.4. Van't Hoff type plot. $R \ln\alpha_{RS}$ versus 1/T

In addition, as follows from the Gibbs-Helmholtz relationship, there exists a certain temperature, called isoenantioselective temperature, T_{iso}, at which Δ_{RS} (ΔG) is zero with no enantiomer resolution.

$$T_{iso} = \Delta_{RS} (\Delta H) / \Delta_{RS} (\Delta S) \qquad (2.20)$$

At temperatures higher than T_{iso}, the enantiomer elution order should be reversed. Evidence for the reversal of enantioselectivity has been found in gas chromatographic enantiomer separations. Below T_{iso}, enantiomer separation is enthalpy (Δ_{RS} (ΔH)) controlled and the R enantiomer is eluted after the S enatiomer while above T_{iso} enantiomer separation is entropy (Δ_{RS} (ΔS)) controlled and S enantiomer is eluted after the R enatiomer (reversal of the elution order, peak inversion).

2.5.2 Enatioseparations in Capillary Electrophoresis Techniques

Electrophoretic techniques (CE) are capable of separating analytes due to the different mobilities of charged analytes in an electric field. On the one hand, the mobility is determined by the size and charge of the analyte, and on the other hand by the strength of the electric field and the mobile phase.[134]

Separation of enantiomers in CE is based on the difference in effective mobilities of enantiomers in a chiral background electrolyte (BGE), resulting from different stabilities of transient complex of enantiomers with the chiral selector (C). When a charged solute form a complex with a chiral selector, its charge/mass ratio, and thus its mobility decreases. The free or uncomplexed enantiomer migrates as it would in absence of chiral selector. The mobility's of each enantiomer, free or complexed, are identical to one another. Differences in the equilibrium constants determine the ratio of free/complexed material. If the equilibrium constants are sufficient different between enantiomers, separation will occur. The electrophorectic mobility μ of free enantiomers and of the complexes enantiomer-chiral selector and the concentration of chiral selector [C] are correlated by the following equations:

$$\mu_{R\,app} = \mu_{eo} + \frac{\mu_R + \mu_{Rc} K_R [C]}{1 + K_R [C]} \qquad (2.21)$$

$$\mu_{S\,app} = \mu_{eo} + \frac{\mu_S + \mu_{Sc} K_S [C]}{1 + K_S [C]} \qquad (2.22)$$

where: μ_{Rapp} and μ_{Sapp} are the apparent mobilities of R, S enatiomers,

μ_R and μ_S are the mobility of free enantiomers,

μ_{Rc} and μ_{Sc} are the apparent mobility's of complexed R, S enatiomers,

μ_{eo} is the electro-osmotic flow,

K_R and K_S are the equilibrium constants.

Since the mobility of free and complexed enantiomers are the same for R and S enantiomers, their apparent mobility is influenced by the proportion of time spent as complexed material, $m_{Sc}K_S[C]$.

The difference between the electrophoretic mobility's of the two enantiomers, taking into account that $\mu_R = \mu_S = \mu$ and $\mu_{Rc} = \mu_{Sc} = \mu_c$, can be calculated as:

$$\Delta m = \frac{(m - m_c) \cdot (K_S - K_R) \cdot [C]}{1 + (K_S + K_R) + K_R K_S [C]^2} \qquad (2.23)$$

From eqn. 2.23 it can be seen that if $K_S = K_R$ (both enantiomers have the same equilibrium constant) then $\Delta\mu = 0$ (no separation).

It was found that enantioselectivity in CE is also temperature depended.[141,151] Besides a decrease of buffer viscosity, and thus a decrease in migration time, an increase in temperature can strongly influence the complex stability between the enantiomers and the chiral selector. Therefore, enantioselctivity in CE is also thermodynamic governed and by enthalpy/entropy controlled. In consequences, all the thermodynamic relationships previously described for classical chromatographic methods can be applied also in capillary electrophoresis techniques

2.6 Retention factor, Separation factor, and Resolution

The *retention factor, k,* can be defined, from a practical approach, as the ratio of the amount of time a solute spends in the stationary and in the mobile phase. Since all solutes spend the same amount of time in the mobile phase, the retention factor is a measure of retention by the stationary phase. The retention factor do not provide absolute retention information but relative retention information:

$$k = \frac{t - t_M}{t_M} = \frac{t'}{t_M} \qquad (2.24)$$

where: t = retention time,

t'=adjusted retention time,

t_M= retention time of unretained compound.

t and t_M are easily obtain from a chromatogram.

From Eqn. 2.21, Eqn. 2.14 the *separation factor* α_{RS} of the enantiomers becomes:

$$a_{RS} = \frac{k_R}{k_S} = \frac{t'_R}{t'_S} \qquad (2.25)$$

α_{RS} is related to enantioselectivity and is thus a temperature dependent quantity. α_{RS} can, therefore, not be defined in temperature – programmed runs.[115]

The ultimate goal of successful enantiomer separation is resolution in a short time. Unfortunately, the *peak resolution* R_S, is not always taken into account. R_S is another measure of how well the enantiomers have been separated and it is important for assessing the efficiency and the selectivity in the same time:

$$R_S = 2\frac{t_R - t_S}{W_R + W_S} \qquad (2.26)$$

where: t_R, t_S = retention times of the R, S enantiomes,

W_R, W_S = peak widths at base of the two enantiomers,

when R enantiomer is eluted after the S enatiomer.

3. CYCLODEXTRINS

One widely used group of chiral selectors is represented by cyclodextrins (CDs). Cds are extensively used as stationary bonded phase (CPS) in high-performance liquid chromatography (HPLC) and gas chromatography (GC), or as mobile additives in HPLC and capillary electrophoresis (CE). CDs impart enantioselectivity to the chromatographic system by formation of transient diastomeric complexes with the analyte enantiomers.[134]

In 1891, Villiers described for the first time the cyclodextrins. He was able to isolate a small amount of crystalline substance by its physical proprieties (solubility and others) from a medium of *Bacillus amylobacter* grown on starch. Villiers called his crystalline product cellulosine because of its apparent similarity to cellulose.

In 1904 Schardinger was the first to characterize CDs as *cyclic oligosaccharides*. This is why CDs, especially in the older literature, are sometimes called “Schardinger dextrins”. He succeeded in isolating a bacillus, which he named *B. macerans*, as the microorganism responsible for the formations of CDs from starch. *B. macerans* is a common source for the enzyme which is used in the production of CDs. Other bacteria that have this enzyme have been identified as well.

For 25 years between 1911 and 1935, Pringsheim in Germany was the leading researcher in this area, demonstrating that these oligosaccharides forme stable aqueous complexes with many other chemicals. By the mid 1970's, each of the natural cyclodextrins had been structurally and chemically characterized and many more complexes had been studied.[154, 157]

3.1 Native cyclodextrins

CDs (also known as cycloamylases, cycloglucans, or cycloglucopyranoses) are natural macrocyclic polymers of glucose that contain from six to twelve D-(+)-glucopyranose units which are bonded through a-(1,4)-linkages. The smallest is the a-CD (six glucose units), followed by ß-CD (seven units), and ?-CD (eight units). They are chiral, toroidal shaped molecules with all the glucose units in a $^4C_1$ chair conformation (Figure 3.1).[111]

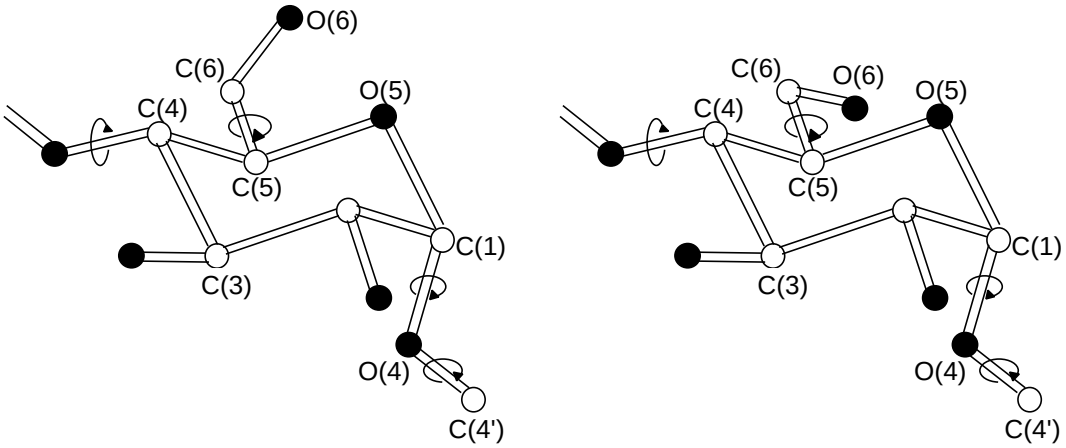


Figure 3.1. Glucose $^4C_1$ chair conformation with O6-H in (-) (left) and (+)(right) orientations. Bonds about which rotations are likely to occur are indicated by circles.[111]

For different reasons (prices, availability, cavity dimensions in which most of common solute can fit closely, etc.) ß-CD represents 95% of all produced and consumed CDs.[134] The structure of ß-CD is shown in Figure 3.2.

The mouth of the molecule has the larger circumference of the openings. It contains the secondary hydroxyl (OH) groups attached on C-2 and C-3 of the glucose unit. The primary OH groups attached to C-6 of the glucose unit are on the opposite end of the CD, forming a smaller opening. Thus the CD molecule is shaped like a truncate cone with the secondary hydroxyl side more open than the primary hydroxyl

side. While the primary hydroxyl groups (C-6) on the truncate end can rotate, partially blocking the opening of the cavity, the secondary hydroxyl groups (C-2 and C-3) are fixed in space with all of the C-2 hydroxyl groups pointed in a clockwise direction and all of the C-3 hydroxyl groups pointed in a counter-clockwise direction (Figure 3.2 and 3.3).

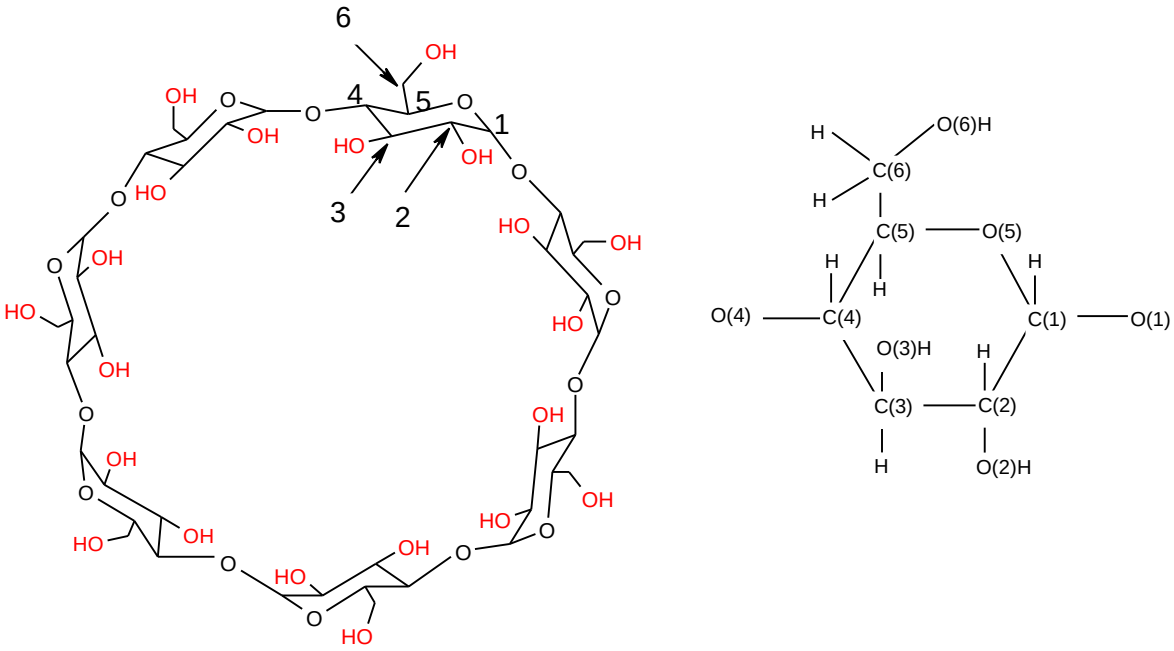


Figure 3.2. (left) Chemical structure of ß-cyclodextrin; and (right) atom numbering scheme of a glucose unit.

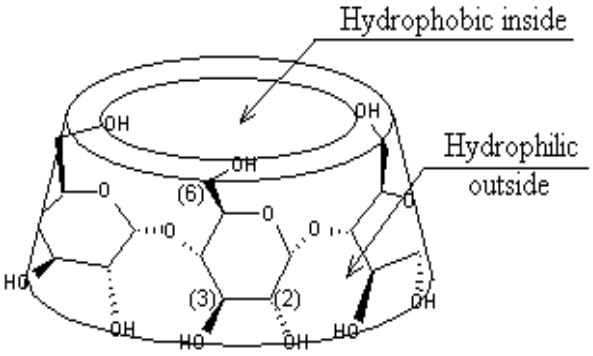


Figure 3.3. Schematic 3D representation of the hydrophobic and hydrophilic regions of a CD.

Each glucose unit contains 5 stereogenic centers; hence there are 30, 35, and 40 stereogenic centers in a-CD, ß-CD, and ?-CD respectively. The interior of the cavity consists of two rings of C-H groups (C-5 and C-3) with a ring of glucosidic oxygen between. Therefore, the interior is relatively hydrophobic in comparison with polar solvents such as water, while the mouth and exterior of the cavity is hydrophilic.[157]

Some of the important physiological parameters for the three native CDs are depicted in Table 3.1.

Table 3.1: Physical characteristics of the three common cyclodextrins.

	Cyclodextrin		
Properties	**a-CD**	**ß-CD**	**?-CD**
No. glucose units	6	7	8
Empirical formula (anhydrous)	$C_{36}H_{60}O_{30}$	$C_{42}H_{70}O_{35}$	$C_{48}H_{80}O_{40}$
Cavity depth, Å	7.8	7.8	7.8
External diameter, Å	13.7	15.3	16.9
Cavity diameter, Å	5.7	7.8	9.5
Heat capacity (anhydrous solid), J mol^{-1} K^{-1}	1153	1342	1568
Heat capacity (infinite dilution), mol^{-1} K^{-1}	1431	1783	2070
pKa (25°C)	12.33	12.20	12.08
Solubility (water 25°C), mol L^{-1}	0.114	0.016	0.179
Molecular weight (anhydrous)	972.85	1134.99	1297.14

Source: data from Ref. 157

Because of differences in the amount of strain in the rings of the a-, ß- and ?-CD, the orientation and degree of hydrogen bonding between the hydroxyl groups on C-2 and C-3 atoms of adjacent glucose molecules is different in each of the CD's. The hydroxyl groups on C2 and C3 of the adjacent glucose units of ß-CD are orientated so that they interact very strongly with each other. As a result, they do not interact

with the bulk solvent water molecules in order to solvate the CD molecule. However, with the increased strain in the ring of a-CD, these hydroxyls groups are positioned so that they interact with each other less that they do in ß-CD, and can thus interact more with water molecules. As a result, a-CD is more soluble in water than ß-CD. ?-CD is less constrained, with an even smaller degree of interaction between the hydroxyl of the adjacent molecules in the ring. Hence, the hydroxyl groups in ?-CD interact much more freely with water molecules. This results in an even greater solubility in water than a- and ß-CD.[159]

3.2 Inclusion Complex Process and Chiral Recognition by CDs

A variety of water soluble and insoluble compounds can fit into the cavity of CD to form inclusion complexes.[134, 153] Inclusion (host-guest) complexes are formed by the entrapment of guest species by the host molecule of CD. Host-guest complexation does not involve covalent bonding, but a combination of various other (hydrogen bonding, electrostatic, hydrophobic, and van der Waals) interactions. It is the structure of the CDs that gives rise to their remarkable ability to form inclusion complexes with various molecules of a polar and un-polar nature, also with ions. These CD complexation processes are highly stereoselective and can be considered as the method of choice for resolution of various structural, geometrical, diastereoisometric, and enantiomeric isomers.

Complexation will occur if:

i. there is a steric compatibility between the CD cavity and the guest molecule (but only a part of the guest molecule can be also included)
ii. the affinity of the guest molecule for the CD cavity is higher than for the other compound present (i.e., solvent);
iii. Host – guest hydrophobic interactions, Van der Waals forces and hydrogen bonding occur independently or in combination.

The stoichiometry of inclusion compound is usually 1:1 (host – guest polar ratio). However, complexes can be made of two or more guests (especially with the large γ-CD cavity) or of several CD molecules by inclusion of different parts of a large molecule (Figure 3.4).[134, 160]

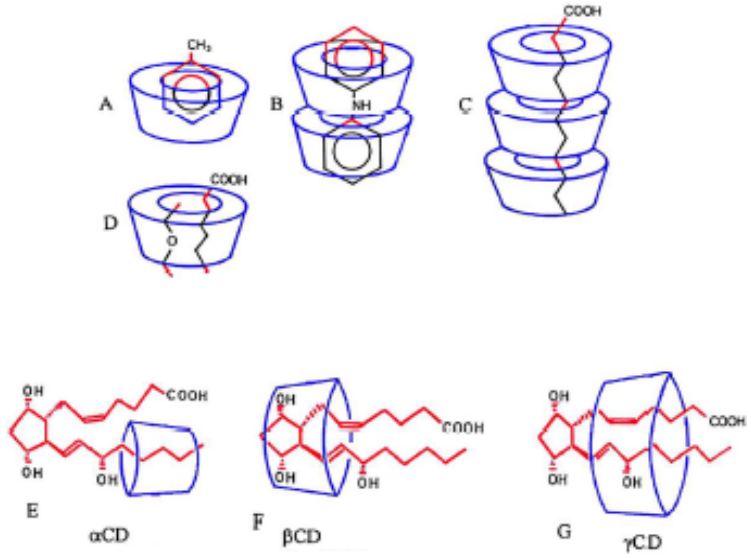


Figure 3.4. Examples for CD-complexes: the same CD with different guests, and the same guest with different CDs. Toluene/β-CD (A), diphenylamine/β-CD (B), long-chain fatty acid/CD (C), short chain fatty acid + diethyl ether ternary β-CD complex (D), prostaglandin E2/α-CD (E), prostaglandin E2/β-CD complex (F), prostaglandin E2/γ-CD (G).

Inclusion complex formation and the size of a solute's binding constant to CD are determined by several factors. These include the 'hydrophobic effect', which induces the apolar portion of a molecule to preferentially reside in the relatively apolar CD cavity; hydrogen bonding between appropriate polar segments of the guest molecule and the secondary hydroxyl groups at the mount of the CD cavity; Van der Waals interactions; release of high energy water from the CD cavity and a change in ring strain upon complexation. In most cases, a combination of these factors is operative; however, the first two seem to be most important. It is apparent that the size and geometry of the guest molecule, in relation to that of the CD cavity, is an important factor in inclusion complex formation (see Figure 3.4).[154]

The main factors and having crucial importance for CD inclusion compounds formation and their stability are hydrophobicity and the shape of the guest compounds. Moreover, as CDs are composed of D-glucose units they are themselves chiral and therefore represent a potential tool for formation of diastereoisomeric complexes with other chiral compounds of different chemical natures, including those that are difficult to transform into diastereoisomers (e.g. hydrocarbons).

Many chromatographic methods and techniques benefite from CDs, particularly gas chromatography, classical column liquid chromatography, high performance liquid chromatography, capillary electrophoresis, capillary electrochromatography, and thin layer chromatography.

Other properties of CDs that especially promote their use and significance in chromatographic separations are as follows:[154]

2. the CD inclusion processes in solutions are not only stereoselective but also reversible;
3. the equilibration in solution is relatively fast since the rate constants of complexation are usually of the same order as those of diffusion controlled processes;
4. CDs are stable within a large range of pH;
5. they are light resistant and do not absorb in the full UV range commonly used for chromatographic detection; and
6. CDs are not toxic.

The broad chiral feature of CDs is based on several phenomena.

CDs have numerous chiral centers – five in every glucose unit. In a glucose unit, every chiral center has a different orientation and it is at different distance from its

neighboring atoms. Moreover, the shape of the glucose units do not repeat themselves from unit to unit, so β-CD has 35 different chiral recognition centers. This twisted shape explains why the CDs have broader recognition spectra than linear oligoglucosides, where all glucose units have identical shape.

CDs are very flexible and prone to “induced-fit” structural changes. They can change their shape to interact intimately with analytes. The great variety of chiral centers and “induced-fit” produce the multimodal characteristic of CDs, which can yield more than one mode of interaction with an enantiomer pair. Of the particular significance is the clockwise versus counterclockwise orientation of the secondary hydroxyl groups on the upper rim of CD that influences strongly the ability of the host molecule to form hydrogen bonds with the guest molecules. For the less tightly bound enantiomer, only single intermolecular hydrogen bonds form between host and guest, but for the more tightly bound enantiomer multiple-contact (simultaneous) intermolecular hydrogen bonds form. Moreover, the ratio of multiple-contact hydrogen bonding (leading to preferential enantiomer stabilization) to single hydrogen bond is about 2:1.[36, 80]

Inclusion complexation alone is not sufficient and, in several cases, even not necessarily for chiral recognition. The least voluminous chiral molecules (branched aliphatic hydrocarbons) have been separated on CSP of derivatizated γ-CD in GC, while inclusion complex formation of these enantiomers are expected mainly with α-CD. Enantiomers of several compounds have been separated with all sizes (α, β, γ) of CDs, and these findings also contradict the requirement for inclusion complex formation in chiral recognition. In capillary GC literature, no evidence has yet been published for the key role of inclusion in the chiral separation. In CE, some chiral separations have also been explained by mechanisms other than inclusion, even though the separations were made in water-based media.

Of course, inclusion complex formation might be a key interaction in chiral recognition, but not necessarly. It is beneficial (and perhaps essential) that the "chiral center" or other substituents from "chiral center" are near and interact with the mouth of the CD cavity. The 2- and 3- hydroxyl groups located at the mouth of CD cavity appear to be particularly important in chiral recognition. Thus, hydrogen bonding and/or steric interactions external to the CD cavity should be present (Figure 3.5).[36]

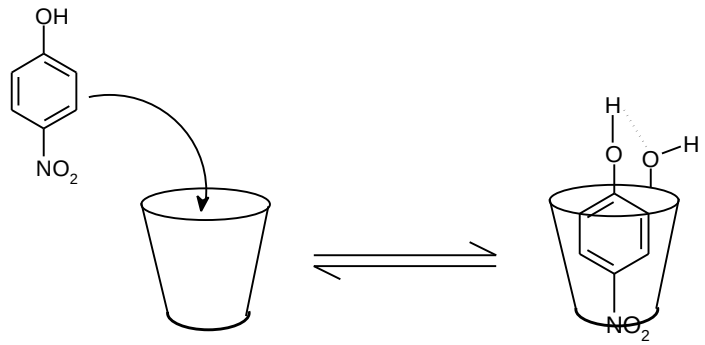


Figure 3.5. Schematic showing the reversible inclusion complex and hydrogen-bonding formation between analyte and CD.

Important contributions to the molecular modeling of CD-mediated separations were made by Lipkowitz.[80] He stressed the dichotomy between the location of the preferred binding site of a selectand within the cavity and the location of the optimum chiral discrimination domain, which are a priori not necessarily identical. It was also proposed that short-range dispersion forces are important as intermolecular forces.

It should be noted that a strong molecular association is not always a prerequisite to efficient chiral discrimination. Often a weak selectand-selector interaction can lead to appreciable chiral recognition. It may even be predicted that one enantiomer is included in the CD cavity while the other enantiomer is excluded due to steric reasons, thereby producing a very high separation.[49]

3.3 Modified cyclodextrins

In every CD, primary and secondary hydroxyl groups can be modified by substituting the hydrogen atom or hydroxyl group by a wide variety of hydrophobic (e.g., methyl, propyl) or hydrophilic groups (hydroxypropyl, sulfate, phosphate, quaternary amine).

The aim of such derivatizations may be:

i. to improve the solubility of the CD derivatives;
ii. to improve the fit and/or the association between the CD and its guest,
iii. to attach specific groups to the binding site;
iv. to form insoluble, immobilized CD-containing structures, polymers, for chromatographic purposes;
v. to increase the number of stereogenic centers;
vi. to enhance selectivity toward certain analytes.

In α-, β-, γ-CD there are 18, 21, and 24 respectively hydroxyl groups which can be modified by substituting the hydrogen atom or the hydroxyl group by a large variety of functional groups (Figure3.6).

A great variety of neutral derivatives of CDs such as heptakis -O-methyl-CD (M-CD), heptakis (2,6-di-O-methyl) CD (DM-CD), heptakis (2,3,6-tri-O-methyl) CD (TM-CD), hydroxyetyl-CD (HE-CD), and hydroxypropyl-CD (HP-CD) have been synthesized and applied to a great variety of compounds.

The substitution is described by a term called degree of substitution (DS), which gives the average number of substitutes per CD molecule.
Derivatizing CDs significantly changes their physicochemical properties as well as their complexation behaviour. Modified β-CDs are better soluble in water. It is well

described in literature that methylation of the parent β-CD result in a dramatic increase in water solubility.[79] *Per*(2,6-di-O-methyl)-β-CD has thus an aqueous solubility 30 times greater than of the parent β-CD and still retains efficiency for complexation.

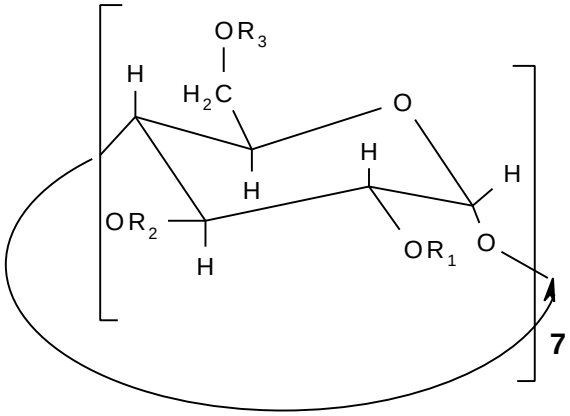


Figure 3.6. β-CD and its derivatives:

(n = DS)

$R_3 = R_1 = R_2 = H$	native β-CD
$R_3 = R_1 = R_2 = H$ or $= -[CH_3]_n$ n = 1, 2, ...	methylated β-CD
$R_3 = R_1 = R_2 = H$ or $-[CH_2CH(OH)CH_3]_n$ n = 1,2,…	hydroxypropylated-β-CD
$R_1 = R_2 = H$; $R_3 = -[CH_2COOH]_n$ n = 1, 2,…	carboxymethylated-β-CD
$R_3 = R_1 = R_2 = H$ or $= -[COCH_3]_n$ n = 1, 2,…	acetylated-β-CD
$R_2 = H$; $R_1 = R_3 = -CH_3$	2,6-di-O-methyl-β-CD
$R_3 = R_1 = R_2 = -CH_3$	2,3,6-tri-O-methyl-β-CD

CD derivatives have more flexible and/or twisted shapes than native CDs. Attachment of methyl or hydroxypropyl groups to the CD extends the cavity of the CDs. Thus, these CDs have much more important hydrophobic domains and cavity volume approximately 10-20% larger than native CDs due to the increase in the height of the CD torus. This gives rise to the CDs that accommodate more easily highly hydrophobic compounds.

Some substitutions (like hydroxypropyl group) add more chiral centers to the CDs, thus broadening recognition spectra.

3.4 Surfactant behaviour of cyclodextrins

Because natural CDs are typically surface-inactive organic compounds, they have not attracted a surface chemical interest. Instead, some modified CDs tend to be surface active.

It was previously pointed out that β-CD presents a surprisingly low solubility in water. It has been observed however that almost any substitution of any hydroxyl group result in a dramatic perturbation of its interaction with the network of water molecules, inducing increased solubility and thus, decreasing the surface tension of water, even the substituted group is hydrophobic (as in the case of methylated β-CD). Some modified β-CDs (both with hydrophobic and hydrophilic type groups) are more or less adsorbed onto the gas/water interface and occupy larger areas than the wider rim of β-CD[55] but little is known about their molecular orientation at the air-water interface. It was suggested that the type and the dimension of the substituents influence the spatial arrangement and density of the modified CDs at the interface[94]. Parallel and perpendicular orientation of CD's molecule at the air/water interface were proposed (Figure 3.7)[54]

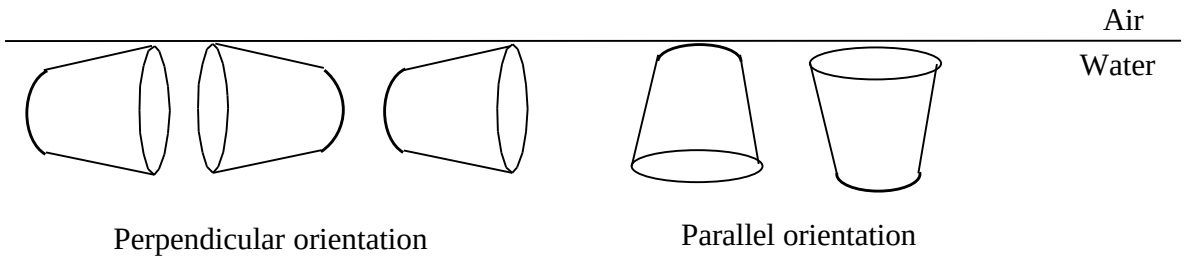


Figure 3.7. Possible perpendicular and parallel orientations of Cyclodextrins at the air/water interface

From the pressure-area isotherm, it was concluded that CDs derivatizated with hydrophobic large moieties (like cholesterol) are positioned toward the water subphase, with their molecular axis predominately parallel to the interface.[140]

In aqueous solution above a critical concentration, they are able to self-assemble in spherical micelles-like aggregates.[19, 79] In these aggregates the CD moiety is exposed to the aqueous medium, the CD keeping the ability to include guest molecules in the cavity.

Modified CDs that posses surface-active proprieties behave as conventional surfactants.[19,55,94] This is the physical basis for using modified CDs as *surface active chiral collectors* in conjunction with a foam separation technique.

3.5 Other applications of cyclodextrins

The ability of CDs to hold various guest molecules in the hydrophobic cavity makes them important excipients in many areas. Because CDs are practically nontoxic, they have widespread applications in foods, pharmaceuticals, cosmetics, and agriculture, for examples, solubility enhancement, stabilization of labile drugs, control of volatility and sublimation, physical isolation of incompatible compounds, long-term protection of colour, door, and flavour, suppression of bitter tastes of drugs, etc.[55,143]

Less than 10% of all produced cyclodextrins, cyclodextrin derivatives are consumed by the pharmaceutical industry. The largest cyclodextrin consumers are the food and the cosmetic industry. In the pharmaceutical industry, CDs have mainly been used as complexing agents to increase the aqueous solubility of poorly water-soluble drugs, and to increase their bioavailability and stability. In addition, they can be used to reduce or prevent gastro-intestinal or ocular irritation, reduce or eliminate

unpleasant smells or tastes, prevent drug-drug or drug-additive interactions, or even to convert oils and liquid drugs into micro crystalline or amorphous powders. The ability of CDs to form complexes with a variety of organic compounds helps to alter the apparent solubility of the molecule, to increase stability in the presence of light, heat and oxidizing conditions and to decrease the volatility of compounds. CDs can also be used as processing aids to isolate compounds from natural sources and to remove undesired compounds such as cholesterol from food products.

The dose of environmental polluting agrochemicals synthetics (insecticides, herbicides, fungicides, etc., from which many are still used as racemate) could be significantly reduced when complexed by cyclodextrins, and/or their effectivity could be enhanced.[160]

Cyclodextrins have also wide fields of utilization in sensors, in diagnostic kits, and in analytical chemistry, particularly in the chromatographic techniques. The analytical applications of CDs refer mainly to the application of cyclodextrins in gas chromatography, in high performance liquid chromatography, and in capillary zone electrophoresis, but some papers are dedicated to thin-layer chromatography, to enhancement of UV-VIS absorption, luminescence/phosphorescence by CDs and to increasing the sensitivity of the related analytical methods. Apparently, it is difficult to find a separation problem on analytical scale, which could not be solved by using the appropriate CD.

4. CHIRAL COMPOUNDS USED IN FOAM FRACTIONATION

The compounds used in this work were chosen based on the following criteria:

1. CDs were used as chiral selector; therefore the enantiomers should have chiral affinity to the CDs used, namely:
   - 2-Hydroxypropyl-β-cyclodextrin (HPβCD)
   - Random methylated β-CD (MβCD) and
   - Heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (TMβCD).
2. GC was used for analytical quantification of foam fractionation resulting samples; therefore the enatiomers should be volatile enough to be separated in GC.
3. The solubility in water of the compounds should be different; therefore soluble to insoluble compounds were chosen.
4. In order to study the chiral discrimination of CDs function of molecular structure of selectands, compounds with different structures and dimensions were chosen.

Dichlorprop, mecoprop, and dichlorprop methyl ester

Anionic phenoxy acid herbicides 2-(2,4-Dichlorophenoxy)propionic acid (dichloroprop) and 2-(4-Chloro-2-methylphenoxy)propionic acid (mecoprop) (Figure 4.1) have the chiral carbon close to the aromatic group.

Dichlorprop and mecoprop are systemic herbicides and act as auxins.[1] They were introduced in the 1940s and 1950s to control broadleaf weeds in agriculture, lawn pastures and industry. Dichlorprop and mecoprop, often applied in formulation with other herbicides, are among the most widely used herbicides in the world. Racemic mecoprop is also used to control the growth of weeds in building materials.[92]

Since 1953, it has been known that only the (R)-enantiomers show herbicidal activity. Nevertheless, the racemic mixtures were and still are applied, thereby

introducing large amount of isomeric ballast into the environment. In many countries, dichlorprop and mecoprop are nowadays also sold as enantiomerically pure compounds (named dichlorprop-P and mecoprop-P).

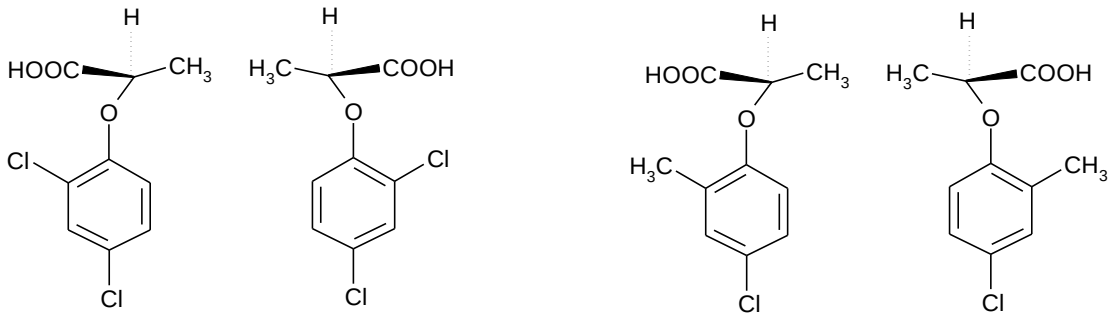


Figure 4.1. Structural formulas of dichlorprop and mecoprop.

Dichlorprop and mecoprop are quite soluble in water and strong acids. Since they are mostly present in the dissociated (anionic) form in the environment, they do not adsorb onto soil increasing the risk for contamination of aquatic systems.[1] Their biological degradation in environment is, in most cases, enantioselective.

Dichlorprop methyl ester (Figure 4.2), the neutral form of diclorprop, is less soluble in water.

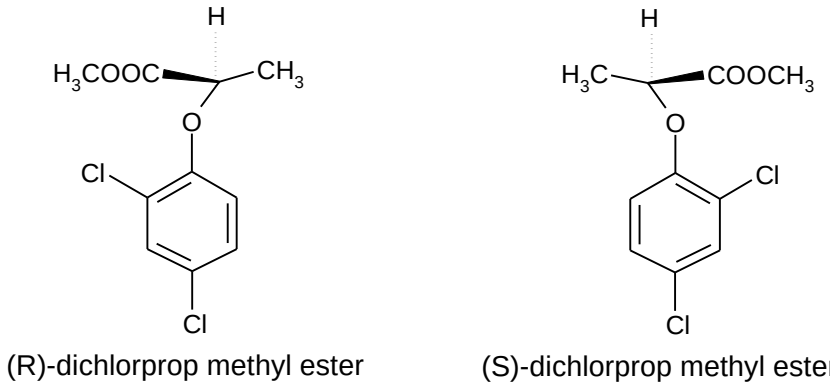


Figure 4.2. Structural formula of dichlorprop methyl ester

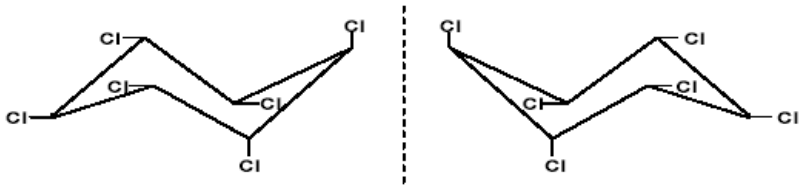
It is known from literature that the enantiomers of dichlorprop and mecoprop were already separated in HPLC with permethylated α-CD as stationary phase[1] and in CE with TMβCD (base line separation) and HPβCD (not base line separation) as additive in the running buffer but dichlorprop methyl ester could not be separated with these two cyclodextrins[146,138.] It was shown that the phenyl moiety of the corresponding phenoxy acid is involved in the formation of inclusion complexes with the cyclodextrins; the size of the cavity of cyclodextrins as well as that of the phenyl moiety governed the chiral separation. For successful chiral recognition with cyclodextrins, hydrophobic interactions between the cavity interior and the analytes are assumed, whereas hydrogen bonding at the cavity edge determines a compound's access to the cavity entrance.[114] Partitioning of the methyl ester to the cyclodextrin is probably of a different nature than that of the corresponding acid; the alkyl moiety of the ester may also be involved in the inclusion complex with or without possible chiral recognition.

After derivatization, both phenoxy acids can be enantiomerically resolved in GC.

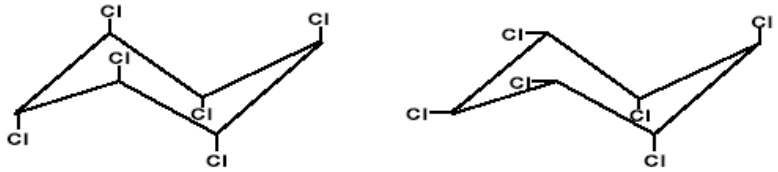
a-Hexachlorocyclohexane

Hexachlorocyclohexane (HCH) belongs to the organochlorine compounds and used to be one o the most widely applied insecticides. It was introduced during world war II and was used mainly in forestry, agriculture, and as a wood preservative. HCH comprises eight isomers, which differ in their axial-equatorial substitution pattern around the ring and from which only α-HCH is chiral (Figure 4.3). Technical grade HCH consist typically of 60-70% α-HCH, 0-15% γ-HCH (the only isomer with insecticidal properties), 12% β-HCH, and 6-10% δ-HCH, whereas the commercial insecticide market today comprises 99% γ-HCH. In 2000, in Europe, the use of this insecticide was completely banned for all agricultutal and gardening applications.

Although the total amount of applied HCH was reduced globally, it is still a contaminant of a great concern in many countries (e.g., in India).[1]



The enantiomers of α-HCH



β-HCH (achiral) γ-HCH (achiral)

Figure 4.3. Chemical structure of some HCH isomers.

HCHs are moderately hydrophobic, semi-volatile compounds and have a low tendency to accumulate in soils. They are transported by water and air, and today, have accumulated in regions where HCH was never used, such as Artic and Baltic Sea. Enantioselctive degradation was observed, the (+)-α-HCH being converted preferentially. The calculated half-lives for (+)-α-HCH and (-)-α-HCH are 5.9 and 23.1, respectively.

5. RELATIONS USED IN DATA QUANTIFICATION

5.1 Foam Fractionation Evaluation

The following performance parameters were used to characterize the efficiency of the foam fractionation process:

Enrichment ratio (E_R) is defined as the increase in the concentration of the compound in the foam (C_f) relative to the initial solution (C_i):

$$E_R = \frac{C_f}{C_i} \qquad (5.1)$$

Recovery (R_P) is defined as the ratio of mass of compound in the foam (m_f) and in the initial solution (m_i):

$$\%R_P = \frac{m_f}{m_i} \cdot 100 = \frac{c_f \cdot V_f}{c_i \cdot V_i} \cdot 100 \qquad (5.2)$$

5.2 Quantification of mixtures of enantiomers

There are many different ways to express chiral composition.

Until recently, the *enantiomeric ratio* (ER) was the most frequently used descriptor for the relative abundance of one enantiomer over the other. ER is defined as:[27]

$$ER = \frac{A_+}{A_-} \qquad (5.3)$$

where A_+ and A_- correspond to the peak areas (or heights) of the (+) (major enantiomer) and (-) enantiomers (assuming equal molar response factors), respectively.

Thus, a racemic mixture has an ER of 1.00. However, there are several limitations in using ER. When used graphically the ER gives a misleading representation of data. Because of the way it is defined, the ER can range from 0 to infinity. Therefore, a unit change in ER away from unity in the downward direction (i.e. < 1) is not equivalent to the same unit change in the opposite direction. Complications may also arise when ER is employed in mathematical expressions.

A better representation of the chiral signature is the *enantiomer fraction* (EF):

$$EF = \frac{A_+}{A_+ + A_-} \quad \text{or} \quad EF_X = \frac{A_1}{A_1 + A_2} \qquad (5.4)$$

where A_1 and A_2 are the peak areas (or heights) of the first and last eluting enantiomers on chiral column 'X' when the identity of the (+) and (-) forms is not known.

The EF can only range from 0 to 1.0 with EF=0.5 representing a racemic mixture. Each unit of deviation from the racemic value (0.5), both in the upward and downward direction, is equivalent. Because it is a proper fraction, the EF can also be applied more naturally in mathematical fate expressions.

Enantiomeric excess (ee) of one enantiomer over the other is defined as:

$$ee = \frac{A_+ - A_-}{A_+ + A_-} \quad \text{or} \quad ee = \frac{A_1 - A_2}{A_1 + A_2} \qquad (5.5)$$

where A_1 and A_2 are the peak areas (or heights) of the first and last eluting enantiomers on chiral column 'X' when the identity of the (+) and (-) forms is not known.

In practice, ee is often quoted as a percentage:

$$\%ee = \frac{A_1 - A_2}{A_1 + A_2} \cdot 100 = \%A_1 - \%A_2 \qquad (5.6)$$

The magnitude of ee extends from ee=0 for racemic mixture to ee=1 (100%) for pure one single enantiomer.
Unless negative values are accepted for ee, the major enantiomer is not identified by the ee definition.

In principle, all three terms are useful for the quantitation of a mixture of enantiomers but %ee is the term of choice for this work and negative values for ee were accepted.

6. MATERIALS AND METHODS

6.1 Chemicals and Solvents

Chiral compounds and chiral selectors

- 2-(2,4-Dichlorophenoxy)propionic acid
  (dichlorprop) (**DCPP**) (99.0% purity; M = 235.07),
- 2-(2,4-Dichlorophenoxy)propionic methyl-ester
  (dichlorprop methyl-ester) (**DME**) (97.0 % purity; M = 249.09),
- 2-(4-Chloro-2-methylphenoxy)propionic acid
  (mecoprop) (**MCPP**) (98.8% purity; M = 214.65),
- 2-(2,4,5-Trichlorophenoxy)propionic methyl-ester
  (fenoprop methyl-ester) (**FME**) (98.5% purity; M = 283,54),
- α-Hexachlorocyclohexane (a-**HCH**) (99.5% purity; M = 290.08),

were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) as racemate.

- 2-Hydroxypropyl-β-cyclodextrin (**HP**b**CD**) (standard quality)

(molar substitution (per anhydro glucose unit): 0.6 - 0.9; M(average) = 1380 – 1500; used in this work: M = 1400)

was purchased from Wacker - Chemie (Munchen, Germany) and

- Heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (**TM**b**CD**)

(90% purity, desiccated; M = 1429.6),

from Sigma-Aldrich (Germany).

Other reagents and solvents

▪ Hexachlorobenzene (**HCB**)	available in CTA in concentration 1000 ng/µl
▪ BF_3-methanol complex	20% solution; Merck
▪ Methanol (**MeO**)	p.a.; Merck
▪ Tetrahydrofurane (**THF**)	99.9%; Aldrich
▪ Diethylether	in house (CTA) distillation system
▪ Cyclohexane	in house (CTA) distillation system
▪ H_2SO_4	96%, Aldrich
▪ HCl	p.a. Fluka (1M)
▪ Bi-distilled/de-ionized water	("Milli-Q_{185} Plus", Millipore)

6.2. Equipments

6.2.1. Gas Chromatography

For dichlorprop, dichlorprop methyl-ester, and a-Hexachlorocyclohexane:

GC:	Chrompack CP-9002
Detector:	ECD, 220°C, Make-up gas N_2
Chiral column:	BGB 176 SE, *BGB-Analytik AG*; Adliswil, *Switzerland, 20% 2,3-dimethyl-6-tert-buthylmethylsilylated -bCD dissolved in SE-5,2, fused silica,* 30 m length, 0.25 mm ID, 0.35 mm OD, 0.25 µm film thickness;
Pre-column:	deactivated 10 m, fused silica, *BGB-Analytik AG; Switzerland*
Carrier gas:	H_2, 150 kPa
Split/split less injector:	230°C, split 1:20 2 µl injection volume for DCPP and DME 1 µl injection volume for α-HCH
Temperature:	150°C isotherm for DCPP and DME 157°C isotherm for α-HCH
Software:	Maestro II Version 2.3 – Chrompack

For *mecorprop methyl ester*

GC:	Chrompack CP-9002
Detector:	FID, 250°C, Make-up gas N_2
Chiral column:	CP-Chirasil-Dex CB, *Permethyl 2,3,6-tri-O-methyl-bCD on polysiloxane backbone, fused silica,* 25 m length, 0.25 mm ID, 0.39 mm OD, 0.25 μm film thickness; *Chrompack*
Carrier gas:	H_2, 125 kPa
Split/split less injector:	250°C, split 1:20; 2 μl injection volume
Temperature:	150°C isotherm for MCPP
Software:	Maestro II Version 2.3 - Chrompack

6.2.2. Foam Fractionation Unit – experimental set up

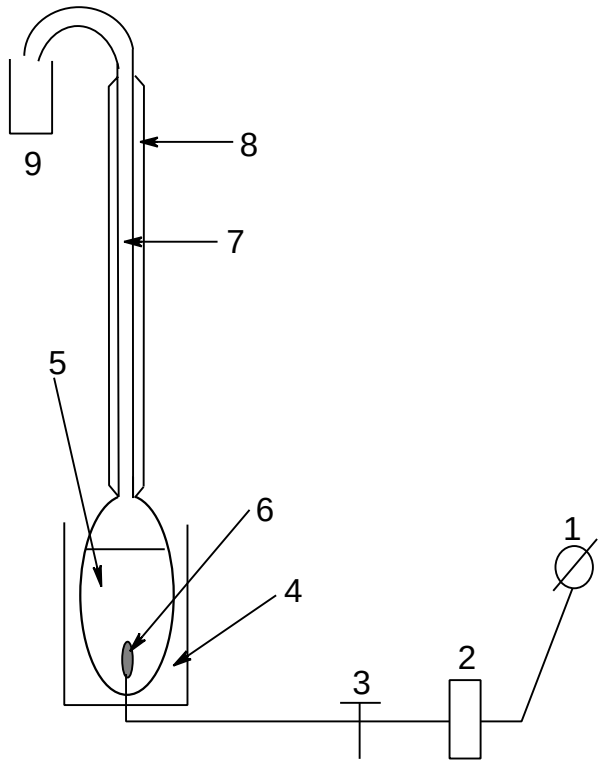
Foam fractionation system (Figure 6.1) was fabricated in the glassworks of the Institute. It consist of three basic parts:

1. the gas section,
2. a sample reservoir, for the aqueous solution containing the racemate and the chiral collector,
3. a detachable jacketed column.

Compressed nitrogen was passed through a digital flow meter (with a control valve incorporated) and directed, via a two-way stopcock, into the bottom of the sample reservoir. The gas was introduced into the solution through a porous glass frit por.4 (Robu Glas filter-Geräte). The three detachable columns used (20, 40, 60 cm x 1.2 cm i.d.) were, together with the sample reservoir, kept at same temperature by means of a water-cooling bath coupled to the water jacket around the column.
The produced foam was collected in a glass vessel from the top of the column.

Figure 6.1. Diagram of apparatus used for foam fractionation:

1. compressed N_2,
2. flow meter with control valve incorporated,
3. two-way stopcock,
4. water cooling bath,
5. sample reservoir,
6. glass frit
(por. 4; nominal pore size 10-16 μm),
7. foaming glass column (detachable),
8. water jacket,
9. foam collector vessel



6.2.3. Other equipments

Electronic Balance:	Precisa, 40SM –200A
Flowmeter:	Mass Flow Controller, 0-500 ml/min; Analyt
pH-meter:	inoLab pH Level 1
Rotating Evaporator:	Büchl
Ultra-sonic Bath:	Sonorex super RK 510 H
Water destilation/deionization:	Millipore, Milli-Q Plus 185
Heaters	

6.3.Methods

6.3.1. CD's Foamability and foam stability

Foamability measurements were carried out by shaking, for 3 min, 10 ml of CD solution (10 or 1 mM concentration) placed in a 100-ml graduated cylinder and, in the case when foam was produced, the volume of the foam was recorded immediately after shaking. Because the foam has the same behaviour, collapsing after the shaking, the volume of the collapsed foam was also recorded. The differences between the foam volumes before and after collapsing was taken into account as the foamability ability.

For foam stability measurements, 5 ml of CDs solutions (10 or 1 mM concentration) were placed in small glass column (30 cm x 0,8 cm i.d.) and foamed to the same height (15 cm) by purging gas at 15 ml/min flow rate. The time of foam collapsing to the half of the height was then recorded.

6.3.2. Foaming procedure

Although derivatized CDs present some surface activity, the foam produced is not stable and collapses very fast. Therefore, a high gas flow rate was necessary for maintaining the foam in the column. In many cases the gas flow had to be increased and/or decreased in order to keep the foam at a constant height. Several attempts to foam the mixtures of CDs/chiral compound showed that for every ratio concentration CD/concentration chiral compound the behaviour of foam is different in terms of time necessary for the foam to reach a certain height and of its stability. It was clear that a general optimised foam fractionation method could not be developed and the operating variables of the system had to optimised for every ratio of CD concentration/chiral compound concentration.

Mainly, unless stated otherwise, the foam fractionation experiments were conducted as follows:

Foam fractionation system was operated in simple batch mode. Nitrogen was used as inert gas. With the digital flow meter set at a low value and the two-way stopcock on the position “out”, a total of 50 ml solution was placed in the sample reservoir. The column was attached to the reservoir and the gas directed into the reservoir (the two-way stopcock in position “in”). The gas flow rate was increased so that the bubbles, produced by the introduction of gas through the frit, leave the solution surface as foam. The foam bubbles tended to break on the way up in the column, producing a liquid reflux. The gas flow rate was adjusted to obtain steady countercurrent conditions. The foam was allowed to reach the top of the column and to equilibrate for a period of time (before sample removal). After this time a foam fraction (maximum 3 ml volume; which means that only the upper part from foam height was removed) was colleted by increasing the gas flow rate to force some foam out of the column. All the collected foams collapsed very fast (in a few seconds)

Then the gas flow was cut off (the two-way stopcock in position “out”), the foam in the column allowed to collapse completely and the residual solution (which is now starting solution) foamed again. In some experiments, 1 ml from the residual solution between each consecutive foaming was taken out for analyses.

When experiments were done at temperature lower than room temperature, the reservoir, with the solution inside, was immersed in the water-cooling bath set at the desired temperature. The column jacket was coupled to the cycling system of the cooling bath and the solution and the glass foaming column allowed, prior the experiment, to reach the desired temperature, only then 1 ml from bulk solution was taken out as s0.

When experiments were done at temperature higher than room temperature, water at the desired temperature was placed in the water bath. The reservoir, with the solution inside, was immersed in the water bath, the column jacket coupled to the cycling system of the water bath with only the pump turned on. The solution and the glass foaming column were allowed, prior to the experiment, to reach the desired temperature, only then 1 ml from bulk solution was taken out as s0. During the experiment, the solution temperature decreased only 2°C.

After each foaming experiment, all glass components of the system were soaked in 2-propanol/NaOH bath over night, rinsed extensively with distilled water, kept in citric acid bath for at least 4 hours and rinsed again with distilled water.

6.3.3. Solution preparation for foam fractionation

CDs stock solutions, in different concentrations (ranging from 0.1 to 10 mM), were prepared in water and kept in refrigerator. Fresh stock solutions were prepared for every two experiments.

Stock solutions of *phenoxy acids (dichlorprop and mecoprop)* were prepared by dissolving the appropriate quantity of compound in methanol 10% (first methanol and then water) and kept in refrigerator.

Stock solutions of *dichlorprop methyl ester* were prepared by dissolving the appropriate quantity of compound in THF 2.2% (first THF and then water) and kept in refrigerator.

α-HCH stock solution was obtain as follows: 5 mg α-HCH were dissolved first in 10 ml THF and 500 ml water was gradually added. The solution was kept in ultra-sonic bath for one hour at 40°C. After cooling at room temperature, water was added

till a final volume of 1000 ml and the solution (5 mg α-HCH in 1000 ml THF 1%) was kept again in ultra-sonic bath for another hour at 40°C. After cooling, the solution was not clear as some α-HCH remained in solution as particulate material. This was the maximum concentration that could be obtained for α-HCH in water and it was used as *5 ppm a-HCH water solution.* The stock solution was kept at room temperature.

Before each foaming experiment stock solutions of both compound and CDs were allowed to reach room temperature and then mixed in different volume ratios (v:v) in order to obtain the desired concentration for both compound and CD. In the case of α-HCH, prior to each use, α-HCH solution was kept in ultra-sonic bath for one hour in order to homogenize the solution. The CD was added directly, in different quantities, to 50 ml α-HCH solution (5 ppm), in order to obtain the desired concentration of CD (ranging from 0.1 to 10 mM).

HCl (1 N) was used to adjust the pH value of the solutions.

6.3.4. Sample Preparation for GC analyses

Samples (1 ml) from initial solution, from foam and from residual solutions (between the consecutive foaming and the final one) were prepared for GC analyses.

Dichlorprop and mecoprop

Dichlorprop and mecoprop cannot be analysed directly by GC and must be derivatizated prior to GC analyses.
For the isolation of dichlorprop and mecoprop from aqueous solution, 1 ml from each sample was manually shaken for 2 min, three times, with 4 ml, 4 ml and 3 ml diethylether and the organic phases separated, with a Pasteur pipette, and mixed. The mixture was again shaken for about 1 min and 9 ml from organic phase was carefully transferred to another glass and evaporated to dryness in rotating evaporator under reduced pressure at 50°C. 3 ml of BF_3-methanol complex was added, the mixture kept in a oil bath at 70°C for 30 min, cooled in a cold water bath, 1 ml water and 8 ml cyclohexane were added and after 2 min of manual shaking the organic phase was allowed to separate from aqueous phase. 6 ml from the organic phase was carefully transferred to another glass and evaporated to dryness in a rotating evaporator under reduced pressure and low temperature.

Finally, for *dichlorprop*, 0.5 ml cyclohexane was added and the sample was spiked with 10 µl FME 40 ppm (in cyclohexane) as internal standard (final concentration of FME: 0.78 ppm). A 2 µl volume of the solution was analysed by GC-ECD.
In the case of *mecoprop*, 0.250 ml cyclohexane was added and the sample was spiked with 10 µl DME 1500 ppm (in cyclohexane) as internal standard (final concentration of DME: 57.69 ppm). A 2 µl volume of the solution was analysed by GC-FID.

Dichlorprop methyl ester

8 ml cyclohexane were added to 1 ml of aqueous solution from each sample and after 2 min of manual shaking the organic phase was allowed to separate. CD remained as a separate foam phase between the organic and aqueous phases.

6 ml from the organic phase was carefully transferred to another glass and evaporated to dryness in a rotating evaporator under reduced pressure and low temperature. Finally, 0.5 ml cyclohexane was added and the sample was spiked with 10 µl FME 40 ppm (in cyclohexane) as internal standard (final concentration of FME: 0.78 ppm). A 2 µl volume of the solution was analysed by GC-ECD.

a-HCH

1 ml H_2SO_4 96% was added to 1 ml of aqueous solution from each sample and the mixture was shaken. 8 ml cyclohexane were added and after 2 min of manual shaking the organic phase was allowed to separate from aqueous phase. 6 ml from the organic phase was carefully transferred to another glass and evaporated to dryness in a rotating evaporator under reduced pressure and low temperature. Finally, 1 ml cyclohexane was added and the sample was spiked with 20 µl HCB 5 ppm (in cyclohexane) as internal standard (final concentration of HCB: 0.1 ppm). A 1 µl volume of the solution was analysed by GC-ECD.

Because, for the same compound, the samples preparation for GC measurements (extraction /derivatization) was done in the same manner, the compound's recovery coefficient from water solutions was the same for all the samples (initial solution, foam samples, residual solution). Parameter used to characterize the efficiency of the foam fractionation process, enrichment ratio (E_R) is calculated relative to the

initial solution, and therefore it was not necessary to determine the recovery rate of the compound from water solution.

Since the samples manipulations were performed in an achiral environment, no alteration in enantiomeric excess (ee) could occur during these manipulations and all deviations from an ee=0 (racemic compound) could only arise from enantioselective discrimination during the foam fractionation process.

6.3.5. Analytical methods

The concentration and the enantiomeric excess of the chiral compounds were determined with GC. In order to determine the optimum chiral separation conditions, every compound was analysed initially in temperature-programmed runs. The temperature where enantiomeric separation occurs was chosen for the next attempts to obtain base line separation. Several isothermal runs were performed at higher and lower temperatures and different pressure and flow rates of carrier gas (H_2) until a good base line separation of enantiomers was obtained. The GC enatiomeric separation conditions for every compound are presented in section 6.2.1.

In enantiomeric analyses, a linear detector response is indispensable. Thus, for correct determinations, linearity within a concentration range of at least three order of magnitude is required. It is generally accepted that the flame ionisation detector (FID) fulfils this requirement. The linear response of the electron capture detector (ECD) is low in this respect. Therefore, the linear detector response of ECD was verified via dilution measurements from a concentrated solution.

The linear response range of ECD was obtained from triplicate injections of standard solutions of DME and α-HCH in cyclohexane at different concentrations. FME and HCB were used as internal standard for quantification of DME and α-HCH,

respectively. The regression equations and R^2 were obtained by plotting A_1/A_{IS} versus C_1/C_{IS} as well as A_2/A_{IS} versus $C_2/C_{IS,}$ where A_1, C_1, A_2, C_2 represent the peak areas and concentrations of the first and second eluted enantiomer, respectively, and A_{IS} and C_{IS} represent the peak area and concentration of the internal standard.

The concentration linear range of ECD and the regression equations for the respective curves are presented in Table 6.1.

Table 6.1. Linear response range of ECD and the regression equations.

Chiral Compound	**Concentration linear range (ppm)**	**Regression Equation (for linear range)**
Dichlorprop methyl ester	0.05÷50 (racemic) ⇓ 0.025÷25 (enantiomer)	y = 0.0594x + 0.032 R^2 = 0.999
α-HCH	0.008÷6 (racemic) ⇓ 0.004÷3 (enantiomer)	y =0.3919x - 0.2451 R^2 = 0.9922

As a representative example, chromatograms of standard racemate DME and α-HCH at different concentrations are shown in Figure 6.2. It is clear that in both cases there is an excellent enantiomeric resolution between enantiomers.

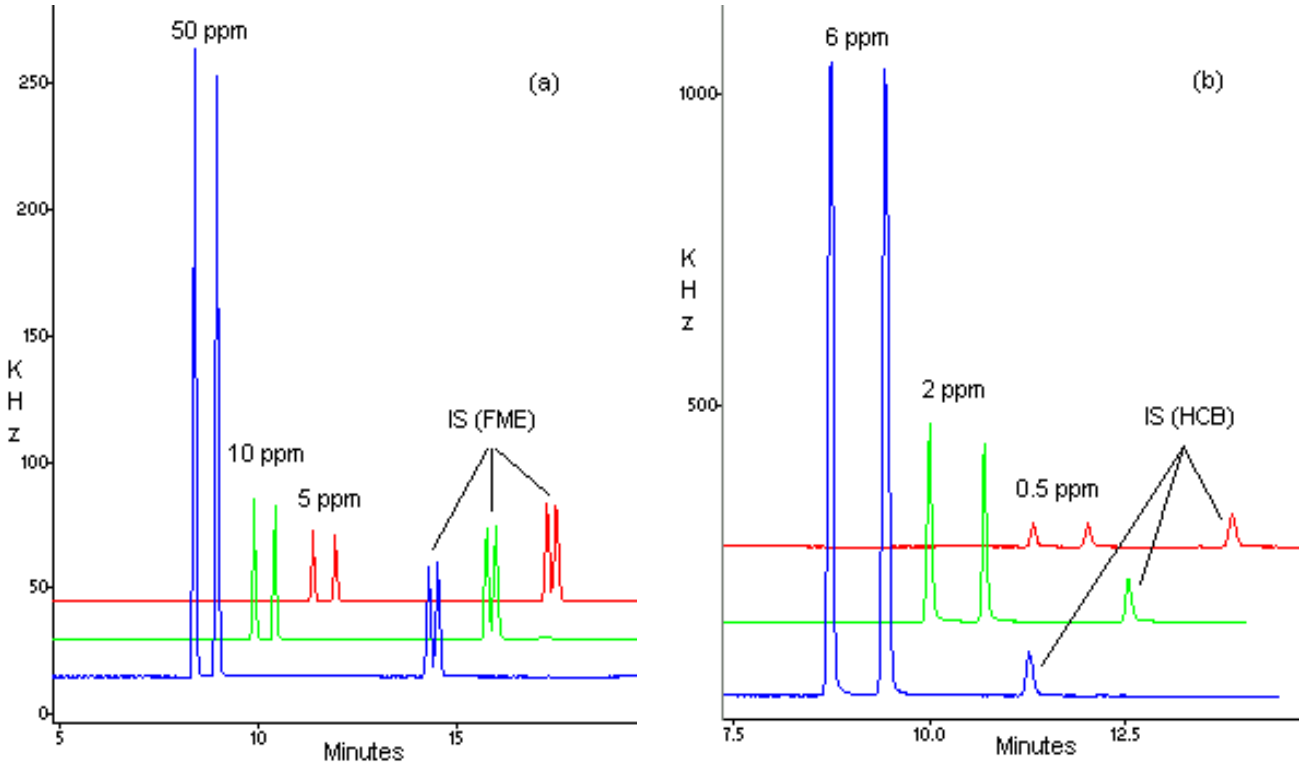


Figure 6.2. GC enantiomeric separation of racemic standards of DME (a), and α-HCH (b).

As it could be expected, concentration of foam samples exceeded the linear range response of ECD and therefore, after a first run, samples were diluted until the concentration of every single sample fall within the linear response range of ECD.

Since, all the GC measurements were done within the linear response range of ECD and peak areas are directly proportional with the concentrations of the samples, all the enrichment ratios (E_R) were calculated using peak areas instead of concentrations, as follow:

$$E_{R_i}(1;2) = \frac{A_i / A_{IS\,i}}{A_0 / A_{IS\,0}} \qquad (6.1)$$

where:

E_{R_i} = enrichment ratio for the foam sample i, and 1 or 2 denotes the first eluted enantiomer or the second eluted enantiomer, respectively,

$A_i / A_{IS\,i}$ = peak area of sample / peak area internal standard (foam sample i),

$A_0 / A_{IS\,0}$ = peak area of sample / peak area internal standard (initial solution, prior foaming).

7. RESULTS

7.1. Foamability and foam stability measurements

Prior to foaming experiments with CDs and chiral compounds, the foamability and foam stability of different CDs were tested.

In order to determine the presence of surface activity, solutions of HPβCD, MβCD, TMβCD, heptakis (2,6-di-O-methyl)-β-cyclodextrin, and α-, β-, γ-cyclodextrin were purged with gas. Only the first three (HPβCD, MβCD, TMβCD) produced foam and these three were the CDs of choice for the foaming experiments.

Solutions of the three CDs (10 mM) were foam fractionated from acidic to basic pH. In all cases the CDs produced foam with almost the same characteristics. It could be concluded that the surface activity of all three CDs is not dependent on the solution's pH.

The main characteristics responsible for foaming performance are the foam ability – the capacity of the continuous phase to include gas, and the foam stability – the ability to retain the gas for a certain period of time.[102] Foaming ability is determined by the increase of the volume, just after the introduction of the gas into solution. The stability of the foam relates to the decrease of foam volume with time.

Foaming ability (or foamability) has been studied using the shaking method. In this method foam is produced quickly by rapid shaking of solutions causing a sudden expansion of interfacial area. Simultaneously, due to vigorous shaking, there is a destruction of foam too. Each CD solution was tested at least five times and the reproducibility was ± 2 ml. The results for the three CDs used are presented in Figure 1.

The foams produced by all three CDs had quite similar behaviour: a first phase of volume growing followed by a fast collapse. At low concentration (1 mM), all three CDs still produced foam but bubbles were large, with interstitial liquid draining back to the bulk solution (so called wet foam). Practically, all the foam collapsed completely in a very short time.

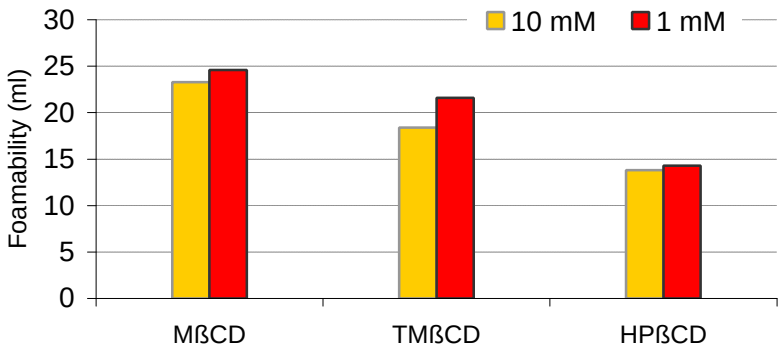


Figure 7.1. Foamability (by shaking method) as function of types of substituents in derivatizated CDs

In the case of foam stability (a more important parameter for foaming experiments) the results are different (Figure 7.2) and do not correlate with the foaming tests. For all three CDs, even when solutions were purged with gas, the foam did not reach steady state: a first phase of foam volume growth was followed by its decrease and then again by its growth. This feature was interpreted as reflecting either a depletion of CD in the liquid solution or as a complexation of CD molecules, leading to changes in surface activity and/or in the orientation of these aggregates at the gas/liquid interface.

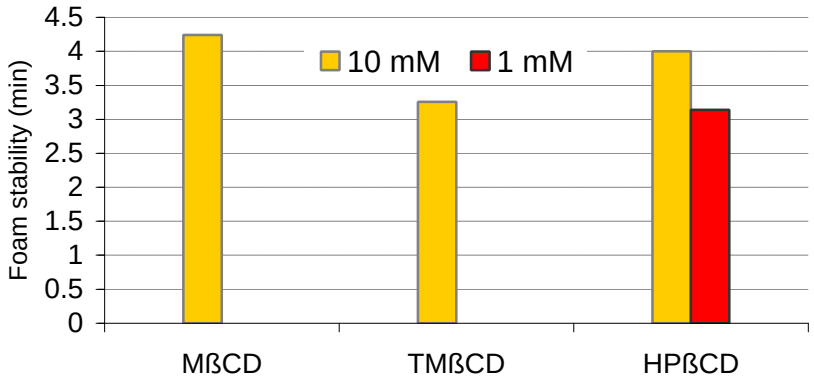


Figure 7.2. Foam stability as function of types of substituents in derivatizated CDs

While at 10 mM, all three CDs produced foam when purged with gas (15 ml/min flow rate), at 1 mM only HPβCD produced foam that reached 15 cm height. It could be observed that both MβCD and TMβCD, even at low concentration, produced foam as long as the solutions were purged with gas but the foam stability was very low. In the case of MβCD (1 mM) foam did not reach 15 cm and bubbles coalesced on the upper part of column and then collapsed and some wet foam was produced again. TMβCD (1 mM) produced dry foam but also did not reach 15 cm. When the gas flow rate was increased up to 30 ml/min, foam rose up and the coalescence phenomena occurred in the upper part of foam column but when the gas flow was stopped the foam collapsed very fast (<10 sec).

These differences in foam stability might arise from the differences in the type of functional groups in the three derivatized CDs: hydrophobic ($-CH_3$) for MβCD and TMβCD and hydrophilic ($-CH_2-CH(OH)-CH_3$) for HPβCD.

Considering the foam stability of the three CDs and the well-known broad chiral recognition spectra of TMβCD and HPβCD, only the last two CDs were used in foam fractionation experiments as chiral surface-active collectors.

7.2. Foam fractionation of chiral compounds

It is known that optimum enantiomeric separation of ionic compounds is occurring at a pH value around their dissociation constant, pK. It is also known that there is a certain optimum ratio concentration chiral selector/concentration selectand at which a base line separation is obtained. This ratio is practically determined by repeated measurements at different chiral selector/chiral selectand concentrations.

In this work, the whole experimental design (in terms of solution conditions and operating variables) was kept as simple as possible in order to determine the basic processes occurring in the system. No any kinds of additives (surfactants, organic or viscosity enhancers) were used. The reason for using simple aqueous solutions, without additives to enhance the foam stability, was the fact that these additives could alter the CD's enantioselectivity toward the chiral compounds. The experiments were conducted step wise, the reasoning and conditions for one experiment being chosen depending on the results from a previous one and the succession of experiments was not the same as presented in the following sections, where the results are grouped in function of the operating parameters that could influence the enantimeric enrichment.

Three glass columns, 20, 40 and 60 cm long, 1.2 cm i.d., were operated at room temperature (~ 24°C ± 2°C), at 5°C and at 55°C. The pH values of the initial solution were acidic or neutral. Measurements of samples' pH after the end of experiments showed that the pH was not changed during the foam fractionation and therefore no buffer solutions were used. Every pair CD/chiral compound was tested by foaming it first in a 20 cm long glass column at room temperature. If the obtained ee values were significantly different from ee of racemates (high chiral enantioselectivity), in order to obtain the maximum ee value, experiments with longer columns and under different conditions were further performed.

Foam samples, after their preparation, were analysed by GC. It was observed that for initial solution samples (prior foaming) there was always a deviation from the expected ee=0 (1:1 enatiomeric ratio) corresponding to a racemic mixture. Also, when experiments for the determination of the linear response range of ECD were carried out using standard solution, deviations from racemic ee=0 ranging from 0.075 to 1.9 (corresponding ER ranging from 1.002 to 1.040) for α-HCH and from 0.049 to 0.458 (corresponding ER ranging from 1.001 to 0.991) for DME were observed. Changing the chromatographic parameters (temperature, carrier gas flow or pressure) these deviations were not diminished. Such deviations from racemic ee=0 of racemates are often reported in literature [26, 12, 71, 72] and possible causes are described. Therefore, it was concluded that the standards purchased were racemates and apparent deviations from ee=0 were due to possible artefacts during the GC separation.

Before proceeding to a detailed discussion about the results obtained, some explanations about the terms and notations used have to be made. In GC, under the same operating conditions, the elution order of the enantiomers of a chiral compound separated with the same chiral column will be the same, irrespective to their concentration. Therefore, for the same compound:

- with c1, c2 will be denoted the concentration (calculated from peak areas) of the first eluted (in GC) enantiomer and second eluted enantiomer, respectively,
- with E_R1 and E_R2 will be denoted the enrichment (calculated from peak areas, cf. eq. 6.1) of the first eluted (in GC) enantiomer and second eluted enantiomer, respectively, (as two distinct compounds) for sample si (i=1, 2, 3,….)
- with s0 will be denoted initial solution sample (prior foaming),
- with si will be denoted foam samples (at different time interval),
- with sr will be denoted residual solution sample (after foaming),
- the term “enantiomeric enrichment” will refer to the enantiomeric excess ee (cf. eq. 5.6) for all samples.

7.2.1. Foam Fractionation of Diclorprop and Dichlorprop methyl ester with HPbCD

7.2.1.1. Preliminary experiments

Foam fractionation experiments started with dichlorprop (DCPP) and HPβCD as surface-active agent and chiral selector.

Solution with initial concentration ratio [HPβCD]/[DCPP](racemate): ***9.8 mM/22.2 ppm*** was foamed at room temperature using 20 cm long glass column. The pH of the solution was adjusted to the value 3 with HCl (pKa of DCPP is 3.1). Initial gas flow rate was 15 ml/min but had to be increased because no foam was produced. At a gas flow rate of 55 ml/min foam started to be produced and the flow rate was slowly increased to 100 ml/min in order to obtain a quasi-stable foam with a constant height at the top of glass column. After 25 min (time necessary for the foam to reach a quasi stable state), the gas flow rate was increased to force some foam out of the column. Three sample were collected one after the other. Then the gas flow rate was decreased until the foam withdrew back in the glass column and the foam height was controlled by adjusting the gas flow rate. The procedure was repeated until no foam was produced. In total 11 foam samples were collected (samples' volume between 2-3 ml). The results are depicted in Figure 7.3.

The first three foam samples were collected after 25 min, one after other. It can be observed that the enrichments (relative to the initial solution), as well as the ee values, are very low in all three samples. Samples s4, s5, s6 were collected after another 15 min and the enrichments increased but the ee values did not change significantly. The last samples were collected at 10 min interval (s7, s8 and s9, 10, s11) and a quite significant change in ee values can be observed.

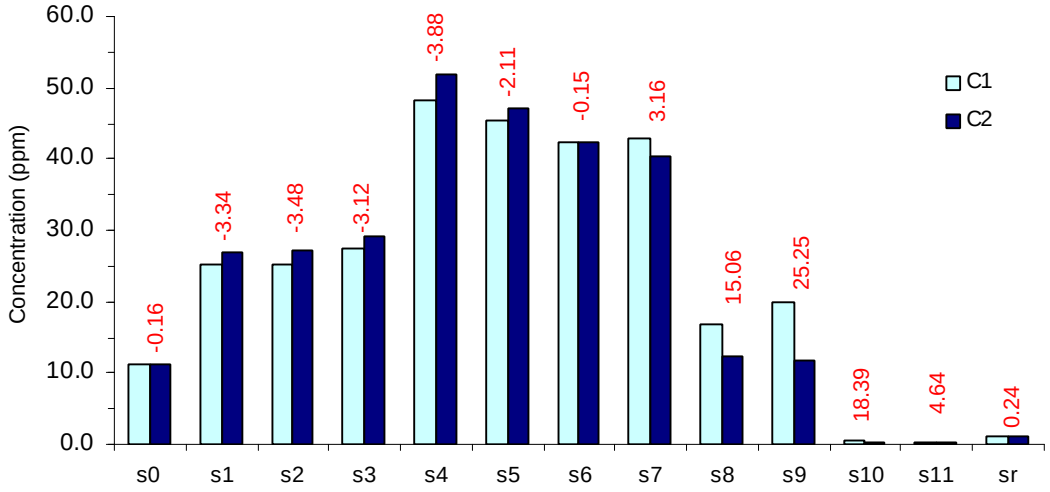


Figure 7.3. Foam fractionation HPβCD/DCPP (racemate) at initial concentration ratio **9.8 mM/22.2 ppm**. Numbers in red represent the ee for respective sample *Experimental conditions*: column 20 cm; pH 3; room temperature.

Table 7.1. Enrichment of dichlorprop in initial solution, foam samples and residual solution using HPβCD as chiral collector.

Sample	Initial solution: HPbCD 9.8 mM DCPP (racemate) 22.2 ppm			
	c1	c2	E_R1	E_R2
s0	11.10	11.10		
s1	25.24	26.90	2.27	2.42
s2	25.36	27.09	2.28	2.44
s3	27.51	29.18	2.48	2.63
s4	**48.17**	**51.89**	**4.34**	**4.67**
s5	45.38	47.18	4.09	4.25
s6	42.27	42.26	3.81	3.81
s7	43.03	40.26	3.88	3.63
s8	16.76	12.33	1.51	1.11
s9	19.95	11.87	1.80	1.07
s10	0.55	0.38	0.05	0.03
s11	0.35	0.32	0.03	0.03
sr	1.06	1.06		

Experimental condition: column 20 cm; pH 3; room temperature.
Note: all E_R are calculated relative to initial solution concentration

Some remarks and conclusion can be drawn:

1. It can be supposed that HPβCD and DCPP are both depleted in the bulk solution after every foam removal and it became clear that foam fractionation performances are improved for low concentrations of HPβCD and/or DCPP in the bulk solution (s4, s5, s6).

2. Although in the first samples ee values are very low (small deviations from racemic ee = 0), a certain enantioselectivity can be observed and the second enatiomer is preferentially enrich in the foam, leading to its higher depletion in the bulk solution comparative to the first enatiomer. Consequently, in the last foam sample the other enantiomer is dominant (the change in the sign of ee). Therefore, it can be supposed that there is a certain ratio (concentration HPβCD)/(concentration DCPP) in the bulk solution at which enantioselective enrichment occurs, and this can be determined only experimentally.

Next step was to decrease the concentration of HPβCD in the initial solution relative to that of DCPP, keeping the other experimental conditions identical. First, solution with initial concentrations ratios [HPβCD]/[DCPP](racemate): ***4.5 mM/22.2 ppm*** was foamed following the same procedure as previously described.

The foam had the same behaviour and the only difference was that the time necessary for the foam to reach a quasi-stable state was shorter (~ 15 min). No significantly differences in ee values and E_R were obtained (Figure 7.4 (a) and Table 7.2). When the concentration of DCPP was also decreased ([HPβCD]/[DCPP](racemate): ***4.9 mM/6 ppm)*** higher enrichment was obtained, ee values remaining in the same range (Figure 7.4 (b) and Table 7.2) and DCPP was completely removed from bulk solution after five samples.

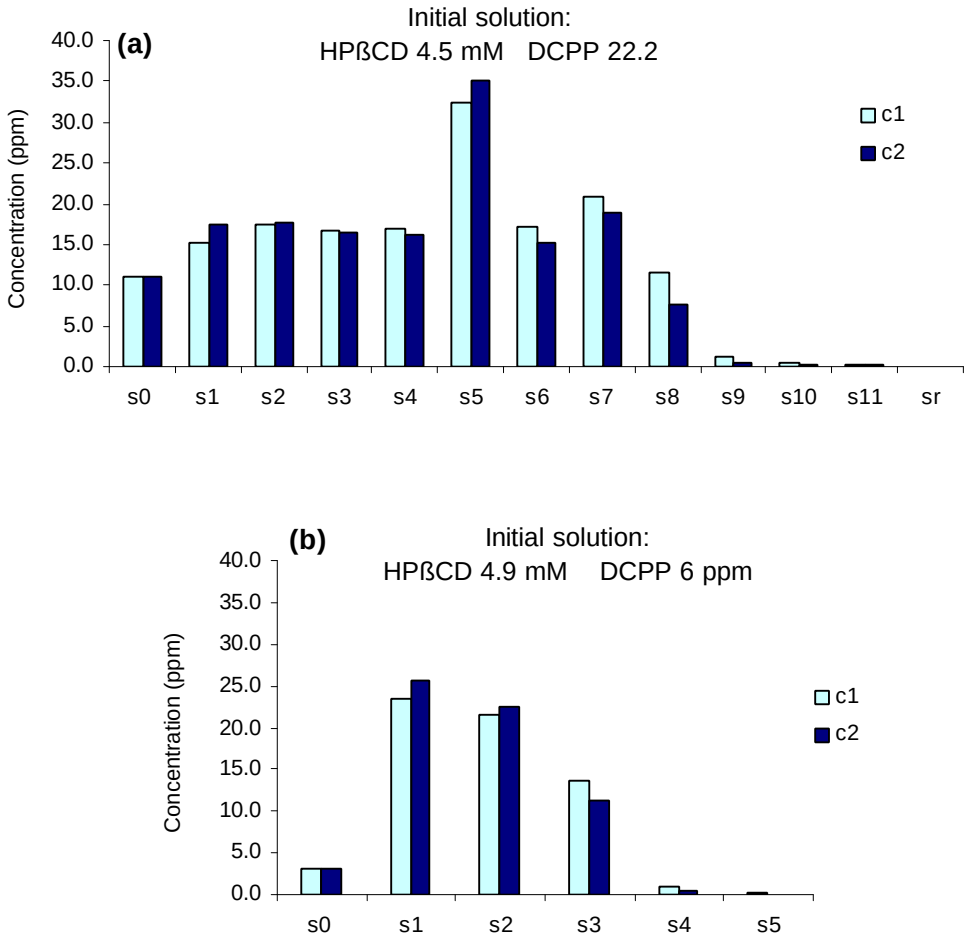


Figure 7.4. Foam fractionation of DCPP (racemate)/HPβCD at two different initial concentration ratios. *Experimental conditions*: column 20 cm; pH 3; room temperature.

Although HPβCD alone produced foam even at low concentration (1 mM; see Fig. 7.1), decreasing the concentration (< 4.5 mM) of HPβCD, in solution with DCPP, leaded to very unstable foam that could not be controlled adjusting the gas flow rate and no foam samples could be collected. It could be concluded that the surface activity of the complex (HPβCD-DCPP) is lower than that of HPβCD alone.

Table 7.2. Enrichment and enantiomeric excess of dichlorprop in initial solution, foam samples and residual solution for two different initial solution concentrations ratio.

Sample	Initial solution: HPbCD 4.5 mM DCPP (racemate) 22.2 ppm					Initial solution: HPbCD 4.9 mM DCPP (racemate) 6 ppm				
	c1	c2	E_R1	E_R2	ee	c1	c2	E_R1	E_R2	ee
s0	11.10	11.10			0.28	3.00	3.00			0.23
s1	15.33	17.52	1.38	1.58	-6.39	**23.37**	**25.60**	**7.91**	**8.53**	-3.29
s2	17.37	17.70	1.57	1.59	-0.66	21.62	22.50	7.31	7.50	-0.71
s3	16.66	16.44	1.50	1.48	0.93	13.62	11.14	4.61	3.71	11.29
s4	16.86	16.31	1.52	1.47	1.93	0.93	0.46	0.31	0.15	**34.52**
s5	**32.42**	**35.07**	**2.92**	**3.16**	-3.65	0.12	0.09	0.04	0.03	**19.14**
s6	17.22	15.30	1.55	1.38	6.17					
s7	20.79	18.79	1.87	1.69	5.34					
s8	11.57	7.52	1.04	0.68	**21.47**					
s9	1.32	0.52	0.12	0.05	**44.02**					
s10	0.38	0.23	0.03	0.02	**24.42**					
s11	0.23	0.20	0.02	0.02	6.93					
sr	0.07	0.10			-15.58					

Experimental condition: column 20 cm; pH 3; room temperature.
Note: all E_R are calculated relative to initial solution concentration

In all three experiments, enrichment and “enantiomeric enrichment” (enantiomeric excess ee) of DCPP did not correlate. A higher E_R was obtained at low concentration of DCPP and HPβCD in bulk solution with low ee values for these samples. Enantiomeric excess in foam samples increased only in the last foam samples (which have very low concentrations) and is due to a depletion of one of the enantiomers in the bulk solution and not due to a change in inversion of CD’s enantioselectivity.

Some attempts to use a longer glass column (40 cm) were done, but no foam could be collected.

In order to compare the enantioselectivity of HPβCD toward the ionic and neutral form of the same compound, dichlorprop methyl ester (DME) was used in ratio[HPβCD]/[DME](racemate): ***9.8 mM/6 ppm***. All the experimental conditions were kept the same as for DCPP/HPβCD (glass column 20 cm long; solution pH 3; room temperature) and the foaming fractionation was done in the same way. Results are shown in Table 7.3. Both E_R and ee values do not differ significantly from those obtained by foaming the HPβCD/DCPP solution.

Table 7.3. Enrichment and enantiomeric excess of dichlorprop methyl ester in initial solution, foam samples and residual solution using HPβCD as chiral collector.

Sample	Initial solution: HPbCD 9.8 mM/DME (racemate) 6 ppm				
	c1	**c2**	**E_R1**	**E_R2**	**ee**
s0	3.00	3.00			**0.47**
s1	4.67	5.08	1.56	1.69	**-3.65**
s2	5.66	6.32	1.89	2.11	**-5.08**
s3	6.47	6.84	2.16	2.28	**-2.28**
s4	5.77	5.99	1.92	2.00	**-1.44**
s5	4.85	4.80	1.62	1.60	**1.01**
s6	**6.90**	**6.79**	**2.30**	**2.26**	**1.32**
S7	1.21	0.81	0.40	0.27	**20.14**
S8	0.53	0.47			**6.67**

Experimental condition: column 20 cm; pH 3; room temperature.
Note: all E_R are calculated relative to initial solution concentration

In all experiments, previously described, foam behaviour was quite the same:

1. wet foam with large bubbles,
2. coalescence phenomenon manifesting in the upper part of foam column leading to alternate phases of foam formation followed by its collapse and, in consequence,
3. the necessity of a strict gas flow control in order to maintain a certain height of the foam,
4. all the collected foam samples collapsed immediately (practically the foam exiting the glass column reached the collector vessel in liquid state),

From this foam behaviour and from the low values obtained for E_R and ee it was concluded that: (1) HPβCD is not the proper chiral selector for DCPP and DME; (2) the surface activity of both complexes HPβCD-DCPP and HPβCD-DME is too low to produce, at least, a quasi stable foam, and, in consequence, (3) the reproducibility of the results is very low.

Therefore, taking also into account that it was difficult to obtain foam with a height of 20 cm (the height of the glass column used) it was useless to continue the foaming experiments with the longer columns or to try with other initial concentration ratios.

7.2.2. Foam Fractionation of Diclorprop methyl ester with TMbCD

7.2.2.1. Preliminary experiments

Foam fractionation experiments of dichlorprop methyl ester (DME) with TMβCD, as chiral collector, started in the same way as for DCPP and HPβCD.

Solutions with initial concentration ratios *[TMbCD]/[DME]*(racemate)*:* ***9.8 mM/6 ppm*** and ***4.9 mM/6 ppm*** were foamed at room temperature.

Solutions' pH was adjusted to the value 3 with HCl and 20 cm long glass column was used. Initial flow rate was adjusted to a minimum value to obtain foam and then slowly increased until the foam reached a constant height at the top of glass column. First sample was collected after 15 min (time necessary for the foam to reach a stable state) and all the other samples were collected at 5 min interval (samples' volume 1-3 ml, all in liquid state). Between consecutive foam sampling the gas flow rate was decreased until the foam withdrew in the glass column and the foam height was controlled adjusting the gas flow rate. The procedure was repeated until no foam was produced. The results are depicted in Figure 7.5 and Table 7.4.

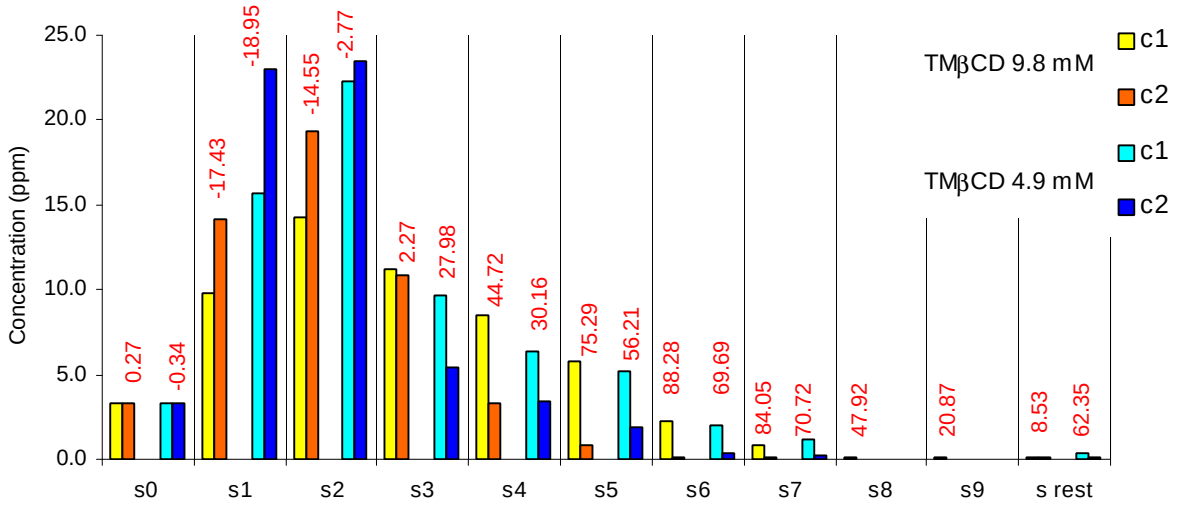


Figure 7.5. Foam fractionation of DME (racemate) **6 ppm** at two different initial concentration of TMβCD. Numbers in red represent ee values for the respective sample. *Experimental conditions*: column 20 cm; pH 3; room temperature.

Table 7.4. Enrichment of dichlorprop methyl ester in foam samples for two different initial concentration of TMβCD.

Sample	Initial solution: TMbCD 9.8 mM DME (racemate) 6 ppm				Initial solution: TMbCD 4.9 mM DME (racemate) 6 ppm			
	c1	c2	E_R1	E_R2	c1	c2	E_R1	E_R2
s0	3.30	3.30			3.30	3.30		
s1	9.83	14.10	2.98	4.27	15.67	23.00	4.75	6.97
s2	14.32	19.35	**4.34**	**5.86**	22.24	23.51	**6.74**	**7.13**
s3	11.20	10.79	3.40	3.27	9.71	5.46	2.94	1.66
s4	8.46	3.26	2.56	0.99	6.33	3.40	1.92	1.03
s5	5.78	0.82	1.75	0.25	5.23	1.92	1.58	0.58
s6	2.21	0.14	0.67	0.04	2.04	0.36	0.62	0.11
s7	0.79	0.07	0.24	0.02	1.14	0.20	0.35	0.06
s8	0.14	0.05	0.04	0.02				
s9	0.09	0.06	0.03	0.02				
s rest	0.07	0.06			0.40	0.09		

Experimental conditions: column 20 cm; pH 3; room temperature.

Note: all E_R values are calculated relative to the initial solution.

It was evident that enantiomeric enrichment of DME is possible using TMβCD as chiral collector (ee values in first foam samples (-17.43, -18.95) differ significantly from ee of the racemic value in initial solution). The complex TMβCD- DME produced quasi-stable foam that could be easily controlled adjusting the gas flow rate. In consequence, a relative high enrichment of enantiomers could be obtained. At lower initial concentration of TMβCD (4.9 mM) the foam became more stable and both E_R and ee are higher. But the 20 cm glass column is too short to allow a complete development of the foam with total reflux.

Therefore, further experiments were done at lower initial concentrations of the chiral collector using longer glass columns (40 and 60 cm) and different experimental conditions (DME initial concentrations, temperature, pH).

7.2.2.2. Influence of foam height on E_R and ee

In order to find out if there is an enhancement in enantiomeric enrichment with an increase of foam height two columns with ***40 and 60 cm*** length were used. Knowing from previous experiments that a higher enrichment and enantiomeric excess could be obtained for lower initial concentrations of TMβCD relative to DME concentrations, solutions with two initial concentrations ratios *[TMbCD]/[DME]* (racemate) were used: ***(0.5 mM/30 ppm)*** and ***(0.1 mM/6** ppm)*. The solutions (pH 3) were foamed at room temperature. After a couple of attempts to control the foam height after every foam sample removal, it became clear that it was not possible to keep the foam at the same height only by increasing/decreasing the gas flow rate. Therefore, after every foam sample removal, the gas flow was cut off and the remaining foam in the column was allowed to collapse completely and then foamed again (as described in 6.3 – Foaming procedure). Foam samples were collected at 10 min after beginning of foaming (time necessary for the foam to reach a stable state) (samples' volume 1-3 ml, which means that only the upper part of foam was collected).

As shown in Figure 7.6 and Table 7.5, the enrichment increased as foam height increases for both initial concentrations ratios [TMβCD]/[DME] (racemate) used. This is due to an increase in the complex (TMβCD-DME) concentration in the foam for greater foam height. The increased complex concentration in the foam is a result of longer drainage time and increased coalescence. Visual observation indicated that foam was more stable for the longer column and coalescence was predominant at the top of the foam columns. The complex lost from the films, as a result of coalescence, drains back through the rising foam and concentrate in the interstitial liquid leading to drier foam in the upper part of foam column and in consequence, promoting the enrichment.

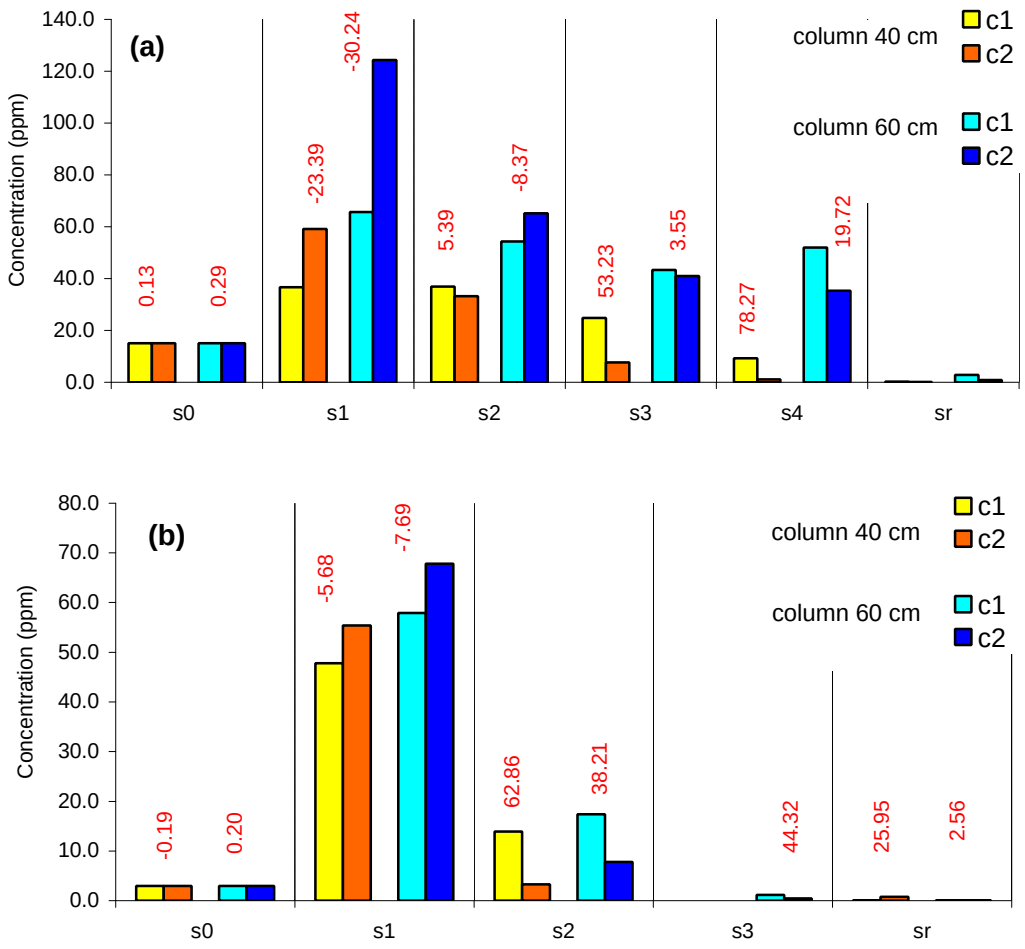


Figure 7.6. Effect of foam height on the enantiomeric enrichment of racemic DME using TMβCD as chiral collector. Numbers in red represent ee values for the respective sample.
Initial concentration ratios [TMbCD]/[DME](racemic): **(a)** 0.5 mM/30 ppm**; (b)** 0.1 mM/6ppm.
Experimental conditions: pH 3; room temperature

Enantiomeric enrichment (enantiomeric excess), for both initial concentration ratios, is also enhanced for higher foam height. This could result from a difference in surface adsorption of TMβCD when associated with one of the enantiomers, from a more effective enantioseparation occurring in drier foam (drier foam on the top of column for longer column), or a combined affect of both.

The change in the sign of ee for the last foam samples result from the different depletion in the bulk solution of the enantiomers. The higher ee values for the last foam samples and residual solution (samples with very low concentrations) result from the numerical definition of ee (ee =$[(A_1-A_2)/(A_1+A_2)]*100\%$), which represent the *relative* abundance of one enantiomer over the other. Lower A_1 and A_2 values will result in higher ee values.

Table 7.5. Effect of foam height on enrichment of DME.

Sample	Column 40 cm				Column 60 cm			
	Initial solution: [TMβCD] / [DME] (racemate): 0.5 mM / 30 ppm							
	c1	**c2**	**E_R1**	**E_R2**	**c1**	**c2**	**E_R1**	**E_R2**
s0	15.00	15.00			15.00	15.00		
s1	36.59	59.08	**2.44**	**3.94**	65.63	124.24	**4.38**	**8.28**
sr	0.18	0.04			2.80	0.82		
	Initial solution: [TMβCD] / [DME] (racemate): 0.1 mM / 6 ppm							
	c1	**c2**	**E_R1**	**E_R2**	**c1**	**c2**	**E_R1**	**E_R2**
s0	3.00	3.00			3.00	3.00		
s1	47.78	55.36	**15.93**	**18.45**	57.87	67.79	**19.29**	**22.60**
sr	0.07	0.04			0.11	0.10		

Experimental conditions: pH 3; room temperature

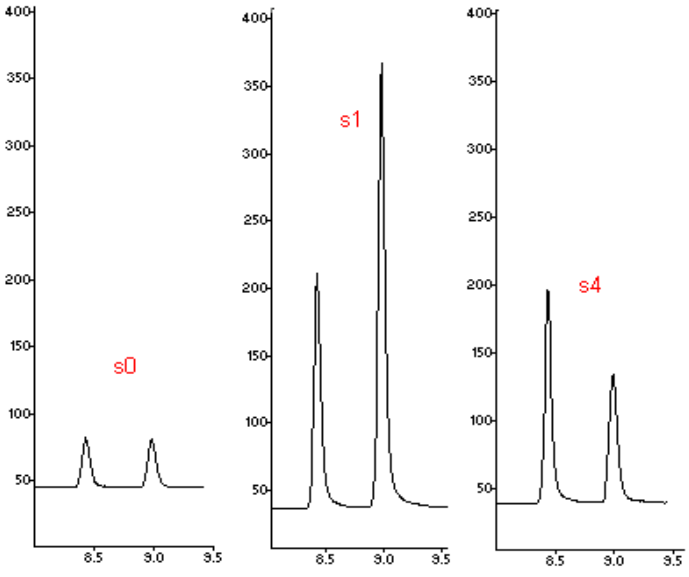


Figure 7.7. Representative GC chromatograms showing the change in ee sign during consecutive foaming of DME using TMβ**CD (0.5 mM)** as chiral collector.
(see Fig. 7.6 (a) for ee values and experimental conditions)

7.2.2.3. *Influence of initial solution concentration ratio on E_R and ee*

On the basis of the results achieved from previous experiments (Fig. 7.6) it could be expected that initial concentrations of both DME and TMβCD significantly influence enantiomeric excess. To gain further insight into the effect of initial concentration ratio [TMβCD]/[DME], two series of experiments were carried out at two initial concentration of TMβCD and different concentrations of DME, using 60 cm long column.

First, for ***0.5 mM*** concentration of TMβCD four concentrations of DME were choosen: ***6, 15, 30, and 60 ppm*** and the solutions were foamed in the same way as previously described (allowing the foam to complete collapse between foam sampling; solutions' pH 3; room temperature). All foam samples were collected at 10 min after the begging of foaming and care was taken that the samples' volume to be approximately the same (between 1-2 ml). Results are depicted in Figure 7.8 and Table 7.6.

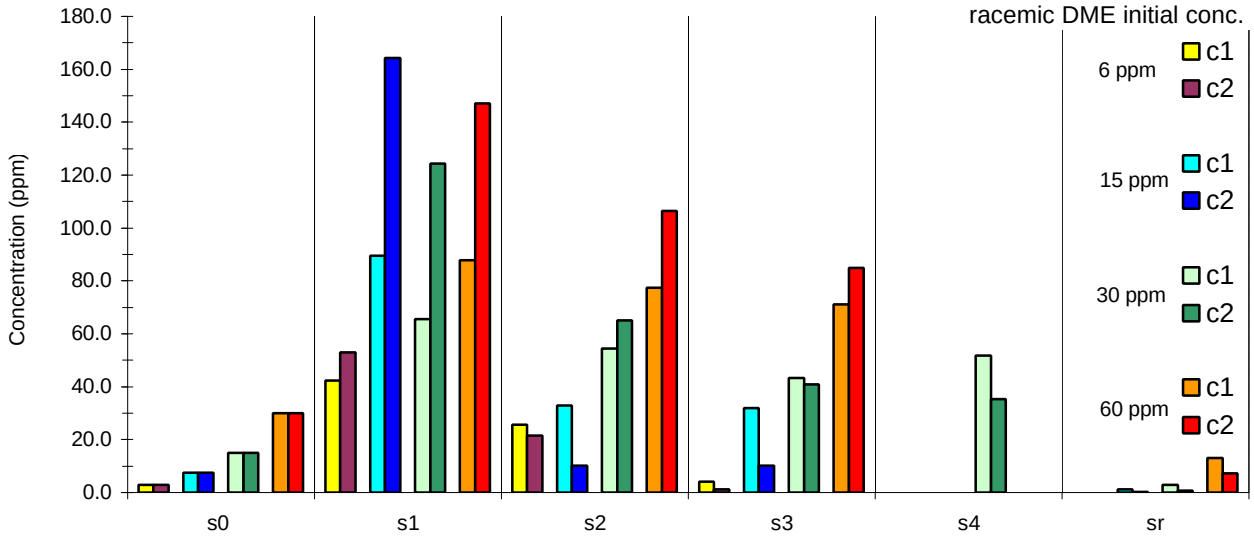


Figure 7.8. Effect of initial concentration of DME (racemic) on the enantiomeric enrichment using TMβCD (**0.5 mM** concentration) as chiral collector.
Experimental conditions: column 60 cm long; pH 3; room temperature, 10 min foaming time before every foam sample removal.

Table 7.6. Enrichment and ee for DME (racemate) as function of initial concentration using TMβCD (**0.5 mM** concentration).

Sample	Initial DME (racemate) concentration:											
	6 ppm			15 ppm			30 ppm			60 ppm		
	E_R1	E_R2	ee	E_R1	E_R2	ee	E_R1	E_R2	ee	E_R1	E_R2	ee
s0			-0.07			0.16			0.29			-0.27
s1	14.12	17.65	-10.37	11.94	21.91	-28.01	4.38	8.28	-30.24	2.92	4.90	-25.53
s2			9.63			54.02			-8.37			-15.99
s3			52.85			53.15			3.55			-9.04
s4			5.88						19.72			
sr			-0.16			73.97			55.28			27.72

Experimental conditions: column 60 cm long; pH 3; room temperature;
10 min foaming time before every foam sample removal.

The increase in DME (racemate) concentration in the initial solution leaded to a significant increase in the ee values and decrease of enrichment. Two main processes concur to give rise to these trends.

Firstly, enantiomeric separation involves formation of a pair of reversible diastereomeric complexes TMβCD - DME. It can be supposed that the stoichiometry of diastereomeric complexes is the same regardless of the DME or TMβCD concentrations. The differences in the stability between these diastereomeric complexes lead to a different enantioselectivity and the enantiomer that forms the less stable complex will be shorter time retained as complex with TMβCD.

Secondly, foam fractionation can be seen as a chromatographic method in which enatiomeric separation occurs in liquid state. During foaming, it can be supposed that only free TMβCD and complexes TMβCD–DME are adsorbed onto the bubbles surface (DME alone do not posses surface activity). As a result of the natural

drainage and coalescence, the bubbles surfaces are already saturated with these two species after a short rising height. Therefore, there are five species in the interstitial liquid which flow down and permanently interact: the two complexes TMβCD–each DME enantiomers, free TMβCD, and the two free DME enantiomers.

Visual observation indicated that coalescence was predominant at the top of the foam column. The species lost from the films, as a result of coalescence, drain back through the rising foam and concentrate in the interstitial liquid leading to very dry foam in the upper part of foam column. In consequence, at a certain time, the enantiomer that forms less stable complex with TMβCD will, preferentially, be free to drain back through the rising foam and concentrate in the interstitial liquid and, in consequence, the removed upper part of the foam will be enriched predominantly with the strongly bonded enantiomer resulting in enantiomeric separation.

Foam stability increased as DME concentration decreased. This could be a result of the fact that, for all DME concentrations, there was an excess (molar ratio) of TMβCD over the DME and this excess decreases as DME concentration increase. In consequence, higher amount of free TMβCD would contribute to more stable foam.

On the other hand, higher concentration of DME will provide for a higher amount of DME to be refluxed and, consequently, for more reversible complexes with TMβCD to be formed.

These two phenomena together lead to an increase in enantiomeric enrichment as the concentration of DME increases relative to that of TMβCD

The sign of ee in the first foam sample is the same for all initial concentrations of DME. For low concentrations of DME (6 and 15 ppm), the sign of ee changes already in the second foam sample while for high concentrations of DME (30 and 60 ppm) ee keeps the same sign even for the third and fourth samples (in the case of 60 pmm), which means that enantiomeric separation continue to occur.

This can be explained as following:
With every foam sample removal a certain amount of TMβCD and DME enantiomers is removed from bulk solution and, in consequence, less amounts of the two enantiomers and TMβCD will be available for the next foaming sequence. Due to the fact that, between every foam removal, the foam was allowed to completely collapse, every consecutive foaming of residual solutions can be seen as an independent foaming process, but with lower initial concentrations of TMβCD and DME. Consequently, in the case of low concentration of DME in the beginning of experiment, there is not enough DME left in the residual solution for the enantioselective process to occur and in the next foaming and therefore, the enantiomer that was less enriched in the first foam will be dominant. For higher initial concentrations, the amount of DME left in the residual solution is enough to further allow the enantioselective enrichment to occur.

Evidently, enrichment of both enantiomers in the first foam sample decreases with increase in initial concentration of DME. This results from the decreasing in foam stability with increasing of DME concentration. The coalescence and drainage is more rapid in a less stable foam leading to more dry foam at the top of foam height and promoting the enrichment. On the other hand, enrichment is defined as relative to the initial solution concentration and therefore, for low values of initial concentrations E_R will be higher.

In order to prove the above statements regarding processes which could lead to enantiomeric enrichment, a second set of foaming experiments were carried out, keeping the experimental conditions unchanged and decreasing the concentration of TMβCD to ***0.1 mM***. Two concentrations of DME were chosen: ***6ppm*** and ***30 ppm*** (for which lowest and higher ee values were obtained) and the solutions were foamed in the same way as previously described. The results are shown in Figure 7.9 and Table 7.7.

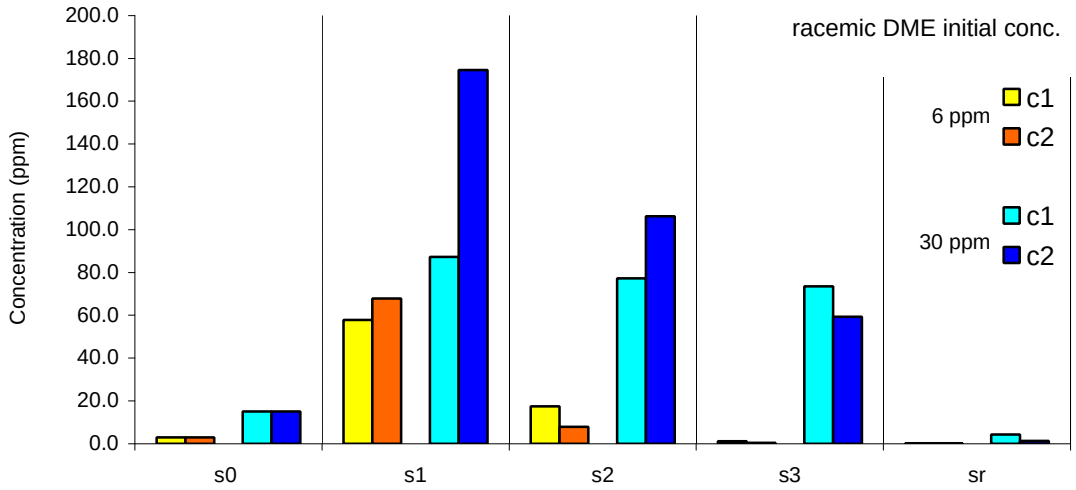


Figure 7.9. Effect of initial concentration of DME (racemic) on the enantiomeric enrichment using TMβCD (**0.1 mM** concentration) as chiral collector. *Experimental conditions*: column 60 cm long; pH 3; room temperature, 10 min foaming time before every foam sample removal.

Table 7.7. Enrichment and ee of DME as function of initial concentration (racemate) using TMβCD (**0.1 mM** concentration).

Sample	Initial DME (racemate) concentration:					
	6 ppm			30 ppm		
	E_R1	E_R2	ee	E_R1	E_R2	ee
s0			0.20			0.42
s1	19.30	22.60	-7.69	5.82	11.64	-31.99
s2			38.21			-14.31
s3			44.32			12.25
sr			2.56			54.35

Experimental conditions: as described in Fig. 7.9.

The values for E_R and ee are in the same range as for 0.5 mM initial concentration of TMβCD and, although no significant improvement was obtained, the trend was the same: there is an increase in enantiomeric enrichment (ee) for higher initial concentrations of DME correlated with a decrease in enrichment (E_R).

These results, correlated with the fact that higher enantiomeric enrichment was obtain for longer foam column (7.2.3.2), lead to the conclusion that enantiomeric enrichment is enhanced for higher concentration of DME relative to TMβCD concentration and the separation of enantiomers occurs mainly in the upper part of foam column where the foam is drier.

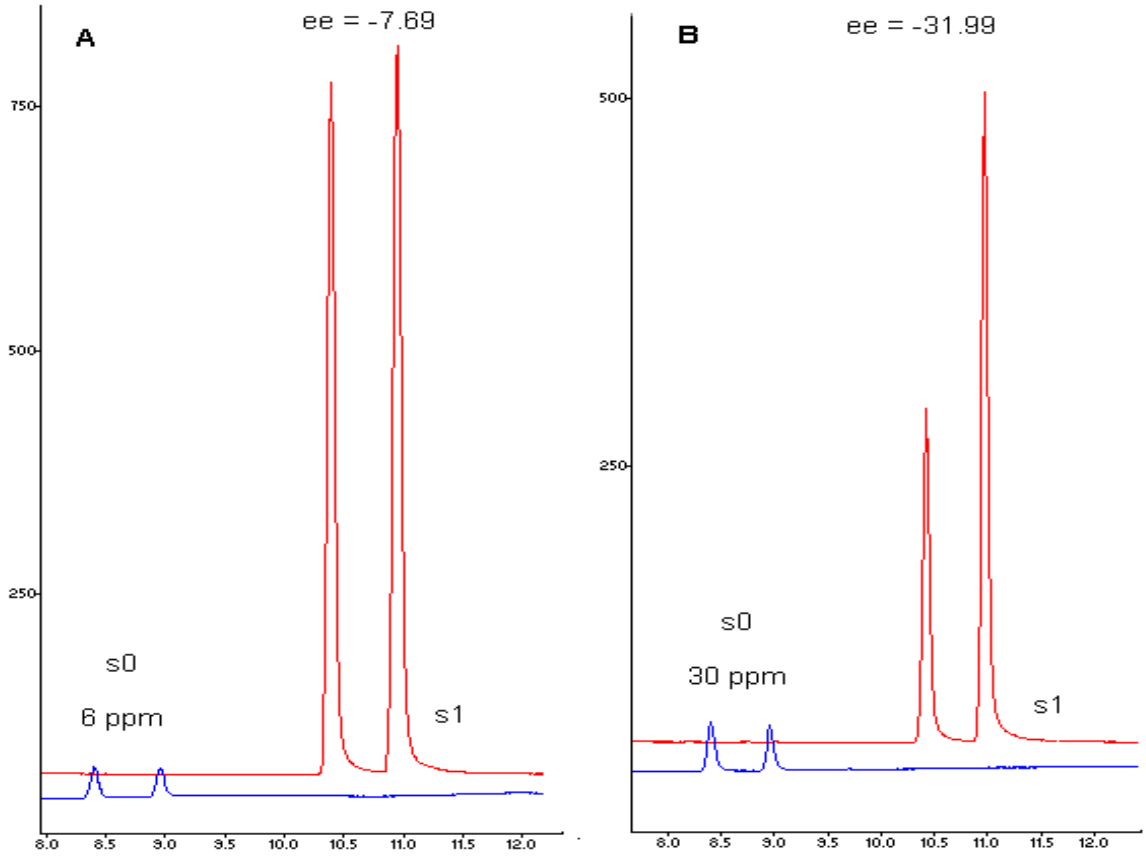


Figure 7.10. GC representative chromatograms showing the increase in ee values with increase in DME initial concentration. TMβCD (initial concentration 0.1 mM) was used as chiral collector. *Experimental conditions*: as described in Fig. 7.9.

Note: samples represented in **B** have different dilutions comparative to those in **A** (1:10).

7.2.2.4. Influence of temperature on E_R and ee

Temperature is an important operating variable for the cases where the foam stability is different at different temperatures. Temperature can also strongly influence the complex stability between the enantiomers and the chiral selector.

In order to see if there is a dependence of enantiomeric enrichment on the temperature, foaming experiments were carried out at temperatures higher and lower than room temperature (~ 24°C) as follow:

- Solution with initial concentration ratio [TMβCD]/[DME] = **[0.5 mM]/[30 ppm]** was foamed at 5°C using 40 cm long glass column;
- Solution with initial concentration ratio [TMβCD]/[DME] = **[0.5 mM]/[15 ppm]** was foamed at 55°C using 60 cm long glass column.

Foaming procedure was as described in section 6.3.2. In the case of foaming at 5°C the first foam sample collected at 15 min after the foam reached the top of glass column (the time necessary for the foam to reach a quasi-stable state).

As it was expected, the increased solution viscosity at low temperature (5°C) leaded to a slow drainage between bubbles resulting in more stable foam, with the same wetness along the column height. Consequently, the enantiomer that form less stable complex with TMβCD does not drain back to the bulk solution as fast as in the case of foaming the same solution at room temperature (when a fast drainage occurred).

On the other hand, it can be supposed that the complex stability between DME and TMβCD was affected by the lower temperature, but no any kind of affirmation, whether there is an increase or decrease in complex stability, can be advanced.

As the enantiomeric enrichment, in the conditions of foam fractionation, is the result of interdependent enantioselective and adsorptive processes, the lower ee value obtained at 5°C (Figure 7.11 (a)) is a combined effect of both processes.

At high temperature (55°C), there was an evident deterioration of foam properties, which was very unstable, with a very fast drainage and coalescence occurring all along the foam height. As result, the foam started to collapse very fast and a high gas flow rate was necessary to maintain the foam at the top of column. The interstitial liquid was practically pushed up in the column, affecting the natural drainage. In this case, due to a very low foam stability, the conditions for the enantiomeric enrichment to occur are not attained and, as the foam samples contained a high amount of interstitial liquid, the ee values, as well as E_R, were low (Figure 7.11 (b) and Table 7.8)

Table 7.8. Temperature influence on enrichment of DME using TMβCD (**0.5 mM**) under different foaming conditions.

Sample	DME (racemic) initial concentration: 30 ppm; column 40 cm long							
	Room temperature (24°C)				5°C			
	c1	c2	E_R1	E_R2	c1	c2	E_R1	E_R2
s0	15.00	15.00			15.00	15.00		
s1	36.59	59.08	**2.44**	**3.94**	39.32	49.99	**2.62**	**3.33**
	DME (racemic) initial concentration: 15 ppm; column 60 cm long							
	Room temperature (24°C)				55°C			
	c1	c2	E_R1	E_R2	c1	c2	E_R1	E_R2
s0	7.50	7.50			7.50	7.50		
s1	89.57	164.35	**11.94**	**21.91**	21.54	26.37	**2.87**	**3.52**

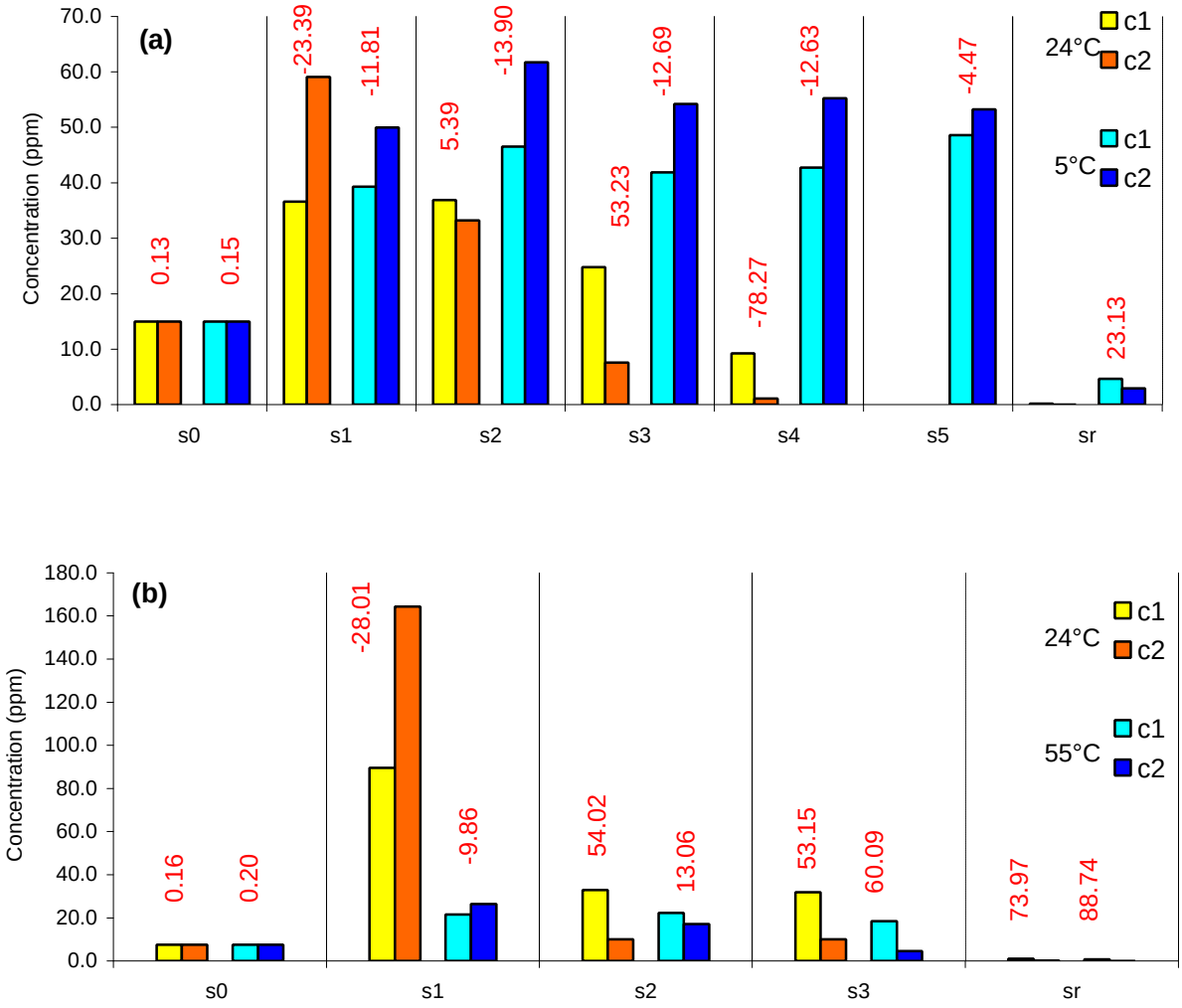


Figure 7.11. Effect of temperature on the enantiomeric enrichment using TMβCD as chiral collector. Numbers in red represent the ee value for the respective sample.

(a): *Initial concentration ratio* [TMβCD] / [DME] (racemate): **0.5 mM / 30 ppm;** *Experimental conditions*: column **40 cm** long; pH 3;

(b): *Initial concentration ratio* [TMβCD] / [DME] (racemate): **0.5 mM / 15 ppm;** *Experimental conditions*: column **60 cm** long; pH 3.

7.2.2.5. Influence of foaming time on E_R and ee

During the experiments it was observed that the time necessary for the foam to reach a quasi-stable state was depended on the chiral collector / chiral compound pair and its initial concentration, column length, and temperature. In the case of TMβCD/DME foamed at room temperature, the necessary time for the foam to stabilize after it reached the top of glass column was of, approximately, 10 min for all initial concentration ratios used. Therefore, the foaming time (the time between the moment when the foam reached the top of glass column and the moment of foam sample removal) was 10 min. When the foaming experiments were carried out at 5°C, the necessary time for the foam to stabilize, due to the increased solution viscosity, was of, about, 15 min.

To find out if a changing in the foaming time will result in a changing in enantiomeric excess, solutions with initial concentration ratio [TMβCD]/[DME] that gave the highest ee value (0.5 mM /30 ppm) were foamed as following:

- At 5°C, using 40 cm long glass column, and allowing the foam to stabilize for 30 min (comparative to 15 min in a previous experiment),
- At room temperature, using 60 cm long glass column, and allowing the foam to stabilize for 20 min (comparative to 10 min in a previous experiment).

Solutions were foamed as described in section 6.3.2. Care was taken that sample's volume did not exceed 2 ml.

By extending the foaming time, the foam did not reach a steady state: a first phase of foam volume growing was followed by a quasi-stable phase, in which the foam equilibrated (15 min, at 5°C, and 10 min, at room temperature, respectively) under the reflux conditions (collapse and drainage) and having a constant height, and then

the foam started to collapse. To maintain the foam at the top of column, the gas flow rate had to be increased, which leaded to less dry foam.

Therefore, after the foam has equilibrated for a period of time (15 min, at 5°C, and 10 min, at room temperature, respectively) there was no benefit (no further enantiomeric enrichments) in extending the foaming time, as shown in Figure 7.12.

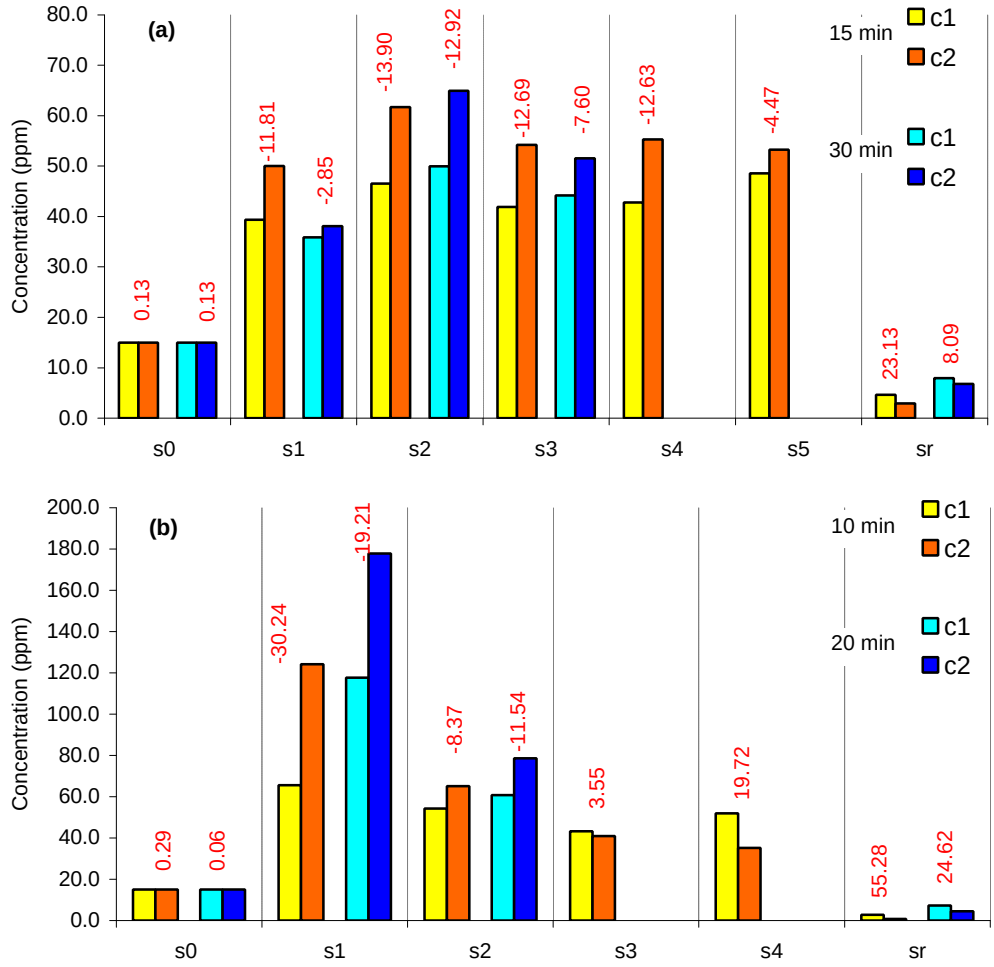


Figure 7.12. Effect of foaming time on the enantiomeric enrichment of DME using TMβCD as chiral collector. Numbers in red represent the ee value for the respective sample. *Initial concentration ratio* [TMβCD] / [DME] (racemate): **0.5 mM / 30 ppm;**

(a): *Experimental conditions*: column 40 cm long, temperature: 5°C, pH 3;

(b): *Experimental conditions*: column 60 cm long, room temperature (~24°C), pH 3.

7.2.2.6. Influence of pH on E_R and ee

Another factor that could affect the enantiomeric enrichment is the pH of the initial solution. Therefore, solution with initial concentration ratio [TMβCD]/[DME] 0.5 mM/15 ppm and neutral pH 6 (no HCl addition) was foamed at room temperature using 60 cm long column. Foaming procedure was as described in section 6.3.2 and the foaming time before the samples removal was 10 min.

Figure 7.13 shows that DME is more enantiomeric enrich at pH 3 that at neutral pH. The foam produced at pH 3 had almost the same characteristics with the one produced at neutral pH and therefore, it can be suppose that the difference in ee values are mainly due to a change in the nature of enantioselective complexation of DME with TMβCD.

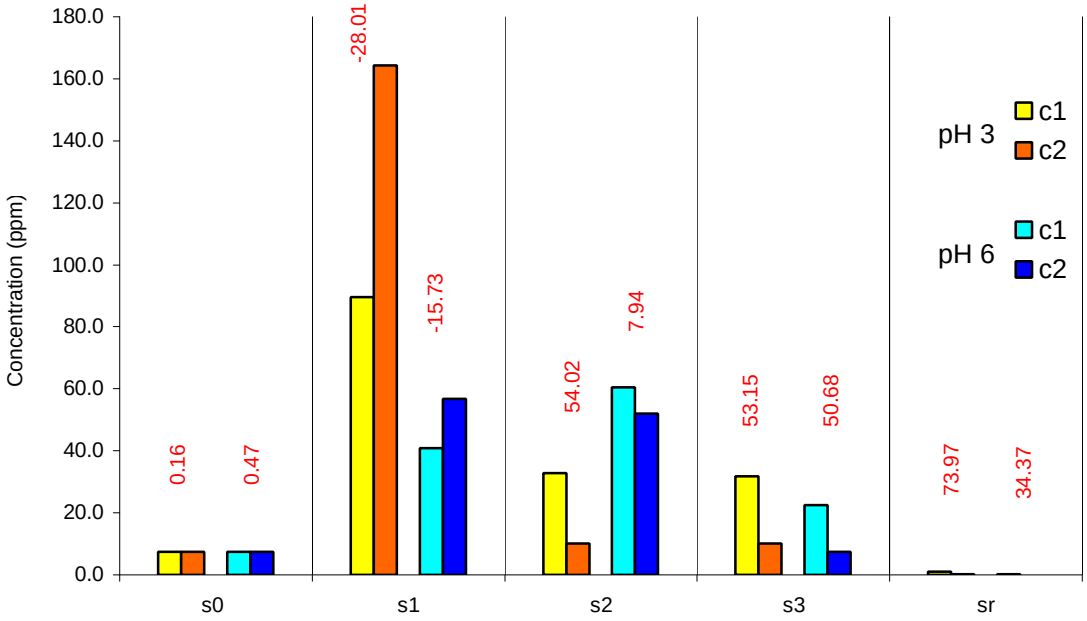


Figure 7.13. Effect of pH on the enantiomeric enrichment of DME using TMβCD as chiral collector. Numbers in red represent the ee value for the respective sample.
Initial concentration ratio [TMβCD] / [DME] (racemate): **0.5 mM / 15 ppm;**
Experimental conditions: column 60 cm long, room temperature

7.2.3. Foam Fractionation of a-HCH with HPbCD

7.2.3.1. Preliminary experiments

Foam fractionation experiments of α-HCH with HPβCD, as chiral collector, were conducted in the same way as for DCPP and DME.

Solutions, with concentration ratios [HPβCD]/[α-HCH]: **1 mM/5 ppm**, were prepared by direct adding of HPβCD to 50 ml α-HCH solution 5 ppm. The pH of the solution was adjusted to the value 3 with HCl and foaming experiments were carried out at room temperature using 20 cm and 40 cm long glass columns. Initial flow rate was 50 ml/min but had to be increased because no foam was produced. Foam started to be produced at 80 ml/min gas flow rate and the flow rate was slowly increased to 110 ml/min in order to obtain a quasi-stable foam with a constant height at the top of glass column. After 20 min (time necessary for the foam to reach a quasi stable state), the gas flow rate was increased to force some foam out of the column. After every foam removal (samples' volume between 1-3 ml) the gas flow rate was decreased until the foam withdrew back in the glass column and the foam height was controlled adjusting the gas flow rate. The procedure was repeated until no foam was produced. The results are depicted in Figure 7.14 and Table 7.9.

The stability of foam was very low, the bubbles coalesced all along the foam height and a strict gas flow control was necessary in order to maintain a certain height of the foam. The collected foam samples collapsed immediately (practically the foam exiting the glass column reached the collector vessel in liquid state) and contained high amount of interstitial liquid resulting in low values for E_R and ee.

It was concluded that: (1) HPβCD is not the proper chiral selector for α-HCH and (2) the surface activity of the complex HPβCD - α-HCH is too low to produce, at least, a quasi stable foam. Therefore no further studies were done.

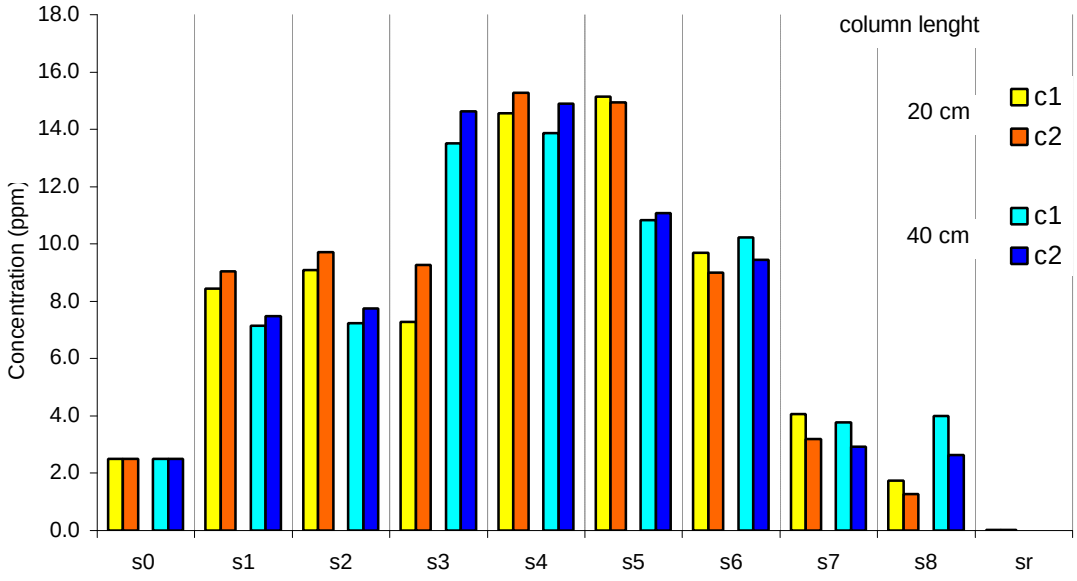


Figure 7.14. Foam fractionation of α-HCH using HPβCD as chiral collector.
Initial concentration ratio [HPbCD]/[a-HCH] (racemate): **1 mM/5 ppm**
Experimental conditions: columns 20 cm and 40 cm long; pH 3; toom temperature.

Table 7.9. Enrichment and enantiomeric excess of α-HCH in initial solution, foam samples and residual solution using HPβCD as chiral collector at initial concentration ratio *[HPbCD]/[a-HCH]* (racemate): 1 mM/5 ppm.

Sample	Column height: 20 cm					Column height: 40 cm				
	c1	c2	E_R1	E_R2	ee	c1	c2	E_R1	E_R2	ee
s0	2.50	2.50			0.47	2.50	2.50			0.84
s1	8.44	9.03	3.38	3.61	-2.91	7.14	7.49	2.85	2.99	-1.56
s2	9.10	9.71	3.64	3.88	-2.79	7.24	7.75	2.90	3.10	-2.55
s3	7.27	9.27	2.91	3.71	-11.60	13.52	14.64	5.41	5.86	-3.13
s4	14.56	15.28	5.82	6.11	-1.96	13.87	14.91	5.55	5.96	-2.77
s5	15.14	14.94	6.06	5.98	1.14	10.84	11.09	4.34	4.43	-0.29
s6	9.69	8.99	3.88	3.60	4.22	10.22	9.45	4.09	3.78	4.77
s7	4.07	3.20	1.63	1.28	12.51	3.78	2.92	1.51	1.17	13.68
s8	1.75	1.26	0.70	0.51	16.56	3.99	2.64	1.60	1.06	21.11
sr	0.02	0.01			17.60	0.00	0.00			0.00

Note: all E_R are calculated relative to initial solution concentration

7.2.4. Foam Fractionation of a-HCH with TMbCD

7.2.4.1. Preliminary experiments

Solutions, with initial concentration ratios *[TMbCD]/[a-HCH]:* ***0.1 mM / 5 ppm***, and ***1 mM / 5 ppm,*** were prepared by direct adding TMβCD to 50 ml α-HCH solution 5 ppm. The pH of the solution was adjusted to the value 3 with HCl and foaming experiments were carried out at room temperature using 20 cm long glass column. Initial flow rate was adjusted to a minimum value (100 ml/min) at which the foam started to be produced and then slowly increased until the foam reached a constant height at the top of glass column.

Foaming time before first foam sample removal was 20 min for 0.1 mM TMβCD initial concentration and 10 min for 1 mM TMβCD initial concentration, (time necessary for the foam to reach a stable state). All other foam samples were collected at 10 min foaming time. Between consecutive foam sampling the gas flow rate was decreased until the foam withdrew in the glass column and the foam height was controlled adjusting the gas flow rate. The procedure was repeated until no foam was produced. The results are depicted in Figure 7.15 and Table 7.10.

The complex TMβCD - α-HCH produced stable, dry foam that could easily be controlled adjusting the gas flow rate. Visual observation indicated that foam was more stable for higher initial concentration of TMβCD (1 mM) with coalescence occurring predominantly at the top of the foam columns. The complex lost from the films, as a result of coalescence, drains back through the rising foam and concentrate in the interstitial liquid in the lower part of foaming column leading to drier foam in the upper part of foam column and in consequence high values of E_R (see Table 7.10) were obtained.

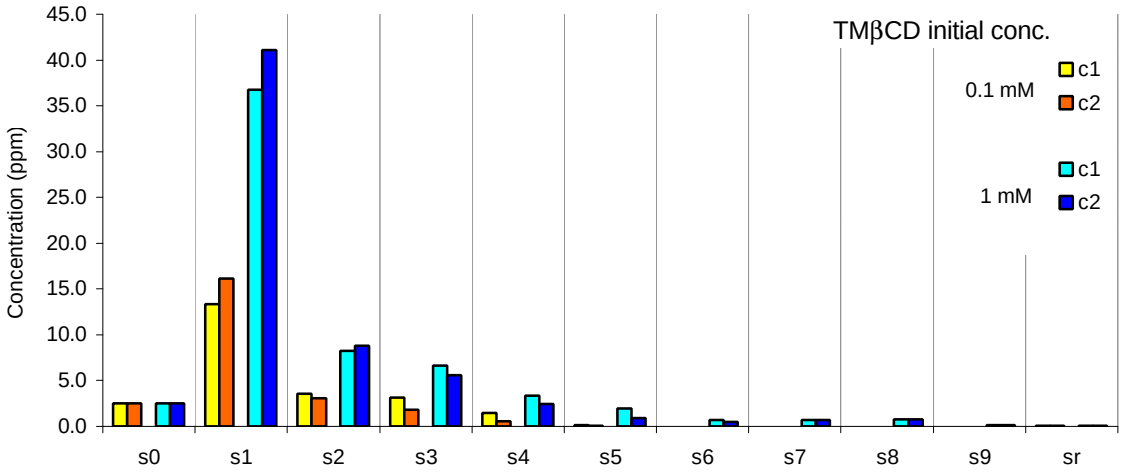


Figure 7.15. Foam fractionation of α-HCH (racemate) **5 ppm** at two different initial concentration of TMβCD. *Experimental conditions*: column **20 cm**; pH 3; room temperature.

In the case of 0.1 mM initial concentration of TMβCD, the foam produced was steady, dry foam uniform with large bubbles all along its height with no reflux occurring (after a first phase of foam volume growth with a low drainage there was no bubbles coalescence and no countercurrent flow in the column). In consequence, lower enrichment of enantiomers was obtained.

Only six foam samples could be collected (after the sixth foaming no foam was produced anymore) comparative to nine foam samples in the case of 1 mM initial concentration of TMβCD (see Table 7.10). This feature reflects a fast depletion of TMβCD in the bulk solution.

Table 7.10. Enantiomeric enrichment of α-HCH (**5 ppm** initial concentration) for two different initial concentration of TMβCD.

Samples	TMbCD initial concentration:									
	0.1 mM					1 mM				
	c1	c2	E_R1	E_R2	ee	c1	c2	E_R1	E_R2	ee
s0	2.50	2.50			0.36	2.50	2.50			0.83
s1	13.33	16.15	5.33	6.46	-7.72	36.76	41.10	14.70	16.44	-3.47
s2	3.54	3.09	1.42	1.23	8.74	8.26	8.78	3.31	3.51	-0.95
s3	3.11	1.83	1.25	0.73	27.70	6.61	5.59	2.64	2.24	10.38
s4	1.47	0.53	0.59	0.21	48.53	3.38	2.44	1.35	0.98	18.17
s5	0.14	0.08	0.06	0.03	30.09	1.93	0.91	0.77	0.36	37.87
s6	0.01	0.01			15.70	0.68	0.52	0.27	0.21	14.99
s7						0.72	0.69	0.29	0.28	4.24
s8						0.77	0.76	0.31	0.31	2.34
s9						0.16	0.16	0.06	0.06	3.54
sr						0.08	0.08			0.65

Experimental conditions: column **20 cm**; pH 3; room temperature.

Note: all E_R are calculated relative to initial solution concentration.

Enantiomeric enrichment (ee) in the first foam samples increased for lower initial concentration of TMβCD (due to the drier foam produced) (See Table 7.10). Although the obtained ee values did not differ significantly from the ee of racemate in the initial solution, it was evident that there is enatiomeric separation occurring under the foam frationation conditions. Therefore, further experiments were done using longer glass columns (40 and 60 cm) and different experimental conditions (temperature, pH). Unfortunately, due to the fact that the maximum concentration of α-HCH that could be obtained was 5 ppm, the possibilities to test the influence of initial concentration ratio on the enantiomeric enrichment were limited.

7.2.4.2. Influence of foam height on E_R and ee

Solutions with initial concentration ratio *[TMbCD]/[α-HCH]* (racemate) ***(1 mM/5 ppm)*** (pH 3) were foamed at room temperature using columns with ***40 cm*** and ***60 cm*** length. After every foam sample removal, the gas flow was cut off and the remaining foam in the column was allowed to collapse completely and then the residual bulk solution foamed again (as described in 6.3 – Foaming procedure). Initial flow rate was adjusted at 100 ml/min then slowly increased (to 140 ml/min in the case of 40 cm long column and to 150 ml/min in the case of 60 cm long column) until the foam reached a constant height at the top of glass column and after 10 min increased again until some foam was forced out of the column (samples' volume between 1-2 ml). Results are shown in Figure 7.16 and table 7.11.

Some attempts were done with 0.1 mM initial concentration TMβCD, but the steady, dry foam produced reached only about 30 cm height. The gas flow rate was slowly increased (to more than 250 ml/min) but the foam collapsed and no foam could be collected.

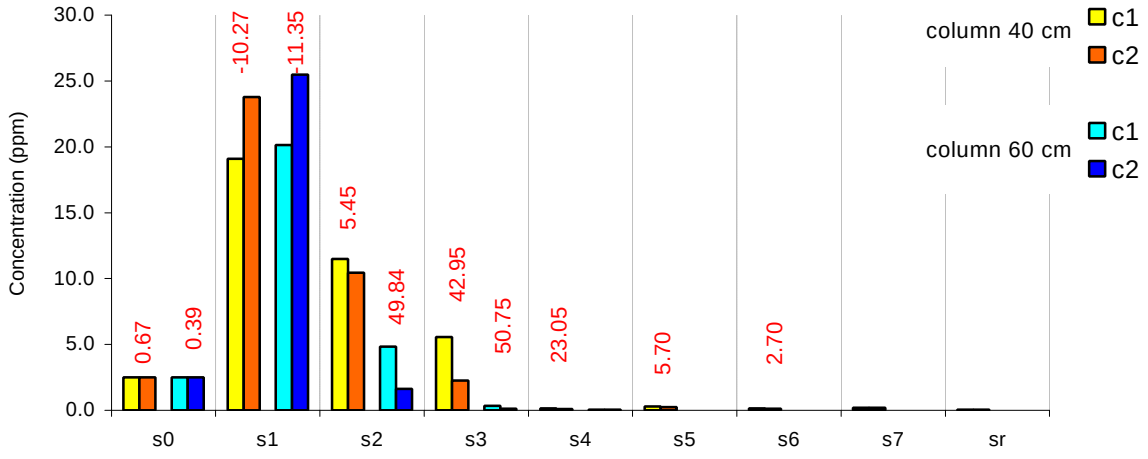


Figure 7.16. Effect of foam height on the enantiomeric enrichment of racemic α-HCH using TMβCD as chiral collector. Numbers in red represent ee values for the respective sample.
Initial concentration ratios [TMbCD]/[α-HCH](racemic): **1 mM/5 ppm;**
Experimental conditions: pH 3; room temperature

Table 7.11. Enantiomeric enrichment of α-HCH using TMβCD as chiral collector
Initial concentration ratio [HPbCD]/[a-HCH](racemate): 1 mM/5 ppm.

Sample	Column 40 cm				Column 60 cm			
	Initial solution: [TMβCD] / [α-HCH] (racemate): 1 mM / 5 ppm							
	c1	c2	E_R1	E_R2	c1	c2	E_R1	E_R2
s0	2.50	2.50			2.50	2.50		
s1	19.10	23.79	**7.64**	**9.52**	20.12	25.47	**8.05**	**10.19**
sr	0.04	0.03			0.00	0.00		

Experimental conditions: pH 3; room temperature

As the gas flow rate was increased, foam rose up in the column till a certain height (about 30 cm) and kept this height. At this moment, only few bubbles from the top of the foam coalesced resulting in a low drainage. In order to obtain foam at the top of column, the gas flow rate was further increased which affected the stability of foam. Due to the high flow rate the foam coalescence was increased leading to more "dynamic" foam with faster drainage. The fast drainage allowed the less stable complexed enantiomer to be displaced from the top of foam and to flow down with the interstitial liquid. This alteration of steady state of the foam resulted in higher ee values for higher foam column (-10.27; -11.35 comparative to -3.47 obtained using 20 cm long column) but detrimental to the enrichment (see Table 7. 10 and Table 7.11).

As the foam characteristics were the same in both experiments (40 cm; 60 cm long columns), the small difference in ee values, as well as in E_R values, are due, most probably, to the differences in samples' volume.

7.2.4.3. *Influence of initial solution concentration ratio on E_R and ee*

The low water solubility of α-HCH restricted the study of initial concentration ratio [TMβCD]/[α-HCH] influence on enantiomeric enrichment to few choices. On the basis of the results achieved from experiments done with TMβCD/DME, it was known that there is a certain initial concentration ratio at which the best enantiomeric enrichment (separation of enantiomers) will be obtained. As the concentration of α-HCH could not be increased, the only possibility was to decrease the concentration of TMβCD. Also, in previous experiments (see 7.2.5.2 – foaming with 40 cm long column) no foam could be collected for 0.1 mM TMβCD initial concentration. Therefore, solutions (pH 3) with:

- Initial concentration ratio [TMβCD]/[α-HCH]: **0.5 mM/5 ppm**, and
- Initial concentration ratio [TMβCD]/[α-HCH]: **0.5 mM/2.5** ppm

were foamed at room temperature.

Foaming procedure was as described in section 6.3 - Foaming procedure, the foaming time before the samples removal being 10 min and the samples' volume between 1-2 ml. The results (together with those obtained from previous experiment in which solution with initial concentration ratio [TMβCD]/[α-HCH]: 1 mM/5 ppm was foamed in the same conditions) are depicted in Figure 7.17 and Table 7.12.

Table 7.12. Enrichment and ee of α-HCH (racemate) as function of initial concentration ratios using TMβCD as chiral collector.

Sample	Initial concentration ratio [TMbCD]/[a-HCH] (racemate):								
	1 mM / 5 ppm			0.5 mM / 5 ppm			0.5 mM / 2.5 ppm		
	E_R1	E_R2	ee	E_R1	E_R2	ee	E_R1	E_R2	ee
s0			0.39			0.18			0.35
s1	8.05	10.19	-11.35	17.16	21.12	-10.16	11.67	11.56	0.49
s2			49.84			60.08			56.08
s3			50.75			70.88			
s4			5.37						

Experimental conditions: see Fig. 7.17

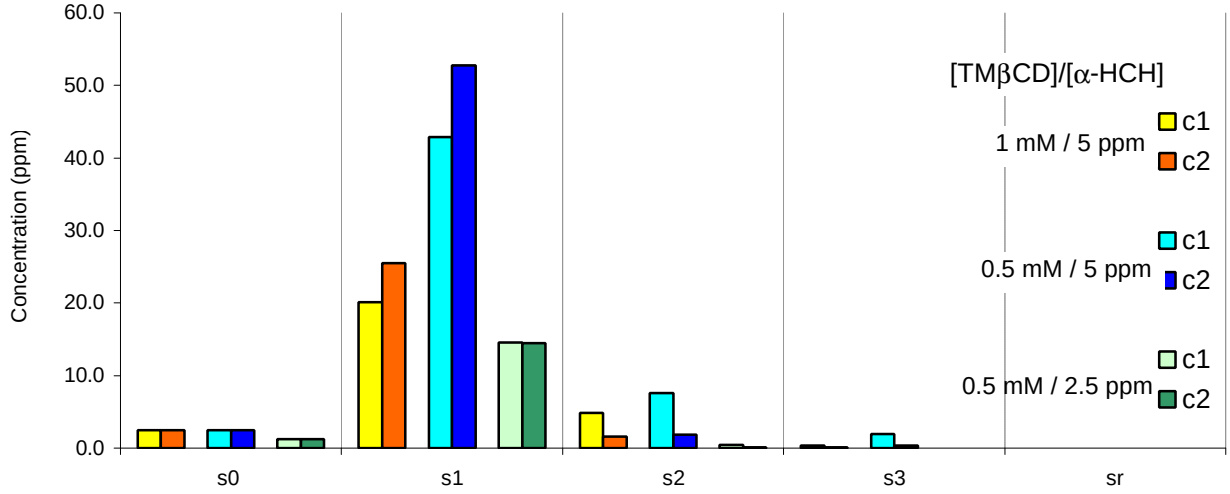
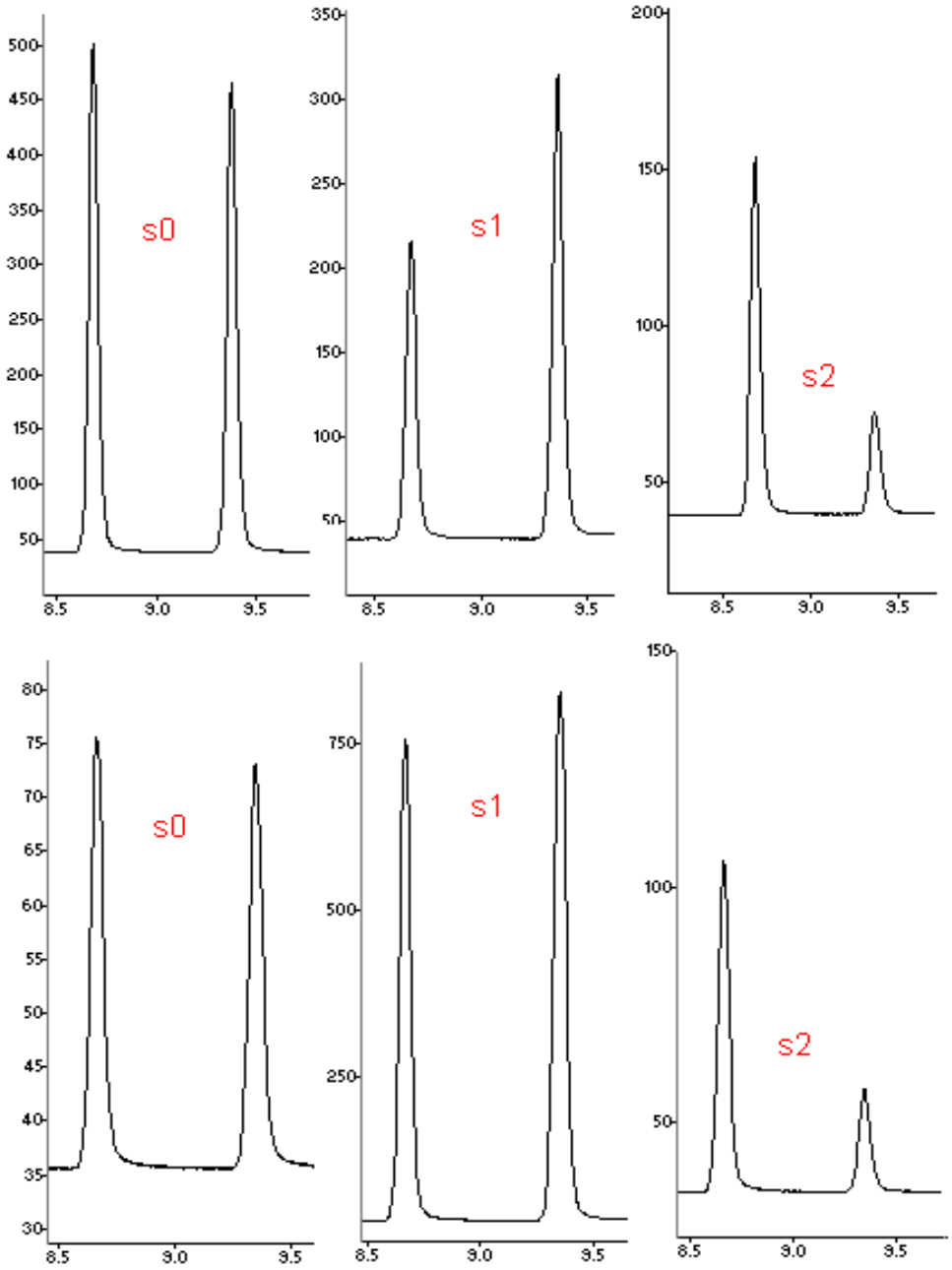


Figure 7.17. Effect of initial concentration ratio [TMβCD]/[α-HCH] on the enantiomeric enrichment.

Experimental conditions: column **60 cm** long; pH 3; room temperature, 10 min foaming time before every foam sample removal.

Decreasing the concentration of TMβCD from 1 mM to 0.5 mM, there was no improvement in enantiomeric separation (ee values are very close). Correlating these ee values with E_R values and with the fact that the foam characteristics were the same for both TMβCD concentrations, it can be supposed that the small difference in enatiomeric enrichment is due only to the different volume of foam samples and not to changes in separations conditions. Decreasing also the concentration of α-HCH to 2.5 ppm the enrichment of both enantiomers was on the same level but without their discrimination (no enantiomeric separation).

These results are in concordance with the trends obtained foaming TMβCD/DME with different initial concentration ratios (section 7.2.3.3).



A

[TMβCD]/[α-HCH]:

1 mM/5 ppm

B

[TMβCD]/[α-HCH]:

0.5 mM/5 ppm

Figure 7.18. GC representative chromatograms for two different initial concentration ratios [TMβCD]/[α-HCH] (see Fig. 7.17 and Table 7.12 for e*xperimental conditions* and ee values).

Note: all samples represented in **A** and **B** have different dilutions.

It could be supposed that increasing the concentration of α-HCH a better separation of the enatiomers would occur. Therefore, 0.012 g α-HCH were added to 100 ml THF 1% to obtain a saturated solution. The resulting solution was a precipitate as a part of α-HCH remained in solution as particulate matter. This solution was foamed with TMβCD (0.5 mM) in the same way as for previously experiments (consecutive foaming at room temperature and pH 3).

The first foam sample was a precipitate and the residual solution a clear solution. Certainly, besides the adsorption of (TMβCD-α-HCH) complex onto the bubbles surface, there was also a mechanical transport of the undissolved α-HCH in the foam.

It was expected that due to the decreasing of α-HCH concentration in bulk solution, during the consecutive foaming, the enatiomeric separation would occur. Unexpectedly, in spite of its high initial concentration, α-HCH could be detected only in four samples (Figure 7.19) (as in previous experiments when α-HCH 5 ppm was foamed with TMβCD 0.5 mM (see Fig. 7.17 and Table 7.12)). As result of high depletion of α-HCH and TMβCD in the bulk solution after the first foam removal, there was too less chiral compound and/or cyclodextrin for the enantiomeric separation to occur (ee values in all foam samples did not differ significantly from ee of racemate, as shown in Figure 7.19).

Also, it could be observed that during the first foaming the the stability of foam was altered by the presence of the precipitate, phases of very fine foam production being followed by its completely collapse.

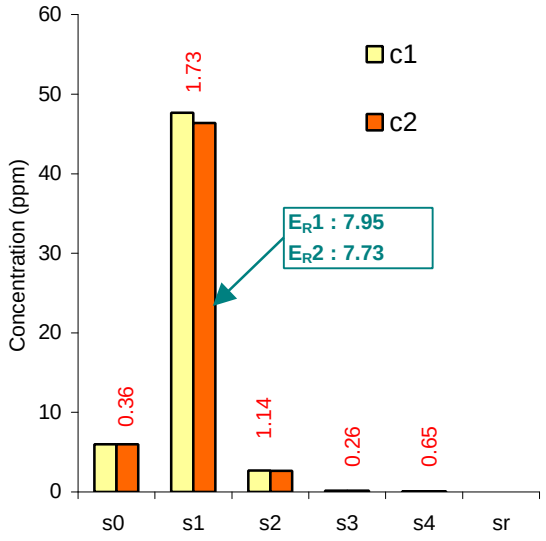


Figure7.19. Enantiomeric enrichment of α-HCH saturated solution **(0.012 g/100 ml)** using TMβCD **(0.5 mM)** as chiral selector.

Experimental conditions: column 60 cm long; pH 3; room temperature; 10 min foaming time before every foam sample removal.

7.2.4.4. Influence of temperature on E_R and ee

To study the effect of temperature on the enantiomeric enrichment of α-HCH, foaming experiments were carried out at temperature lower than room temperature (~ 24°C) as follow:

- Solution with initial concentration ratio [TMβCD]/[α-HCH] = [1 mM]/[5 ppm] was foamed at 5°C using 40 cm long glass column;
- Solution with initial concentration ratio [TMβCD]/[α-HCH] = [0.5 mM]/[5 ppm] was foamed at 5°C using 60 cm long glass column.

Foaming procedure was as described in section 6.3.2. In the case of foaming at 5°C the first foam sample was collected at 15 min after the foam reached the top of glass column (the time necessary for the foam to reach a quasi-stable state).

Attempts to foam the solutions with the same initial concentration ratios at higher temperature (55°C) leaded to excessive instability of the foam with repeated collapsing along the column. High flow rates were necessary to obtain foam at the

top of column, but at such high flow rates, the bulk solution was practically pushed up in the glass column and there was a mixture of foam with liquid. Therefore, the experiments, as well as the possible results, were considered as being not conclusive for this study.

The increased solution viscosity at low temperature (5°C) leaded to a slow drainage between bubbles resulting in stable foam, with the same wetness and bubbles' dimension all along the column height. As a result of the foam stability, ee values and the enrichments were lower (Figure 7.20 and Table 7.13) than those obtained foaming the solution with the same initial concentration ratios at room temperature.

Table 7.13. Temperature influence on enantiomeric enrichment of α-HCH using TMβCD (**0.5 mM**) under different foaming conditions.

Sample	[TMbCD] / [a-HCH] (racemate): 1 mM / 5 ppm; column 40 cm long									
	Room temperature (24°C)					5°C				
	c1	c2	E_R1	E_R2	ee	c1	c2	E_R1	E_R2	ee
s0	2.50	2.50			0.67	2.50	2.50			0.48
s1	19.10	23.79	7.64	9.52	**-10.27**	12.86	13.03	5.14	5.21	**-0.01**
	[TMbCD] / [a-HCH] (racemate): 0.5 mM / 5 ppm; column 60 cm long									
	Room temperature (24°C)					5°C				
	c1	c2	E_R1	E_R2	ee	c1	c2	E_R1	E_R2	ee
s0	2.50	2.50			0.18	2.50	2.50			0.69
s1	42.90	52.79	17.16	21.12	**-10.16**	17.82	21.15	7.13	8.46	**-7.85**

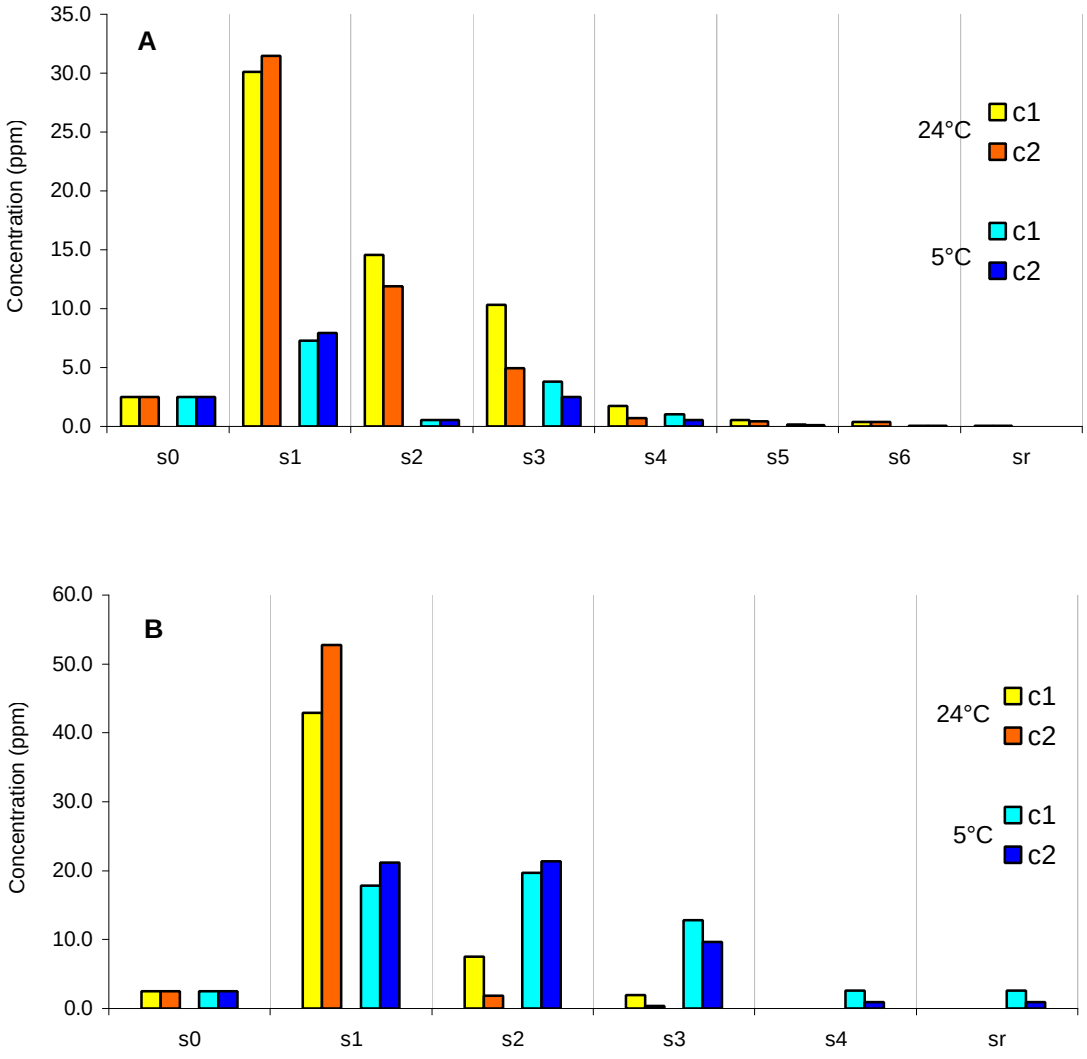


Figure 7.20. Effect of temperature on the enantiomeric enrichment of α-HCH using TMβCD as chiral collector.

A: *Initial concentration ratio* [TMβCD] / [α-HCH] (racemate): **1 mM / 5 ppm;** *Experimental conditions*: column **40 cm** long; pH 3;

B: *Initial concentration ratio* [TMβCD] / [α-HCH] (racemate): **0.5 mM / 5 ppm;** *Experimental conditions*: column **60 cm** long; pH 3.

7.2.4.5. Influence of pH on E_R and ee

A solution with initial concentration ratio [TMβCD]/[α-HCH]: **1 mM/5 ppm** and neutral **pH 6.8** (no HCl addition) was foamed at room temperature using 60 cm long column. Foaming procedure was as described in section 6.3.2 and the foaming time before the samples removal was 10 min.

The foam produced at pH 6.8 presented a high instability, with excessive coalescence occurring all along the foam column leading to alternative phases of foam volume grow and partially collapse. To obtain foam at the top of column the gas flow had to be increased to more than 200 ml/min (comparative to ~160 ml/min in the case of foaming at pH 3) and the foam samples contained high amount of interstitial liquid. Consequently, both ee and E_R values are lower than those obtained at pH 3 (Figure 7.21). It seems that the differences in ee and E_R values are mainly due to the alteration in the surface activity of the complex at neutral pH but also there could be a change in the nature of enantioselective complexation of α-HCH with TMβCD.

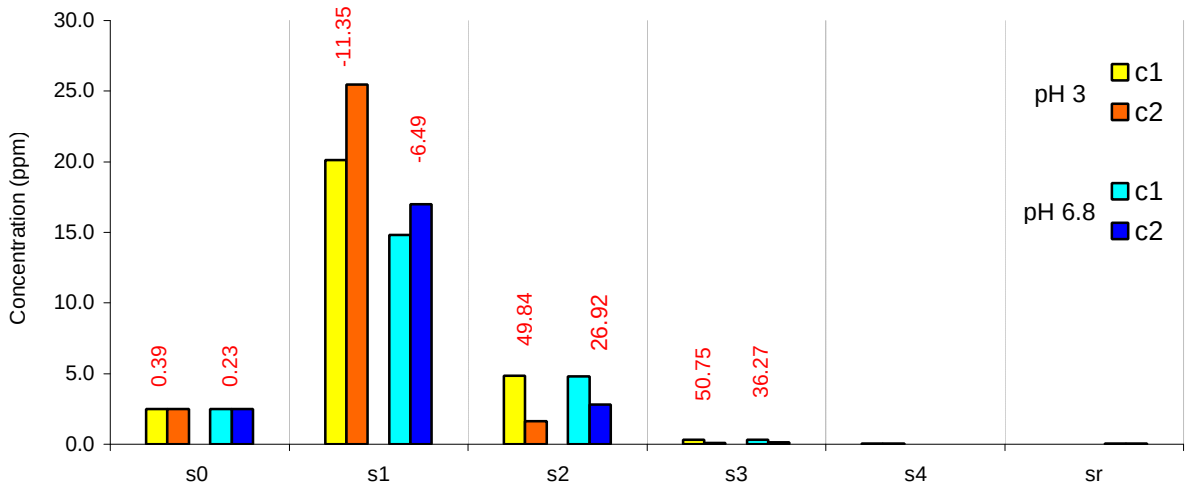


Figure 7.21. Effect of pH on the enantiomeric enrichment of α-HCH using TMβCD as chiral collector. Numbers in red represent ee values for the respective samples.
Initial concentration ratio [TMβCD] / [α-HCH] (racemate): **1 mM / 5 ppm;**
Experimental conditions: column 60 cm long, room temperature

7.2.5. Foam Fractionation of Mecoprop with HPbCD

7.2.5.1. Preliminary experiments

Initially, a solution with a concentration ratio [HPβCD]/[MCPP] = **1 mM/30 ppm** (pH 3) was foamed at room temperature using 20 cm. Initial flow rate was 20 ml/min but had to be increased because no foam was produced. Foam started to be produced at 80 ml/min gas flow rate and the flow rate was slowly increased up to 120 ml/min in order to obtain foam at the top of column.

All the foam samples were collected at 10 min time interval. After every foam removal (samples' volume between 1-3 ml) the gas flow rate was decreased until the foam withdrew back in the glass column and the foam height was controlled adjusting the gas flow rate. The procedure was repeated until no foam was produced. The results are depicted in Figure 7.22 and Table 7.14.

Foam presented very low stability, with bubbles collapsing all along the foam height and a high gas flow control was necessary in order to maintain a certain height of the foam and to force some foam out of column. Due to the high flow rate, the bulk solution was pushed up in the column and the foam samples contained high amount of interstitial liquid detrimental to the enantiomeric enrichment (Figure 7.22 and Table 7.14)

Because the 20 cm long column was to short to permit the foam to reach an equilibrium, a new attempt was done, and a solution with concentration ratio [HPβCD]/[MCPP] = **10 mM/15 ppm** (pH 3) was foamed at room temperature using 60 cm long column. Initial gas flow rate was 20 ml/min (as in previous experiment) and then slowly increased to about 200 ml/min in order to obtain foam at the top of column. At this gas flow rate, the foam was mixed with the bulk solution pushed up

into the column. To force some foam out of the column, the gas flow rate was increased to 250 ml/min. In these conditions, the foaming experiments cannot be repeated and, although results are not conclusive they are presented in Figure 7.22 and Table 7.14.

It was concluded that: (1) HPβCD is not the proper chiral selector for MCPP and (2) the surface activity of the complex HPβCD - MCPP is too low to produce, at least, a quasi stable foam.

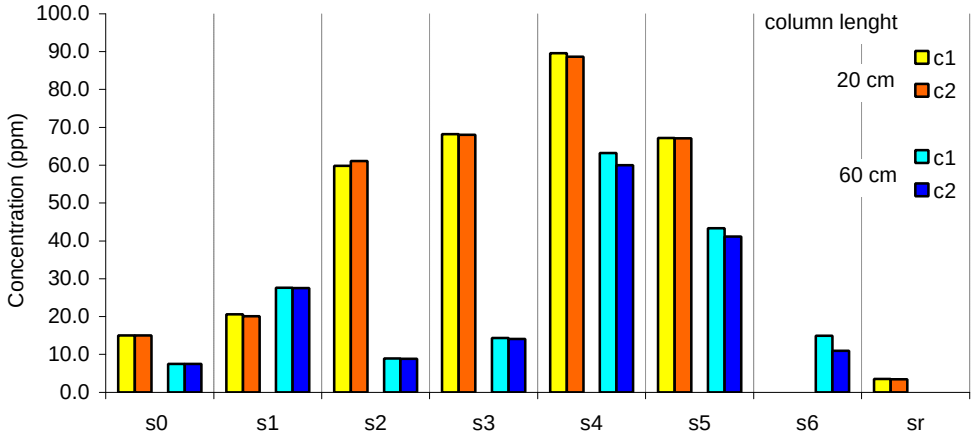


Figure 7.22. Foam fractionation of MCPP using HPβCD as chiral collector.
Initial concentration ratio [HPbCD]/[a-HCH] (racemate):
Column **20 cm** long: **1 mM/30 ppm**
Column **60 cm** long: **10 mM/15 ppm**
Experimental conditions: pH 3; room temperature.

Table 7.14. Enrichment and enantiomeric excess of MCPP in initial solution, foam samples and residual solution using HPβCD as chiral collector .

Sample	Column height: 20 cm *[HPbCD]/[MCPP]:* 1 mM/30 ppm.					Column height: 60 cm *[HPbCD]/[MCPP]:* 10 mM/15 ppm.				
	c1	c2	E_R1	E_R2	ee	c1	c2	E_R1	E_R2	ee
s0	15	15			-0.86	7.5	7.5			-0.65
s1	20.57	20.07	1.37	1.34	0.36	27.61	27.52	3.68	3.67	-0.48
s2	59.85	61.05	3.99	4.07	-1.86	8.97	8.89	1.20	1.18	-0.16
s3	68.21	68.01	4.55	4.53	-0.72	14.36	14.06	1.91	1.87	0.41
s4	89.54	88.68	5.97	5.91	-0.38	63.22	60.00	8.43	8.00	1.97
s5	67.18	67.10	4.48	4.47	-0.81	43.35	41.18	5.78	5.49	1.91
s6						14.97	10.98	2.00	1.46	14.73
sr	3.51	3.48			-0.45	0	0			

Note: all E_R are calculated relative to initial solution concentration

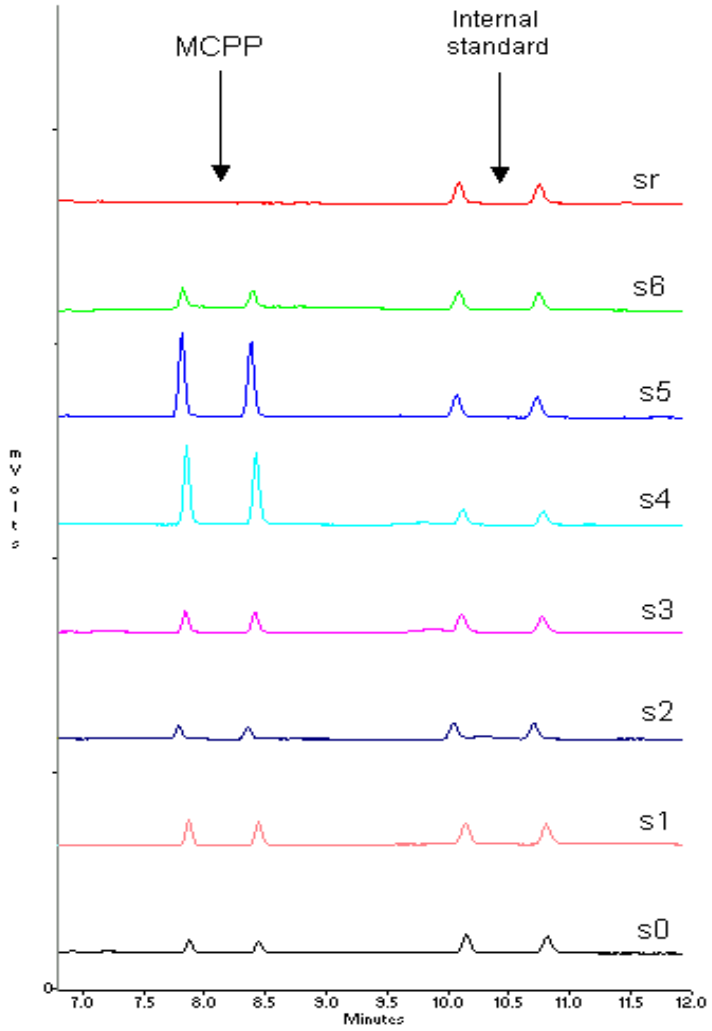


Figure 7.23.

GC representative chromatograms for enantiomeric enrichment of MCPP using HPβCD as chiral collector.

Initial concentration ratios

[HPbCD]/[MCPP]: 10 mM/15 ppm.

Column length: **60 cm.**

(see Table 7.14 for E_R and ee values).

Note: all the samples have the same dilution.

8. DISCUSSION

Foam separation/fractionation techniques have been widely used in the last years in many fields, more or less successful. Different types of classical surfactants have been used and their foaming properties as well as their ability to form complexes with non- or less surface-active compounds were the subject of numerous studies. Theory and practice of foaming methods are understood and established.

The situation is quite different in the field of enantiomeric separation, whether it is done with liquid chromatographic technique, on CSP or CMP, or gas chromatography. In spite of decades of experience, the delicate problem of choosing the proper chiral selector for a given selectand and the technique and optimum conditions for an enatiomeric separation to occur is still a matter of “trial and error”. Yet, some predictions can be made based on characteristics of both chiral selector and selectand.

This work was intended to combine the knowledge and experience in the field of foam fractionation with those in the field of enantiomeric chromatographic separation methods. Up to now, no studies were done in this regard and this work can be considered as a first step in supporting the feasibility of using an adsorptive bubble process to selective enrich enantiomers.

In literature many studies that consider the effect of surfactants properties (structural and interfacial properties) on foam formation and stabilization behaviour can be found, but there is no published information regarding the use of cyclodextrins as *surface-active agents* and *chiral selectors* in foam separation techniques.

There are two basic requirements for the successful foam fractionation of enantiomers using chiral collectors. First, the chiral collectors must be surface active to adsorb onto the gas-liquid interface and to produce foam, at least, partially stable. Second, the enantioselectivity of the chiral collectors toward the enatiomers to be separated must be appreciable, since large numbers of countercurrent equilibrium steps (e.g. theoretical plates) are not available in this technique as they are in HPLC and CE, for example. The ability of a particular surfactant to adsorb at an interface is a function of its physicochemical characteristics and the physical properties of the interface. In the case of cyclodextrins, which were used in this work as surface-active agents and chiral selectors, their physicochemical properties are all important. The cyclodextrins must be:

a) at least partial water soluble,
b) able to produce foam,
c) able to enantioselctively bind to the chosen compounds,
d) able to maintain some surface activity when associating with at least one of the two enantiomers.

Enantioselectivity could results either from a difference in the association energy between the chiral collector and the two enantiomers, from a difference in the surface adsorption of two diastereomeric (collector plus enantiomer) complexes, or from a combination of these two factors.

As it is the case with every new method, in the beginning, choosing the experimental conditions and the process variables was done empirically and there were many attempts to correlate the two processes: adsorption onto the gas-liquid interface and enantiomeric discrimination. As function of the results obtained, the conditions were optimised to obtain better enantiomeric separation.

Next questions which, obviously came during the experiments, were:

- Which of the process are firstly to occur: the adsorption of the surface-active chiral selector onto the gas-liquid interface or the enantioselective complexation with the selectand?
- Where the enantiomeric separation is more likely to occur: in the bulk solution or in the foam column?

It is known from the practice of LC and CE with CDs as mobile chiral selectors additives that enatiomeric separation depends on many factors such as: CD concentration relative to the concentration of the chiral compound, temperature, pH for ionic species, presence of other organic additives, presence of other chiral selectors, etc. All these factors might affect also the performances of the foam fractionation. This work examines the effects of foaming column design, ratio of CD concentration relative to chiral compound in initial solution, and solution conditions (pH and temperature) on the foam fractionation performance parameter (E_R) and on the enantioselectivity of the CDs toward different chiral compounds (ee) under the foam fractionation conditions.

Tests of foamability and foam stability of the three CDs chosen for this work, MβCD, TMβCD, and HPβCD, showed that they are surface active but only TMβCD and HPβCD produced dry, quite stable foam when foamed at low concentration. Therefore, only these two were used in foam fractionation.

A number of things are evident from the data presented in section 7.
First and foremost, it is clearly demonstrated that enatiomeric enrichment (enatiomeric separation) is possible using adsorptive bubble processes if the chiral selector presents enantioselectivity toward the chiral selectand. Second, enantiomeric enrichments are easily enhanced modifying certain experimental conditions.

Four chiral compounds were tested: dichlorprop (DCPP) and dichlorprop methyl ester (DME), mecoprop (MCPP) and α-HCH. They have different water solubility, only the first three being quite soluble in water. For α-HCH hardly water solution could be obtained (maximum 5 ppm, precipitate solution). The compounds have also different structures. While DCPP, MCPP and DME are branched molecules and have a chiral centre close to the aromatic group, α-HCH is a compact molecule and presents conformational chirality. For successful chiral recognition with CDs, hydrophobic interactions between the cavity interior and the analytes are assumed, whereas hydrogen bonding at the cavity edge determines a compound's access to the cavity entrance. It was expected that, due to their different structures, the CDs enantioselectivity toward the four compounds would be different.

Foam fractionation of chiral compounds might be seen as a new enatiomeric separation technique in which the chiral stationary, but "mobile" in the same time, phase is represented by the gas-liquid interface (the bubbles) and the surface-active chiral selector represents the chiral mobile additive phase. Therefore, in the case of enantiomeric separation in foam fractionation, both the adsorbing at the interface and enantioselective processes are important and they are interdependent. A chiral selector and a chiral selectand have different properties than the complex chiral selector - chiral selectand. Therefore, it is understood that the surface activity of CDs alone will be different from that of its complexes with a chiral compound. Not only that the possible hydrogen bonds at the cavity edge as well as the orientation of the enatiomers inside the cavity of CDs will modify the whole structure of CDs (and, in consequence its surface activity) but also the orientation of the complex molecule at the gas-liquid interface might be changed. This, in turn, will affect the enantiomeric separation. Since the CD-enantiomer complexation processes in solutions are not only stereoselective but also reversible and equilibration in solution is relatively fast, if the wider rim of CD molecule is positioned toward the

water subphase, further complexation are more likely to occur. If the wider rim of CD molecule is positioned toward the gas subphase, the cavity entrance is blocked and the further complexations hindered.

In this study, HPβCD was used in combination with all four compounds and TMβCD only with DME and α-HCH.

While when foamed alone HPβCD produced the most stable foam from the three CDs, when complexed with the chiral compounds its surface activity was altered and it was difficult to obtain foam with good characteristics. The maximum enrichments and ee values obtained varied function of initial concentration ratios and of column length (see sections 7.2.1.1, 7.2.3.1 and 7.2.5.1 for ee and E_R data).

Although the ee values for all chiral compounds were different from the ee values for racemates, due to the fact that foam stability was low (alternative phases of foam production and foam collapsing) and a permanent control of gas flow rate was necessary to maintain the foam in the column, the repeatability of the foaming experiments was too low.It was clear that repeating the experiments in the same conditions would give different results. Still, some conclusions can be drawn:

No one of the chiral compounds studied are surface active and they do not adsorb onto the gas-liquid interface. Therefore, they can be enriched in the foam only if they are complexed with CD. The E_R values > 1 obtained for both of the enantiomers demonstrated that the diastereomeric complexes are formed but a low differences in the stability between these diastereomeric complexes could lead to a low enantioselectivity (low ee values). Also, the low surface activity of the complex itself (which produced wet, unstable foam) as well as a possible orientation of the CD's wider rim toward the gas subphase that could hinder further complexations might also contribute to the low separation. It is not sure which one of the above

described phenomena is more responsible for the low enatiomeric separation but with certitude they reciprocally influence.

While foaming the chiral compounds with HPβCD as chiral collector not appreciable high ee values were obtained, foam fractionation of DME and α-HCH with TMβCD leaded to enhanced enantiomeric enrichments.

Factors as experimental design (in terms of foaming column length) and experimental conditions (initial concentration ratios CD/chiral compound, temperature and solution pH) were shown to influence the foam properties and, in consequence, the enatiomeric separation for both DME and α-HCH.

Initially, both DME and α-HCH were foamed with TMβCD using a short column (only 20 cm long). The first direct observation was the higher stability and dryness of the foam comparative to those of foam produced with HPβCD/chiral compound. The ee values were higher and significantly different from ee of racemates (see sections 7.2.2.1 and 7.2.4.1 for ee and E_R data). It could be observed that decreasing the initial concentration of TMβCD relative to the chiral compound resulted in better enantiomeric separation for both DME and α-HCH (higher ee values). The 20 cm long column was too short to allow the foam to equilibrate and therefore, further experiments were carried out using longer columns (40 and 60 cm) and lower initial concentrations of TMβCD.

An increase in column length from 20 to 40 and 60 cm leaded to remarkable increase in ee and E_R values for DME. In the case of α-HCH the differences were not too high, still an increase in ee values was obtained (see sections 7.2.2.2 and 7.2.4.2 for ee and E_R data). Since all the other experimental conditions (temperature and pH which could affect the complexes stability) were the same when foaming using the three columns, the better enantiomeric separation is a result of improved foam

characteristics for higher foam height and not of any changes in the complexation stability of the two enantiomers with the CD.

Visual observations indicated that coalescence was predominant in the upper part of foam height. As the foam rise up in the column, the bubbles start to coalescence leading to longer and faster drainage. The enatiomer that form less stable complex with CD predominantly drain back in the interstitial liquid through the rising foam and concentrate in the lower part of the foam, promoting enatiomeric separation at the top of column. Consequently, an increase in column length from 40 to 60 cm leaded in significantly increased ee value and E_R as well (specially for DME).

Initial concentration of CD relative to that of chiral compound strongly affected the enatiomeric enrichment. Solutions with different initial concentration ratios were foamed keeping all the other conditions (column length, temperature, pH) the same. The low water solubility of α-HCH limited the study to few options (see sections 7.2.2.3 and 7.2.4.3 for the ee and E_R data).

An increase in the concentration of DME relative to that of CD resulted in a significant increase in ee values. It was observed that foam stability increased as DME initial concentration decreased. Since, for all DME concentrations, there was an excess (molar ratio) of TMβCD over the DME, it can be supposed that the free (uncomplexed) TMβCD will provide for higher stability of the foam, which in turn enhances the enantiomeric enrichment. A further increase in DME concentration (for the same TMβCD concentration) resulted in lower ee value (see Fig. 7.8, 7.9 and Table 7.6, 7.7).

Due to the fact that, in every experiment, solutions were consecutivlye foamed (as described in 6.3 – Foaming procedure), only the first foam sample present interest for enantiomeric separation. Using this foaming method, every consecutive

foaming can be seen as an independent foaming experiment with initial concentrations of DME and TMβCD lower than in a previous one. An interesting observation could be made. While for low initial concentrations of DME enantiomeric separation was obtained only in the first foam sample, at higher initial concentration of DME the process occurs in all consecutive foaming. From the HPLC, CE and even GC, it is known that there is an optimum concentration of chiral selector, relative to that selectant, at which separation occurs. It appears that this is also the case in the conditions of foaming method.

In the case of α-HCH, although not too much could be done, the results appear to be in concordance with the trends obtained foaming DME with the same CD as chiral collector: enantiomeric separation was better for longer foaming columns and for higher initial concentration of α-HCH relative to that of CD. An attempt to foam a saturated solution of α-HCH (practically a precipitate) leaded only to higher enrichments of enantiomers due to mechanical transport in the foam of particulate, undissolved α-HCH but without enantiomeric discrimination.

Lowering the temperature of the foaming system to 5°C leaded in a significant decrease in enantiomeric excess for both DME and α-HCH foamed with TMβCD as chiral collector (see section7.2.2.4 and 7.2.4.4 for ee and E_R data). This could result from the excessive foam stability with slow drainage (as effect of increased solution viscosity), from a decrease in the difference in the association energy between the CD and the two enantiomers at low temperature or, most probably, from the combination of these two phenomena.

By increasing the temperature to 55°C there was an evident alteration of foam stability, which, practically, collapsed and formed alternatively. A high flow rate was necessary to keep the foam at a certain height and the bulk solution was pushed up in the column, which resulted in a mixture of solution with foam. In consequence,

both ee and E_R values were low. In the case of α-HCH the foam stability was excessive low and therefore, the experimental results were considered as being non conclusive.

Extending the foaming time after the foam equilibrated (after it reached a quasi-stable state with complete reflux) leaded to a significant decrease of the enantiomeric enrichment (see 7.2.2.5 for ee data) when DME was foamed with TMβCD at room temperature, as well as at 5°C. The foam reaches a steady state when the bubbles are saturated with the surface-active species (the complexes CD-enatiomers) adsorbed onto the interface. After the steady state is attained, and due to the depletion of the surface-active species in the bulk solution, a higher gas flow rate is necessary in order to maintain the foam at a constant height and to extend the process. At high gas flow rate the faster rising foam retards the natural drainage leading to less dry foam at the top of column, which in turn affects the enantiomeric enrichment.

Unexpected results were obtained foaming DME and α-HCH at neutral pH (see section 7.2.2.6 and 7.2.4.5 for ee data). It is known that pH affects the enrichment in foam of ionic species or of those possessing different functional groups Also, is not unusual in HPLC and CE that enatiomeric separations of ionic compounds to be pH depending. Therefore, taking into account that DME and α-HCH are neutral molecules, as well as TMβCD, and the fact that TMβCD is stable over a very large pH range, the low ee values obtained at neutral pH, compared to those obtained at acidic pH, might be only ascribed to a possible change in the nature of enantioselective complexations, which in turn could affect the surface activity of the complexes. But, at this level of knowledge regarding the possible processes involved in enatiomeric separation in the foam, any affirmation would have only empirically character.

Some concluding remarks (strictly for the results obtained in this work) can be draw:

1. It is evident that the functional groups of derivatized CDs play a crucial role not only in the enantioselective complexation nature but also in the surface activity of the formed complexes.
2. Every pair chiral selector/chiral selectand behaves different in the conditions of foam fractionation (in terms of adsorption and enatiomeric separation processes).
3. Foam stability (which determines the mobility of the enantiomers – free and complexed – in the foam phase) seems to control the enantiomeric separation. Enantiomeric separation is enhanced when the foam reach an equilibrium under reflux conditions (when there is a constant countercurrent flow in the column).
4. Due to the differences in the mobilities between the free and complexed enntiomers in the foam phase, the separation of the enantiomers occurs mainly in the upper part of foam height.
5. Changes in the foaming system design affect the foam stability without affecting the differences in the stabilities between the reversible diastereomeric chiral selector-chiral selectand complexes.
6. Factors as initial concentration ratios, temperature, pH affect the foam stability throughout their effects on the nature of diastereomeric complexes and consequently, influencing the adsorbing and/or enantioselective processes.
7. It is understood that any other additives would affect (positively or negatively) the adsorbing and/or enantioselective processes.
8. A multi-stage foaming system (column cascades) might lead to higher enantiomeric enrichment.

9. CONCLUSIONS

CDs are chiral molecules. Therefore, they can act as chiral selectors for a large number of molecules. They are extensively used in enantioselective separation systems. CDs can be part of the separation column (in GC or HPLC, sometimes in CE) or they can be added to the eluent (HPLC) or aqueous buffer (CE). Natural CDs are surface-inactive organic compounds. Instead, some modified CDs (with both hydrophobic and hydrophilic type groups) tend to be surface active and are more or less adsorbed onto the gas/liquid interface.

These two properties of derivatized CDs - their surface activity and their enantioselectivity - were combined in this work to give a totally new approach: the use of CDs as foaming agents *and* chiral selectors in the same time.

It was clearly demonstrated in this work that foam-forming chiral selectors (specifically in this work the cyclodextrins) could be used in conjuncture with an inexpensive glass system to enantiomerically enrich chiral compounds. Different chiral compounds (in terms of their water solubility and structural formula) were foam fractionated in combination with two different derivatised CDs (in terms of their functional groups) HPβCD and TMβCD. The results obtained indicate that foam fractionation of enantiomers is not narrowly applicable.

Although only partial enantiomeric separation was obtained (in chromatographic terms there was no *base line* separation) the results of this work are important because the main mechanisms driving the separation of enatiomers in the conditions of foam fractionation were understood and some trends were observed.

Adsorbing and enantioselective processes cannot be studied separately but only as interdependent processes. Whatever the exact mechanism of separation of enantiomers by foam fractionation is, it is clear that the difference between the CDs-enantiomes complexes stability and their surface activity play central roles by determining the foam stability – the coalescence and drainage. Based upon this study, it seems that the enatiomeric separation is maximized when the foam reach an equilibrium under reflux conditions with a fast drainage (when there is a constant countercurrent flow in the column). Also, it seems that complex interrelationship between CDs-enantiomers complexes adsorbtion and foam drainage is influenced by solution conditions and column operation parameters.

In conclusion, it is indeed possible to selectively enrich the enantiomers and it was demonstrated that foam fractionation can be used as an enantioselective method as long as the complexation is appropriate and, for the first time, the mechanisms involved in separation were studied. The relatively low cost, the simplicity of the foaming system, the ease with which the separations are performed, and the ability to enrich large samples are all important factors in applying this method for large-scale enantiomeric separation. The ease with which the CDs were separated from the foam samples, demonstrated that the chiral collectors, used in this method, can be recycled, which make the method highly cost efficient.

It is hoped that the data presented will stimulate an increased interest in this area of chiral separations.

10. Summary

In recent years, the growing use of pure enantiomers in the pharmaceutical, agrochemical, food, flavours, and flagrances industries calls for search for new chiral separations methods, i.e. methods than can separate and/or distinguish individual enantiomers, applicable on large scales. Adsorptive Bubble Separation (ABS) methods have various field of applicability. Theoretically, with these methods, any dissolved substances can be concentrated and/or separated if an appropriate collector with adequate surface activity can be found. Foam fractionation, one of the ABS method might be an alternative to the classical chiral separation methods as long as at least one of the chiral selector or chiral selectand is surface active and the enantioselective affinity between them is appropriate.

In this work, two properties of some derivatives CDs -their surface activity and their ability to form reversible diastereomeric complexes with chiral analytes- were used to demonstrate the potential of foam fractionation as a new method for enantiomeric separation. To develop a new chiral separation method, it is important that there exist sufficient background knowledge on the potentials of the techniques already available so that a rational choice can be made toward the best possible solution.

Thus, as theoretical background, the processes governing the foam fractionation and the parameters that influence the efficiency of foam fractionation were presented in detail. The subject ‘chirality’ was introduced in general terms. Methods for resolution of optical isomers were reviewed and the mechanisms for chiral recognition as well as the thermodynamics of enantioseparations were described. Finally, a general view of the cyclodextrins as well as their enantioselective and surface-active proprieties were presented.

Dichlorprop (DCPP), mecoprop (MCPP), dichlorprop methyl ester (DME) and α-HCH were used as model chiral selectands and 2-hydroxypropyl-β-cyclodextrin (HPβCD), heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (TMβCD) and methylated-β-cyclodextrin (MβCD) as model surface-active chiral collectors, respectively.
Foamability and foam stability tests of the three CDs showed that all three are surface active but only HPβCD and TMβCD produced foam with good characteristics for foam fractionation of chiral compounds.

Foaming all four pesticides with HPβCD as chiral collector resulted in all cases in low ee values (not significantly different from ee value of racemates). The low enantioseparation obtained for all pesticides was attributed to the structural feature of HPβCD, which could result in a low enatioselectivity toward the chiral analytes and/or to the orientation of the complexes CD-enantiomers at the interface. Foam fractionation of DME and α-HCH with TMβCD as chiral collector resulted in better enantiomeric separation. Thus, it was evident that the functional groups of derivatives CDs play the crucial role not only in the enantioselective complexation nature but also in the surface activity of the formed complexes. Factors as experimental design (in terms of foaming column length) and experimental conditions (initial concentration ratios CD/chiral compound, temperature and solution's pH) were shown to influence the foam properties and, in consequence, the enatiomeric separation for both DME and α-HCH.

On the basis of the experimental data obtained varying these parameters and of the proposed chiral recognition as well as adsorption onto the gas-liquid interfaces mechanisms from the literature, it was possible to (at least partially) unravel the chiral discrimination processes in the conditions of foam fractionation.

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