UNIVERSITY OF
KWAZULU-NATAL

INDUSTRIAL CHROMATOGRAPHIC SEPARATION

OF

MONO-VALENT AMINO ACIDS

By:

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ABBREVIATIONS

HPLC	High performance liquid chromatography
ILE	Isoleucine
AABA	Alpha amino butyric acid
BV	Bed volume

Internationally recognised abbreviations are not included here.

Summary

The aim of the thesis was to optimise the separation and purify an amino acid solution consisting of mono-valent amino acids. Amino acids have historically been separated by chromatographic separation for analytical requirements but separation commercially has not been possible due to the close physical and chemical properties of the amino acids. The study looks at two amino acids, isoleucine and alpha amino butyric acid and used operating and process variables to conclude whether a commercial application is possible.

A 3m glass column operating at atmospheric conditions was set up in the development laboratory at SA Bioproducts for test work. Pulse tests were carried out to evaluate the separation of the individual amino acids and the samples were analysed using HPLC. The column incorporated a jacket which allowed for constant temperature during the experiments.

Experiments started initially with the column being tested for hydraulic consistency and this was done using resin in the Na+ ionic form and passing a NaCl feed stream over the resin as a pulse. A base case was chosen and this using a feed volume of 0.1bed volume, an eluant flow of 1 bed volume per hour, feed concentrations of 2% and an operating temperature of 75°C. The resin was used in the Na^+ ionic form, with a particle size of 320μm, and a height of 1.5m. Results of the base case indicated a low resolution of 38%, and low recoveries at 11.85% at purities of 86%. Evident from the chromatogram was that isoleucine was held more strongly than AABA by the resin as isoleucine was eluted last.

Increasing the concentration of the feed to 4% moved the peaks closer and caused more overlapping thereby decreasing the recovery. Decreasing the concentration resulted in better separation. From the results it was concluded that the separation was dependant on concentration.

The next variable which was studied was the effects of changing the feed pulse volume. For this the feed volume was increased to 0.2BV and this ended up shifting the chromatogram to the right, with lower separation efficiency being measured.

The effects of eluant flowrate were studied next. Decreasing the eluant flowrate to 0.5BV/h resulted in sharper peaks and less overlapping. The conclusion reached was that the higher the contact time between the amino acids and the resin, the greater would be the separation efficiency. Increasing the flowrate of the eluant reduced the contact time and more overlapping was observed.

The effects of temperature were also investigated. The experiment carried out at 90°C, showed very similar separation to the base case with higher recoveries. Decreasing the temperature to 30°C decreased the separation efficiency drastically. The experiments proved that an increase in temperature increased the kinetics, and allowed the amino acids to enter and exit the bead quicker.

Once operating conditions have been evaluated, the resin was converted to the Ca^{2+} ionic form, and all other conditions were run according to the base case. From the chromatogram it was obvious that the amino acids were held more strongly by the resin and the retention time was increased thereby increasing the recovery.

Changing the particle size of the resin from 320μm to 220μm increased the separation efficiency due to the faster kinetics. Increasing the height of the resin was attempted by adding more resin to the column. The separation efficiency was increased.

Finally a optimised experiment was attempted where a 2% feed solution was used, feed volume was 0.1BV, temperature of 90°C, eluate flow of 0.5BV/h, resin in the Ca^{2+} ionic form, with a particle size range of 210–230μm, and resin height of 1.8m. The chromatogram indicated high peak separation efficiencies and high recoveries. The results indicated that the study was feasible.

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1. Introduction

Ion exchange has been used extensively for separating amino acids with large differences in selectivity coefficient (α). When the ratio of the amino acid selectivity for the resin is close to 1, as is the case with most mono-valent amino acids, separation of the amino acids using ion exchange becomes impossible.

In classic ion exchange operations, an ion in solution actually exchanges with an ion on the resin. In contrast to this, ion exchange chromatography uses the ion exchange resin as an adsorption and molecular size separation medium, providing an environment that allows two or more solutes in the feed stream to be separated. The feed solution is added to a chromatographic column filled with ion exchange beads and is eluted with solvent, usually water. The resin shows a selectively for certain solutes, and slows them down, while others are eluted unhindered down the column. The components separate and their individual purities increase. Eventually the solutes appear at different times at the column outlet where each can be drawn off separately.

Chromatographic separation mechanisms can be divided into four groups according to the mechanism of separation. Separation can be achieved due to affinity difference between the molecules, ion exclusion, size exclusion, or ion retardation of the solute.

The aim of this thesis is to use chromatographic separation to separate a mixture of similar amino acids to increase the purity of the more valuable amino acid. Apart from the nature of the amino acids, the separation efficiency of the process largely depends upon the operating or process conditions. Variables include volume of the feed, flow rate of the eluant, relative concentrations of the components in the mixture, temperature, and resin properties i.e. moisture content, particle size and particle size distribution (PSD). Optimisation of these parameters is a prerequisite for the application of chromatography for the separation of amino acids on an industrial scale.

The amino acid of commercial interest to SA Bioproducts is isoleucine, destined for the pharmaceutical industry. Isoleucine is produced by a fermentation process and separated from the fermentation broth using classic ion exchange. During the fermentation process α–amino butyric acid (AABA) and other mono-valent amino acids are formed as by-products. All of these follow the isoleucine through ion exchange and crystallisation, to crystallise out with the isoleucine. Particularly the AABA is extremely difficult to remove once in the crystal and even a re-crystallisation step is insufficient. It is the intention of SA Bioproducts, to use this chromatographic separation project to separate isoleucine from AABA, by scaling up the separation and inserting it into a process flow sheet between ion exchange and crystallisation. The ratio of isoleucine to AABA in the feed stream is approx. 30:1. For the tests a 1:1 ratio of isoleucine to AABA will be used.

2. Literature Review

2.1. Definition of chromatography

Chromatography is a separation method which employs two phases; a stationary and a mobile. Chromatographic methods are classified according to the nature of the mobile and stationary phases used. The term gas chromatography (GC) and liquid chromatography (LC) refer to the nature of the mobile phase. A mixture of solutes is introduced into the mobile phase and carried through the system by the mobile phase. As the mobile phase passes over the stationary phase, the components of the mixture spend time in both phases. A solute which spends a small amount of time in the stationary phase will be swept forward rapidly by the mobile phase. A solute which spends a greater amount of time in the stationary phase will take a longer time to pass through the chromatographic system (Dorfer, 1990). Different solutes are thus separated into bands based on their distribution between the phases.

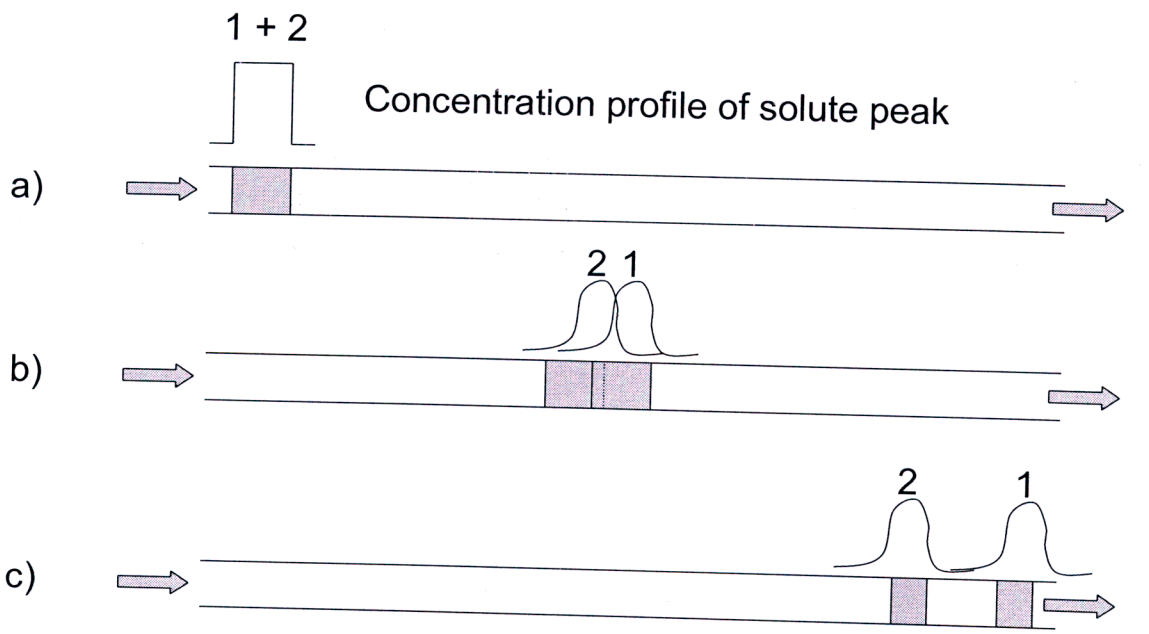


Fig. 1 Separation of a mixture of two solute components 1 and 2 by elution chromatography. a) Shows the solute band immediately after entry of the band into the column. b) and c) show the band separating into two component bands as it passes through the column.

Chromatographic separations are distinguished by their high selectivity that is their ability to achieve separation between components of closely similar physical and chemical properties. Many mixtures which are difficult to separate by other methods can be separated by chromatography. The range of materials which can be processed covers the entire spectrum of molecular weights, from hydrogen ions to proteins.

Chromatographic techniques have been used routinely for chemical analysis since the 1950's and for the automated analysis of process streams in process control since 1961. They have also been extensively developed as rapid and accurate methods for measuring a great variety of thermodynamic, kinetic and physico-chemical properties. The commercial application is often called production or large scale chromatography to distinguish it from smaller laboratory scale preparative and analytical chromatography. Production chromatography is a relatively new entrant to the range of unit operations available to chemical engineers. Its use is increasing as the demand for high purity materials grows (Dorfer, 1990).

2.2. History of Chromatographic Separation

The chromatographic principle was first conceived and tested by operating in a batchwise mode: that is by injecting a small volume of the solution to be separated into a packed column with a continuous flow of mobile phase.

During the 1960's and 1970's the scale up of chromatographic processes favoured the batch mode, mainly due to the simplicity of operation and relative ease in scale up (Siegell, 1982).

Abcor, a development technology company, was very active in the 1960's performing the scale up of batch chromatographic systems. They began by offering standard plants of different sizes with columns up to 1.3m. The stainless steel plants were designed to be as versatile as possible and were adequately instrumented and automated. The system incorporated up to 5 columns which could be interconnected in series to form a batch system. These systems were targeted towards the separation of chemicals that were not readily or economically produced by any other methods, such as flavours and fragrances, petrochemicals and pharmaceuticals.

Another company, Finnish Sugar, in 1962 decided to concentrate on the development of batch chromatographic processes. They designed and built many batch processes throughout the world. Their field of specialisation was the desugarisation of beet molasses, where sucrose is recovered by employing mainly ion exclusion in the Ca^{2+} charged crosslinked polystyrene resins. In 1975 Finnish Sugar installed the then largest batch chromatographic molasses separation plant in the world, consisting of one 2.7m-ID by 6m-high column. This recovered up to 95% sugar in the molasses and obtained sucrose purities up to 92% (Siegell, 1982).

In 1983 a new plant was completed for Amino GmbH in Frellstedt, Germany, for the recovery of beet molasses and purification of amino acids. Seven resin filled columns 3.6m-ID by 12m-high were used in the separation step (Jagsuchies, 1997).

Finish Sugar have also developed preparative and commercial scale systems for the production of mannitol, xylose, betaine, and amino acids such as alanine, valine and leucine. To date their biggest application of chromatographic separation is fructose purification.

Pharmacia Fine chemicals (Sweden) build batch chromatographic columns of various sizes. Packed columns have been used for the purification of milk protein from whey, the fractionation of proteins and amino acids, preparations of enzymes, and the purification of penicillin from high molecular weight impurities (Jagsuchies, 1997).

In 2001 Degussa-Hüls AG and SKW AG, came together to become the new Degussa AG. They were joined in March 2001 by Laporte plc, a major British fine and performance chemicals company, forming a specialty chemicals giant. A core activity of this new company is the Fine Chemicals business unit, which offers fine chemicals and process technologies in the industry. Deggusa is able to run and develop optimum separation processes on laboratory and on pilot scale, and then transfer the process to industrial scale with custom designed separation (Jagsuchies, 1997).

In March 2000, Bayer Central Organic Pilot Plant (ZeTO) in Leverkusen, Germany installed a facility for the synthesis of chiral intermediates using simulated moving bed (SMB) technology (explained in Ch. 2.9.3). It is possible for the plant to continuously achieve a greater than 98% purity. The product streams are concentrated through falling-film evaporators, and the solvents are recycled. The pilot facility is operated continuously, and the capacity - dependent on the product properties – is up to 5 tons per year.

Chromatography is a new process to most sugar technologist and recently there has been interest shown in the move to continuous chromatographic separation (CSEP). The CSEP chromatographic separator, one of the most modern SMB industrial concepts, is uniquely flexible and adaptable to all the challenges in the growing use of chromatography for production scale separations. The technology has already penetrated

the food & sweetener industries on a commercial basis. The CSEP concepts comprise a mechanical and process methodology to efficiently bring into contact liquids and solids as two counter-current contacting streams (explained in greater detail in Ch. 2.9.3).

2.3 Comparison of Chromatographic Separation with other Chemical Separation Methods

Food and chemical process industries use a variety of separation technologies, such as distillation, crystallisation, precipitation, ion exchange, chromatography and others. Each chemical separation method makes use of physical or chemical property differences between the chemicals to be separated in order to effect their separation. To appreciate the advantages of purification and recovery by chromatography it is useful to consider some of these other available approaches.

Fractional distillation is frequently used in situation where there is a large difference in relative volatilities between the components of a mixture. However at relative volatilities below about 1.3, distillation becomes much more costly because more plates or higher columns are required. Thermal sensitivity of the mixture, will also require distillation under vacuum conditions, adds to the cost and complexity of the system.

Crystallisation can be used when the solubilities of the components are sufficiently different, and is a useful way of obtaining very high purity products. However complete separation is rarely if ever possible due to the presence of eutectic composition where other components simultaneously crystallise. This effectively limits the recovery per stage that can be accomplished by crystallisation.

In solvent extractions there are several solvents that accomplish separation by changing the relative activities of the mixture components. These applications generally accomplish class separations by functional group, such as diolefins from mono-olefins and saturates, or aromatics from naphthenes and paraffins. However these systems can rarely discriminate between isomers, which is often critical when the end user requires a specific compound. Also these specialised solvents must be recovered and re-cycled back into the process. (Nicoud, 1991).

Solid adsorbents can be obtained that have a significant affinity or selectivity for one component of a mixture over all others. In the general case all components may enter the

pores but certain ones will be adsorbed more strongly. The relative strengths of adsorption between the desired component and the other adsorbed species in the pore establish the selectivity and thus give a means for the separation

By making deliberate changes to the absorbent, the magnitude as well as the order of selectivity can be adjusted. These changes in selectivity can thus be used to obtain a desired isomer. Adsorptive separation can simultaneously accomplish a class and isomer separation. Adsorption also offers significant improvements in mass transfer efficiency over the conventional equipment used in solvent extraction or extractive distillation. The small adsorbent particles used in the process have high surface area, low diffusion resistance and prevent axial mixing in the continuous liquid phase. As a result efficient mass transfer is accomplished at temperatures that are low enough to avoid degradation of the thermally sensitive feed components (Nicoud, 1991).

In summary the general characteristics favouring a chromatographic method are:

a) It can achieve difficult separations.
b) It can meet high product purity specifications.
c) It can separate heat sensitive compounds.
d) It has relatively low energy consumption.
e) It can split an n-component mixture into n pure components in one column instead of (n-1) columns.
f) Product is usually recovered in a non toxic carrier from which it is easily separated.
g) The technique is very versatile. An appropriate type of chromatography is available for most separations.
h) It is well suited to both low volume/high value separations and high volume/low value separations.

The disadvantages of using chromatographic separation on a commercial scale are:

- It is an extremely difficult process to maintain at steady state. Any changes in process variables (flowrate, temperature, etc) or resin channelling upsets the stability of the profile and the system would need to be restarted. This is overcome with highly accurate and reliable instrumentation which is fully automated.
- Capital investment for a continuous separation unit is very high, especially considering the entire unit needs to be at a constant temperature. The unit becomes more expensive as the temperature is increased.
- One negative aspect which limits the application of chromatographic separation is the dilution of the product. Usually the product collected is diluted to 10% of the original concentration and this in turn requires more vigorous evaporation to achieve a saleable product. In order to decrease the load on evaporation i.e. increase the concentration of the extract, we can manipulate the process variables to give the maximum concentration of components. These include keeping a constant temperature, limiting the linear velocity, maintaining a constant ionic form of the resin and recycling of the dilute tailing portions of the extract (Dorfer, 1990)
- The separation efficiency is as much dependent on the resin packing as it is on the process variables. For large columns compression packing techniques need to be undertaken and these are specialised techniques. If they are not done correctly, separation efficiency is decreased.

Due to the complexity and lack of understanding, chromatographic separation is rarely attempted and the field has been limited to pharmaceutical grade products, and the sugar industry.

2.4. Separation Mechanisms

Chromatographic separation can be accomplished depending on the physical and chemical properties of the solute by one of the following mechanisms. Ionic exclusion together with size exclusion is a separation based on physical properties, while ligand bonding, chiral separation and acid retardation are all based on chemical reactions and properties of the solutes (Dorfer, 1990).

2.4.1. Ionic exclusion

Because of the way water molecules surround metal ions (e.g. Ca^{2+}, Na^{+}, etc,) the surface of a cation exchange resin has a negative charge that is a barrier to negatively charged anions. Since negative charged anions cannot enter the cation exchange resin and since it is required that positive and negative charges be balanced inside the resin, ionic salts are excluded from concentrating inside the resin. Sugars and other small organic molecules which are not electrically charged are free to enter the resin bead. This difference between neutral organic molecules and the charged molecules of salts allows a basis for separation of the organic material from the salt present in a solution.

2.4.2. Size Exclusion

The size difference between large and small molecules may be used to achieve a separation using chromatography. Large molecules can be completely or partially excluded from entering the chromatographic separation media because of its size. Large molecules will be limited in the extent to which they may enter the separation media(resin) and will elute from the column/resin first. Small molecules will more completely diffuse into the channels of the resin. This difference between large and small molecules allows a separation of the different chemical compounds based on size exclusion.

2.4.3. Ligand Bonding (Coordination Complex)

This separation of solutes is based on ligand bond formation between the separation medium with some compounds in a mixture. The separation of fructose and glucose isomers is a good example of this mechanism; the fructose molecule will form a ligand bond with the calcium ion attached to the separation resin. The glucose ion does not form a strong ligand bond because of the different structure of its molecule. This deference in the attraction allows a separation of fructose and glucose to be made with the fructose molecule eluting more slowly from the resin bed.

2.4.4. Acid Retardation

This is a separation based on acid affinity for anion exchange resins. The acid retardation mechanism allows the separation of strong acids from their salts by passing the solution through strong base anion resin in the corresponding salt form. It is also used for acid recovery as a means of effluent control.

2.4.5. Chiral Separations

The chirality of biologically active chemicals is increasingly recognised as important to their activity and toxicology. Pure enantiomers (optical isomers) of pharmaceuticals and agrochemicals can in many cases be made by stereospecific synthesis. An alternative method is to use a less complicated synthesis followed by chromatographic separation of the racemic mixture. Since all the physical properties of two given enantiomers are the same in the absence of a chiral (optical active) medium, their separation is a more difficult chromatographic problem than the relatively simple separation of geometric isomers, stereoisomers or positional isomers. Several methods have been devised. The older technique of indirect resolution required conversion of the enantiomers to diastereoisomers using a suitable chiral reagent, followed by separation of the diasterioisomers in a non-chiral GC or LC stationary phase. This technique is now being rapidly superseded by direct separation, using either a chiral mobile phase (in LC) or a chiral stationary phase.

2.5. General Principles

Three parameters serve to describe the dynamic and chemical process taking place in the column: flow rate, pressure drop and capacity. Before going into details regarding these terms the term bed volume (BV), which is frequently encountered in ion exchange applications, needs to be characterised. It represents the total volume of resin in the column, including both the volume of the resin and the void space between the beads. The void space of closely packed spherical particles is 40% of the total. The term bed volume refers to the total volume occupied by the resin bed after it has been backwashed and settled to achieve complete hydraulic classification (Horvath, 1983).

Another set of terms sometimes misunderstood relates to flow rates. The quantity of liquid flowing through different columns can be expressed in ml/min. A more precise indication of the flowrate should be expressed in ml/cm^2.min. This is in fact the linear velocity of the liquid flowing down the column, in cm/min. It is independent of column size or shape and is one of the most important parameters which define the process. In the laboratory use of columns, it has become common practice to refer to volumes of liquid applied to a column in terms of another column-independent parameter, BV/h. BV/h defines only the volume of eluant relative to the volume of the resin bed, but does not define the linear velocity (Horvath, 1983).

The up flow velocity is also important since it is the characteristic which defines the hydraulic expansion of the ion exchange resin. This is termed backwashing and is done to remove any solids which have become entrapped in the resin as well as any air bubbles. There needs to be enough freeboard to permit approx 40% bed expansion, although exact values are specified by resin manufacturers (Guyot, 1982).

2.6. Characterising Separation Performance

2.6.1. Retention Theory

The theory of retention utilises the time a solute is retained in a chromatographic column between injection and elution. The retention time t_R is defined as the time taken for a solute to travel through the column. Data are most conveniently expressed as a concentration-time plot (the chromatogram). A part of this time t_M is required by the solvent simply to pass through the mobile phase from inlet to outlet. The adjusted retention time $t`_R$ represents the extra retention due to repeated partitioning or distribution of the solute between the mobile and stationary phases as the band migrates along the column i.e. it is the time spent by the solute in the stationary phase (See figure 2 below).

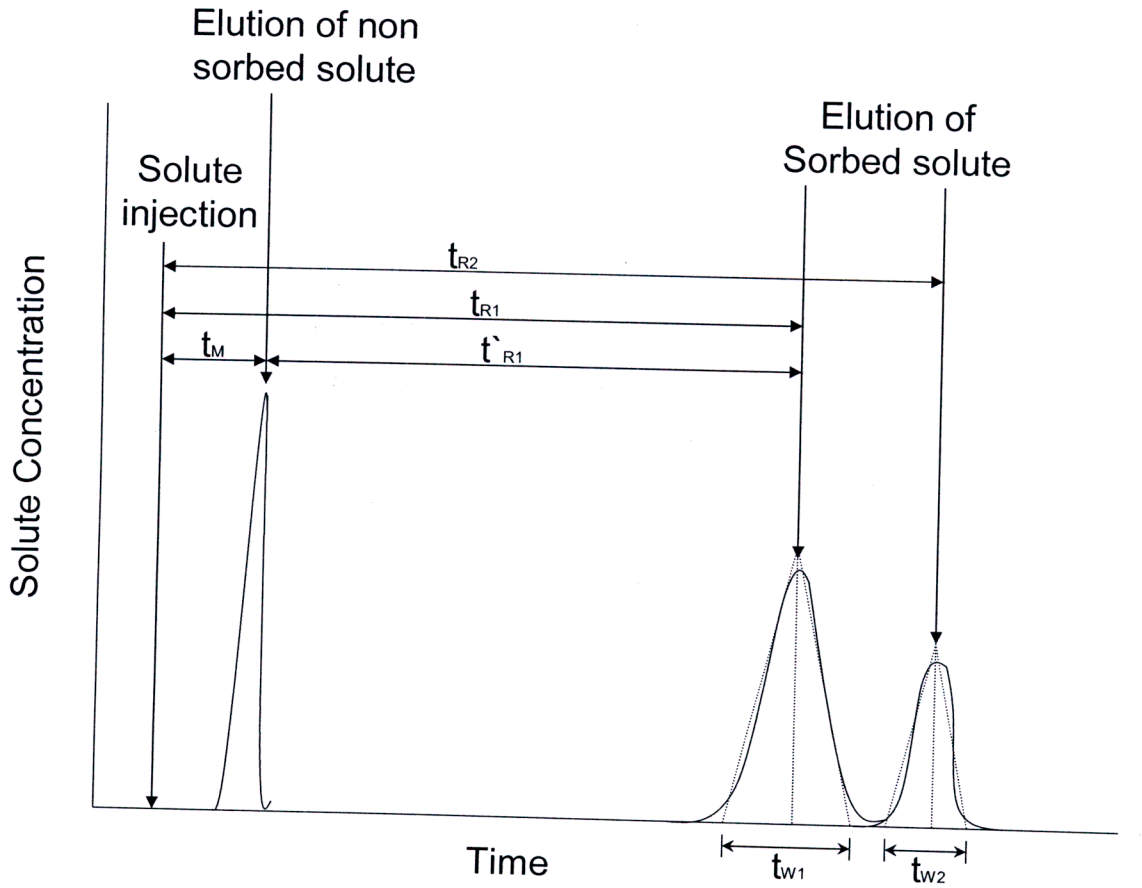


Fig. 2 Elution profiles obtained by chromatography of a mixture of two solutes (Kempe, 1999).

t'_R is equal to $t_R - t_M$. t_W is the width of the solute band at the baseline i.e. the distance between the points of intersection of the baseline with the tangents at the point of inflexion of the sides of the band (Kempe, 1999).

The average molecule of a given solute moves repeatedly in and out of the stationary phase during its passage through the column. The molecule spends some of its time swept along by the mobile phase and some of its time in residence in the stationary phase. If R is the ratio of the total time spent by this average molecule in the mobile phase to the total time spent in the mobile plus stationary phases, then: (Kempe, 1999)

$$R = \frac{t_m}{t_R} = \frac{u_R}{u} \qquad (1)$$

Here u_R is the velocity at which the solute band moves along the column and u is the velocity of the mobile phase is defined as the superficial velocity divided by the fractional volume occupied by the mobile phase ε.

The average molecule is representative of a large number of identical molecules of the solute. Hence R defined above as the fractional time the average molecule spends in the mobile phase, can also be viewed as the fraction of the total number of molecules that are in the mobile phase at equilibrium:

$$R = \frac{n_M}{n_M + n_S} \qquad (2)$$

or:

$$R = \frac{1}{1 + k'} \qquad (3)$$

Where k′ is the mass distribution co-efficient, n_M and n_S are the number of moles of solute in the mobile and stationary phases respectively, in an elemental height of column. Equating the expression from (1) n_S/n_M, and (3) for R:

$$u_R = \frac{u}{1+k'} \qquad (4)$$

or

$$t_R = t_M(1+k') \qquad (5)$$

Equation 5 is the basic retention equation of elution chromatography. It is founded on the assumptions that the distribution isotherm is linear and that equilibrium of the solute between phases is achieved instantaneously throughout the column. This is only true for dilute solutions.

2.6.2. Band Broadening and Separation Efficiency

The equations in the previous section relate only to the average molecule and so describe only the mean retention time of the band. The mean retention time is that of the peak of the band if the band is symmetrical. In practice there is a spread of time about this mean due to several processes which tend to broaden the bands as they migrate through the column, which can be seen below from figure 3 (Pailat, 1999).

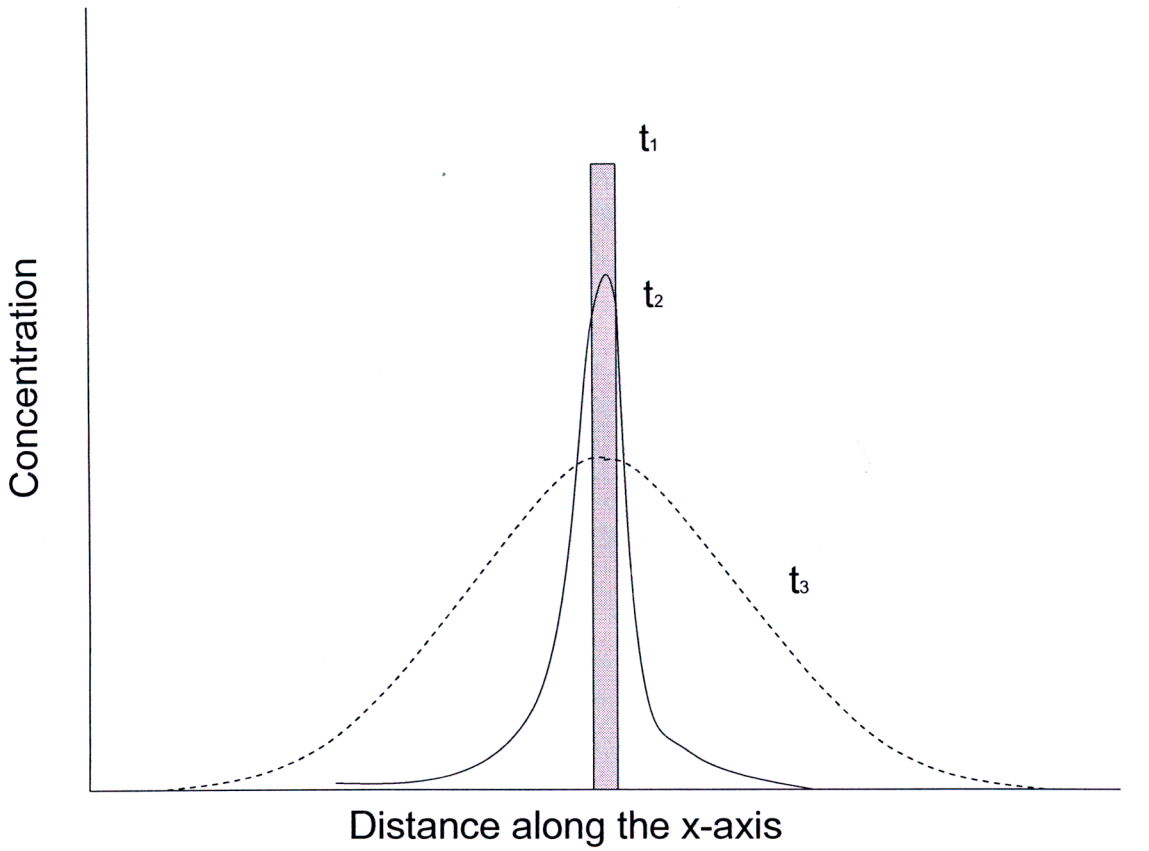


Fig. 3 Zone widening due to diffusion. (D.Pailat, 1999)

t_1 = 2 hours, t_2 = 1 hour, t_3 = 15minutes.

t= time taken for the eluate to pass through the column.

Band broadening can be characterised by theoretical plate height and outline the causes of band broadening. This provides a basis for understanding why modern chromatography is an efficient separation technique.

2.6.3. Plate Height

In an ideal column a solute band would retain its initial profile unaltered as it migrated along the column. In a real (non-ideal) column an initially narrow band broadens by dispersia as it migrates. The band width is proportional to the square root of the distance travelled along the column

The rate at which the band broadens depends on the efficiency of the column. This is more precisely defined as the height equivalent to a theoretical plate (HETP). HETP is defined as a unit of column length sufficient to bring the solute in the mobile phase into equilibrium with that in the stationary phase. Plate models using this concept show that the HETP of a column of length L may be determined by injecting a very small amount of solute onto the top of the column and then measuring its retention time t_R and band width t_W at the column outlet (Nicoud, 1991).

$$HETP = \frac{L}{N} = \frac{L}{16\left(t_R / t_W\right)^2} \qquad (6)$$

The greater the ratio t_R/t_W, the greater the number of plates N in the column.

Small HETP and large N values maximise separation efficiency. In general terms this requires that the process of repeated partitioning and equilibrium of the migrating solute is accomplished rapidly.

2.6.4. Resolution

The resolution between two solute components achieved by a column depends on the opposing effects of

a) The increasing separation of band centres and,

b) The increasing band widths as bands migrate along the column.

The resolution R_S is defined by (Yoritomi, 1981):

$$R_S = \frac{2(t_{R2} - t_{R1})}{(t_{W1} + t_{W2})} \qquad (7)$$

This is an important basic equation for chromatography. The equation needs to cope with the wide, concentrated, feed bands of preparative and production chromatography, it shows how separation is controlled. The great power of chromatography as a separation process lies in the high values the can be achieved in columns (Yoritomi, 1981).

2.6.5. The Separating Power of Chromatography

The selectivity (or separation) factor, α, is a ratio of mass distribution coefficients and so is a thermodynamic rather than a kinetic factor. The value of α depends mainly on the nature of the two solutes, on the stationary and the mobile phase. It is the analogue of the relative volatility in distillation. If a liquid mixture has two components for which the relative volatility is close to one they are difficult to separate by distillation because the relative volatility cannot be controlled without introducing another constituent (as in azeotropic or extractive distillation). The presence of a stationary phase changes relative volatility. Correct choice of the stationary phase (and in liquid chromatography, the mobile phase too) can greatly enhance (α-1) with a corresponding beneficial effect on resolution.

A second reason why very high resolution can be obtained in chromatography is that very large numbers of theoretical plates are readily achieved. If the column is well packed with particles having narrow spread of sizes, the plate height is about twice the particle diameter. A typical large scale GC or LC column will contain 1000 to 10 000 stages (Paananen, 1995).

2.7. *Resin Variables which Influence Separation Performance*

2.7.1. Moisture Content

The moisture content of the resin bead has a direct influence on the separation efficiency achieved, which can be summarised as follows (Jagsuchies, 1997):

- Decreasing resin crosslinkage increases moisture content of the resin as the polymer chains are less tightly bound together.
- Increasing resin moisture content increases resin gel phase diffusion rate.
- Increasing resin moisture content increases the solute holding capacity of the resin.
- Increasing resin moisture content increases resin osmotic loading, which could result in resin breakage.
- Ionic form of the resin affects moisture content due to the change in hydrated radius of the ions exchanged on to the resin (e.g. the separation of sucrose from salt in molasses is negatively affected by calcium ions which displace sodium or potassium ions on the resin). The monovalent elements (K,Na) have the highest moisture content and the divalent elements(Ca) have the lowest.

2.7.2. Bead Size

The resin bead size which is chosen is also very important, as a large beads decrease the separation efficiency. Beads which are too small will give good separation but might result in high pressure drops. The effects of bead size are summarised below.

- Large resin beads have a lower surface to volume ratio than small beads i.e. surface area for mass transfer is smaller.
- Large resin beads have a longer diffusion path to the centre of the resin bead. They are kinetically slower.
- Larger effective bead size at the same particle size uniformity reduces pressure drop.
- Large resin beads are more likely to fracture.

2.7.3. Particle Size Distribution

High particle size distribution increases the variation of lengths that the solute has to travel through the bead. This in turn slows down the rate of diffusion for large beads and quickens up the rate for small beads. The overall effect is poor resolution and increased pressure drop. For good separation the particle size range must be very narrow (Begovich, 1983).

2.8. *Process Variables which influence Separation Performance*

2.8.1. Operating Temperature

The operating temperature of the separation is also very important, and generally a higher operating temperature is favoured due to the following reasons (Moore, 1984):

- Increasing operating temperature increases the rate of diffusion and results in improved mass transfer rates, which sharpens the profile.
- Increasing operating temperature reduces solution viscosity and density resulting in reduced pressure drop through the column.
- Increasing operating temperature may affect distribution of solutes between the stationary phase (resin) and the mobile phase.
- Increasing operating temperature will have the negative effect of degradation of heat sensitive material.
- Increasing operating temperature will result in more rapid resin degradation due to increased rate of oxidation.
- Increasing operating temperature may reduce the growth of troublesome micro organisms.

2.8.2. Feed Concentration

Changes in feed concentration may affect equilibrium distribution of solutes between the stationary and the mobile phase. Changes in feed concentration affect the rate at which the concentration wave fronts move through the separation column. Increasing the feed concentration has the following effects (Moore, 1984):

- Reduces the moisture content of the resin resulting in reduced diffusion rate through the resin bead.
- Increases the viscosity of the mobile phase and results in reduced film diffusion rate. The increased viscosity also results in increased pressure drop through the separation resin.
- Result in an increase in column productivity(production per unit time) since more solute is added to the separation column at the same volume flow rate. This maybe offset by lower product purity and recovery.

2.8.3. Resin Volume

Economic performance of separation systems is typically measured by production rate per unit resin volume. As the productivity per unit resin volume is increased, by higher solvent flows, the separation efficiency is reduced.

2.8.4. Linear flow Velocity

Increased superficial fluid velocity decreases the depth of the boundary layer of fluid surrounding the resin bead. This typically reduces film diffusion mass transfer resistance. Increased superficial fluid velocity results in higher pressure loss through the separation column (Moore, 1984).

2.9. *Commercial Separation System Operations*

Batch chromatography is the simplest form of chromatography so naturally it was the start of the commercial process. These have since been developed into more complex and continuous processes which are moving column technology (CSEP) and simulated moving bed (SMB) technology.

The chemical engineer's preference for operating processes continuously wherever possible has led to the development of alternative modes of operation to cyclic batch chromatography. True moving bed processes which involve movement of resin as packed slurry through a pipe or tank, have shown problems of solids attrition, large axial mixing and mechanical complexity.

2.9.1. Batch Systems

The batchwise route to scaling up the chromatographic process has naturally been the obvious choice and as expected has led the way with many large scale applications. The choice of the type of stationary and mobile phase depends on the type of application and the properties of the mixture to be separated. The eluant used for transporting the feed along the length of the resin column is termed the desorbent. The component of interest is collected together with eluant and is termed the extract; similarly the other solutes are collected with eluant and termed raffinate. (See figure 4 for details (Burke, 1983)).

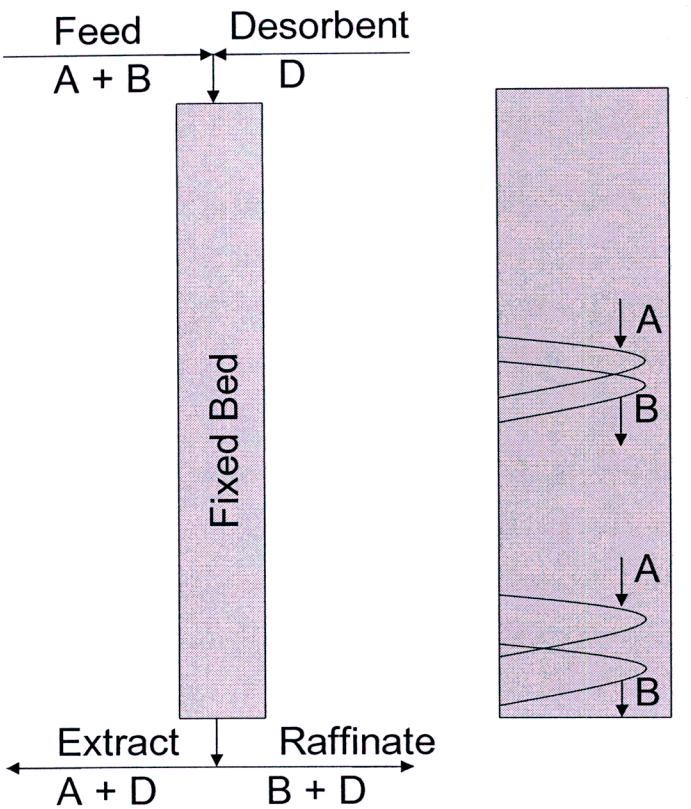


Fig. 4 Batch adsorption conventional chromatographic operation.

More than one feed pulse can be introduced into the column before the components have been eluted. Pulse intervals must allow sufficient time for partitioning each pulse separately. Several products and recycle cuts are taken from the solution exiting the column.

Numerous developments in scale up and application of batch chromatography have taken place in the last 30 years. Large scale batch chromatography is now used increasingly in the biotechnology field.

During scale up from pilot plant to commercial operation dynamic similarity must be maintained between the small and large scale columns. In order to maintain overall dynamic similarity it is important that the resolution be the same. This is achieved by scaling up the system in the following way (Begovich, 1983):

- Keeping the eluant linear velocities identical. If the linear velocity is changed the peaks may show signs of "finger formation" or "tailing".

- Holding constant the physical variables such as the temperature and concentration.
- Ensuring correct packing techniques are used. This was a problem in early 1960's and various baffle systems were incorporated into the column design. The baffle arrangements cleaning and classification of the resin in the column (backwashing) was made difficult and modern chromatography has changed towards a baffle free system. In large scale columns it is difficult to fill columns before settling occurs. For this reason the column should be backwashed to expand the resin adequately once resin is filled. In cases where insufficient head space is available the resin can be physically recycled around the column to ensure perfect classification.

The choice of mean particle size of the stationary phase must take into account the distribution coefficient of the components to be separated, the viscosity of the solution, and the optimisation between maximum throughput and the desired final purity. Generally smaller beads equals better resolution, but higher pressure drops. Flow uniformity is achieved by optimizing the flow rates, temperature and solute concentrations, thus controlling the viscosity and density gradients. Care needs to be taken when designing the column inlet and outlet so as to ensure no hold up areas to allow for back mixing. To increase throughput and optimize the total volume of packing available the repetitive feed injection technique is employed whereby by proper timing the leading edge of the fastest moving component from a following injection comes out just after the tailing edge of the slower moving component of the first injection, or they overlap each other to a predetermined extent. This overlapped fraction is then recycled. Recycling of up to 40% of the feed is commonly practiced (Broughton, 1978).

2.9.2. Continuous Countercurrent Chromatography: Moving Bed/Column Systems

Almost 40 years ago, researchers realised the benefits of developing large scale chromatographic processes operating continuously and employing a counter current contact between and liquid and an appropriate chromatographic packing. Two main schools of thought developed: the moving bed and the moving column systems.

The moving bed appeared first. With this system the packing flowed under gravity while the mobile phase travelled countercurrent in a vertical column. The feed stream was injected into the system continuously at the centre of the column. The least strongly absorbed component was carried upwards with the mobile phase and was withdrawn from the top of the column, while the more strongly absorbed component moved downward with the packing and was stripped at the bottom part of the column. The packing slurry was then pumped back to the top of the column for recycle (Begovich, 1998).

Work carried out on moving bed systems and some large systems have found commercial application. Moving beds have been found to suffer from difficulties in achieving control of the falling solids at increased throughputs, reduced mass transfer and forcing low mobile phase velocities, to prevent fluidisation of the bed (Cores, 1979).

To overcome the foregoing limitations, researchers switched their efforts into developing systems where the whole bundle of columns was rotated past fixed inlet and outlet ports. These moving column systems usually consisted of a circular array of interconnected columns in series rotating at a controlled speed. The columns rotate clockwise and the eluant effectively rotates counter-clockwise (Sayama,1992).

The principal of operation is shown below in figure 5, which represents the total packing length as a circle, arbitrarily divided into eight sections.

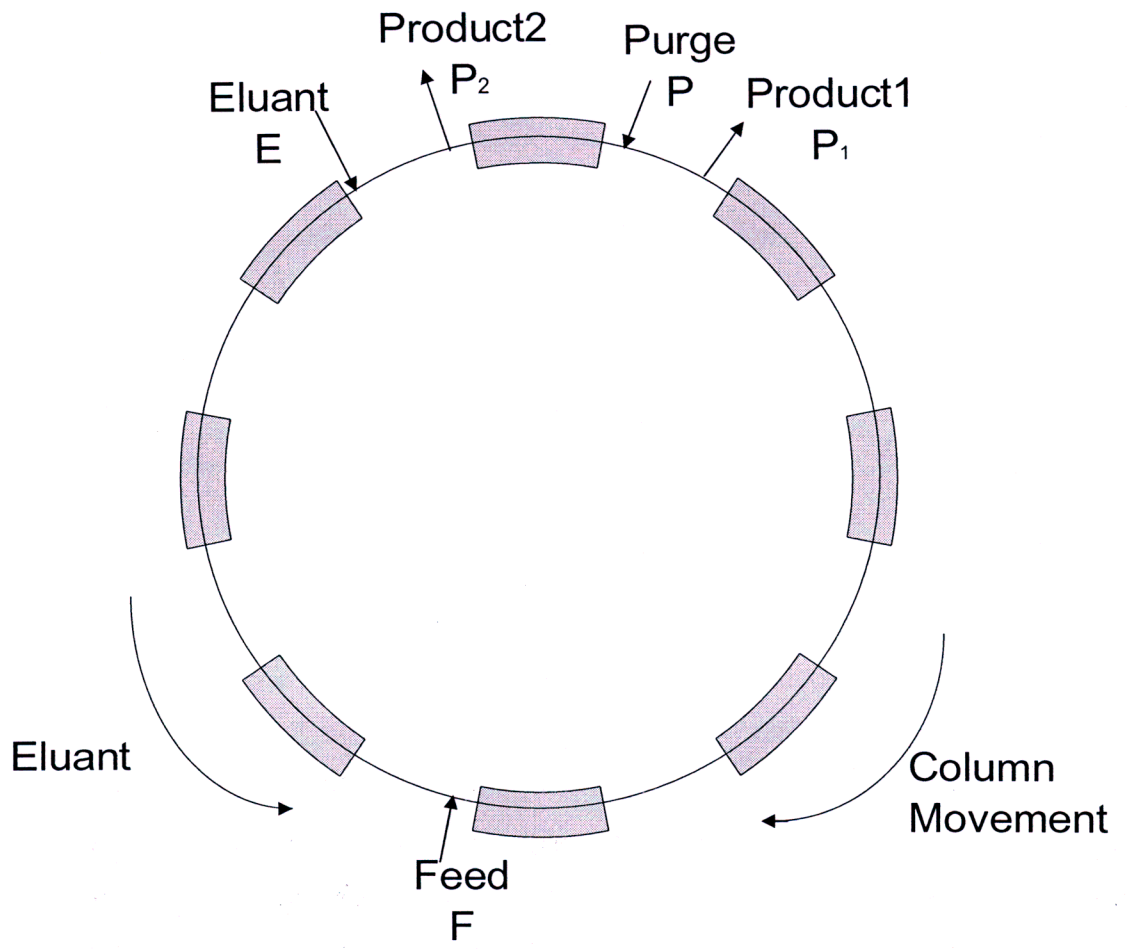


Fig. 5 Diagrammatic representation of continuous Moving Column Operation.

The original designs utilised locking valves between columns, to prevent backflow due to pressure differences. Modern plants have done away with these cumbersome valves by employing better fluid flow designs. The separation of a binary mixture, consisting of components 1(the faster moving component) and 2 (the retarded component) can be described as follows. The feed is injected at port F, between the eluant and the product port. The eluant enters at port E, carrying the faster moving component of the feed in a counter-clockwise direction towards its product port P_1. The slower component will travel clockwise with the resin flow, provided the relative velocities are carefully maintained

The faster moving component 1 is collected from port P_1. The slower moving component 2 moves with the resin and is ejected from the system at product port P_2, using the flow of the purge liquor pumped into port P. Unlike the eluant, the purge stream can be made to carry a low concentration of the resin counter ion, which serves to maintain counter ion integrity on the resin. The controlling parameters in the separation are the mobile phase flow rate (ports E and F) and the rate of movement of the column past the static ports (i.e. the resin flowrate). These variables need to be finely adjusted to ensure that the two components move efficiently in different directions to enable their continuous separation (K.Sayama1992).

2.9.3. Continuous Countercurrent Simulated Moving Bed (SMB)

Simulated countercurrent operation avoids the problems of moving beds by using a fixed bed and moving the positions of entrance and exit ports of the process streams. In this way a solid "stationary phase" can be effectively considered as a moving bed flowing countercurrently to the liquid "mobile phase". The feed mixture to be separated is fed continuously to a point in the middle of the separation profile. The ratio of the feed and desorbent flow rates is chosen so that the velocity of the desorbent lies between the velocities of the fast and slow moving components from the perspective of the feed location: the feed splits into two fractions moving in opposite directions from the feed point. The SMB technology allows continuous feed and desorbent introduction and continuous product and by-product withdrawal with a recirculating fluid flow. In the SMB system product purity in the resin bed increases as the distance from the feed point increases. From a feed inlet reference point, it appears as if the absorbent media is moving upstream and carrying with it the product molecules which it adsorbed, thus the term "Simulated Moving Bed". In this system the "Product" is the slower moving component and the "Byproduct" is the faster moving component.

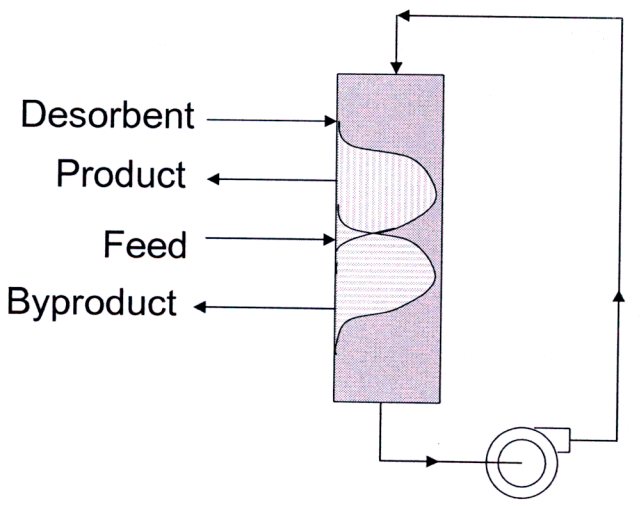


Fig. 6 Simulated moving bed Chromatographic Separation system.

Once a stable concentration profile has been established across the entire length of the separation system it moves slowly down the system with aid of re-circulation flow. As the fast and slow moving components separate in the re-circulating stream the separation profile concentrations are maintained by moving the location of the feed, desorbent, product and byproduct ports down the system at the same rate at which the profile moves (Ganetsos, 1992).

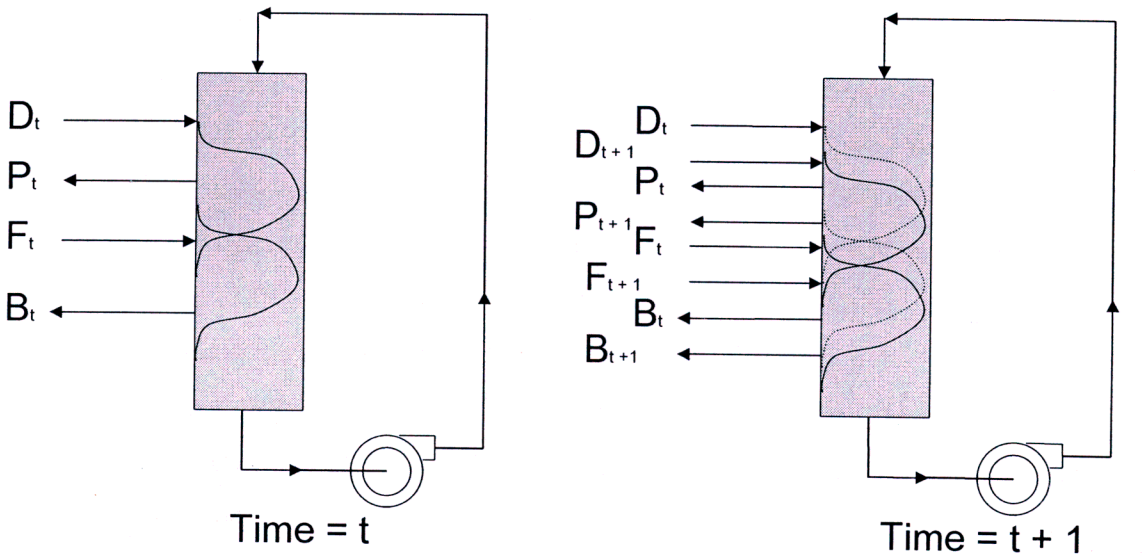


Fig. 7 Adjustments of valves to correspond to moving profiles (G. Ganetsos, 1992)

Movement of the introduction and withdrawal points is accomplished using either multiport valves or multiple manifolds with two position valves. There are auxiliary systems which complement the chromatographic separation system such as degasification of feed and desorbent streams prior to introduction to the resin bed to ensure that minimal oxidation of the resin occurs and ensuring no air bubbles are entrapped in the bed. The recirculating fluid flow can range up to six times greater than the feed flow (Ganetsos, 1992).

The advantages of SMB Technology over Batch Systems are:

- The entire stationary phase is continuously covered with the mixture to be separated which produces a much higher productivity.
- A 90% reduction in the demand for solvent due to solvent recycling.
- High plate counts are no longer required, reducing packing material by 80%.
- Extract and raffinate are extracted (in high concentrations) which make it easier to remove solvent.

2.10. Amino Acid Separation

Amino acids are the general name for chemical compounds having amino groups and carboxylic acid groups in the molecule, and they are differentiated as α, β, γ-amino acids depending on the position of the carboxyl groups.

Over 80 naturally occurring amino acids have been discovered. Amino acids manufactured by chemical synthesis are optically inactive racemic bodies which contain equal parts of L-body and D-body, while the amino acid units of proteins are L-body α-amino acids. Plants and microorganisms are able to biosynthesis all types of amino acids, but since animals are unable to biosynthesis sufficient amounts of them for growth or maintaining health, they have to be taken in as food. These amino acids are known as essential amino acids, and in the case of man they compromise eight types: L-valine, L-leucine, L-isoleucine, L-threonine, L-methionine, L-phenylalanine, L-tryptophan, and L-lysine. Amino acids, therefore, are used not only for increasing the nutrient value of food or livestock fodder but also as seasonings, food improvers and stabilizers, cosmetic additives, drugs and pharmaceuticals, and industrial raw materials.

Amino acids are purified by extraction, synthesis, fermentation and enzyme techniques. In 1908 glutamic acid was extracted and separated from wheat protein for the use as seasoning, and this marked the beginning of the industrial production of amino acids. Around 1955, a fermentation method was developed wherein microorganisms were grown in a culture medium, compromising mainly of sugars as a source of carbon, and ammonia as a source of nitrogen, so as to produce amino acids outside the cell in large quantities. The first amino acid to be produced by this fermentation process was L-glutamic acid. Subsequently with the selection of wild strains, the introduction of mutants and improvement of culture techniques, it has now become possible to manufacture practically all amino acids by fermentation.

Amino acids are all amphoteric electrolytes. If the pH is adjusted to be more acid than a certain value, they dissociate as bases; if on the other hand it is adjusted to be more alkaline than a certain value, they dissociate as acids. There is a certain pH at which

dissociation into cations and anions is equal, and this pH is known as the isolectric point (pI). Isolectric points for amino acid constituents of proteins are given in Table one below.

	Name	Symbol	pI
Basic Amino acids	Arginine	Arg	10.76
	Lysine	Lys	9.74
	Hydroxylysine	Hyl	8.64
	Histidine	His	7.59
Neutral Amino acids	Glycine	Gly	5.97
	Alanine	Ala	6
	Valine	Val	5.96
	Leucine	Leu	5.98
	Isoleucine	Ile	6.02
	Serine	Ser	5.68
	Threonine	Thr	6.16
	Asparagine	Asn	5.41
	Glutamine	Gln	5.65
	Cystine	Cys-Cys	4.6
	Cysteine	Cys	5.07
	Methionine	Met	5.74
	Phenylalanine	Phe	5.48
	Tyrosine	Tyr	5.66
	Tryptophan	Trp	5.89
	Proline	Pro	6.3
	Hydroxyproline	Hyl	5.83
Acidic amino acids	Aspartic acid	Asp	2.77
	Glutamic acid	Glu	3.22

Table 1, Values of pI were taken from "Biochemistry data book"

Amino acids are represented by the general formula R-CH(NH_2)COOH. Their properties are due to differences in the nature of the group R on the side chain, and have classified into groups which are represented below in figure eight.

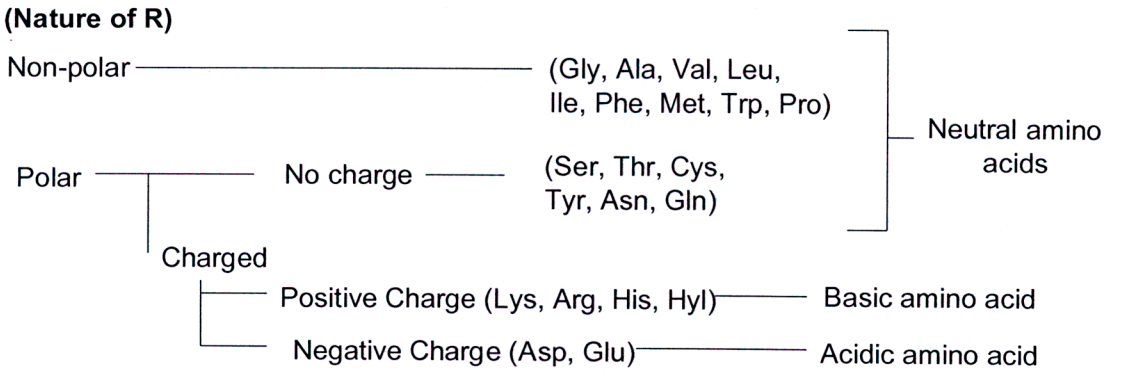


Fig. 8 grouping of amino acids. Separation of neutral amino acids into polar and non-polar amino acids

It can be seen from the pI values that using cationic exchange resins, an impure amino acid solution containing Arginine and valine can be adsorbed at low pH below the pI value. Increasing this pH using NaOH to above 6 but below 10 would cause the valine to be rejected by the resin and keep the arginine adsorbed. Once all the valine has been removed Arginine can be removed by increasing the pH to above 10. This has worked very well commercially to separate amino acids with very different pI values but is not successful in separating similar grouped amino acids, eg. Alanine and valine.

During the last 10 years chromatography has become an integral and indispensable part of the biotechnology industry. Separation of amino acids of similar groupings amino acids are separated at the isolectric point by a combination of mechanisms in chromatographic separation.

Not much literature is published on amino acids separation using chromatographic separation and most applications have been limited to the pharmaceutical industry because of the high purity required, and is usually operated on a preparative scale.

3. Experimental

The quality and design of the equipment is of utmost importance for successful application of chromatographic separation. The heart of the chromatographic system is the column. This is particularly true for larger columns, where the large internal diameter creates specific problems. A very severe problem with large columns is the bed stability. The stability over time depends on many parameters which can be divided into mechanical and chemical. The mechanical ones are related to the redistribution of particles in the column due to the lack of wall support. This phenomenon is connected to the existence of unstable regions formed in the bed during the packing process. They correspond to bridges which can collapse due to shear forces. The particles then redistribute and the entire bed settles, most often forming a void in the column inlet. At this point the column efficiency is reduced, resulting in poor separation. To overcome this problem compression of the bed must also take place.

Although the column is a major piece of hardware, the rest of the equipment has to be designed carefully. The pumping system is important, and also the overall plumbing of the unit, to ensure minimum pressure drop, constant flow and constant temperature of the system.

The packing material also plays a very decisive role. There are two aspects associated with the packing material: physicochemical and mechanical. The physicochemical aspect is related to the retention process (absolute retention and selectivity) and concerns the homogeneity of the surface (specified by the resin manufacturer).

3.1. Equipment Set Up

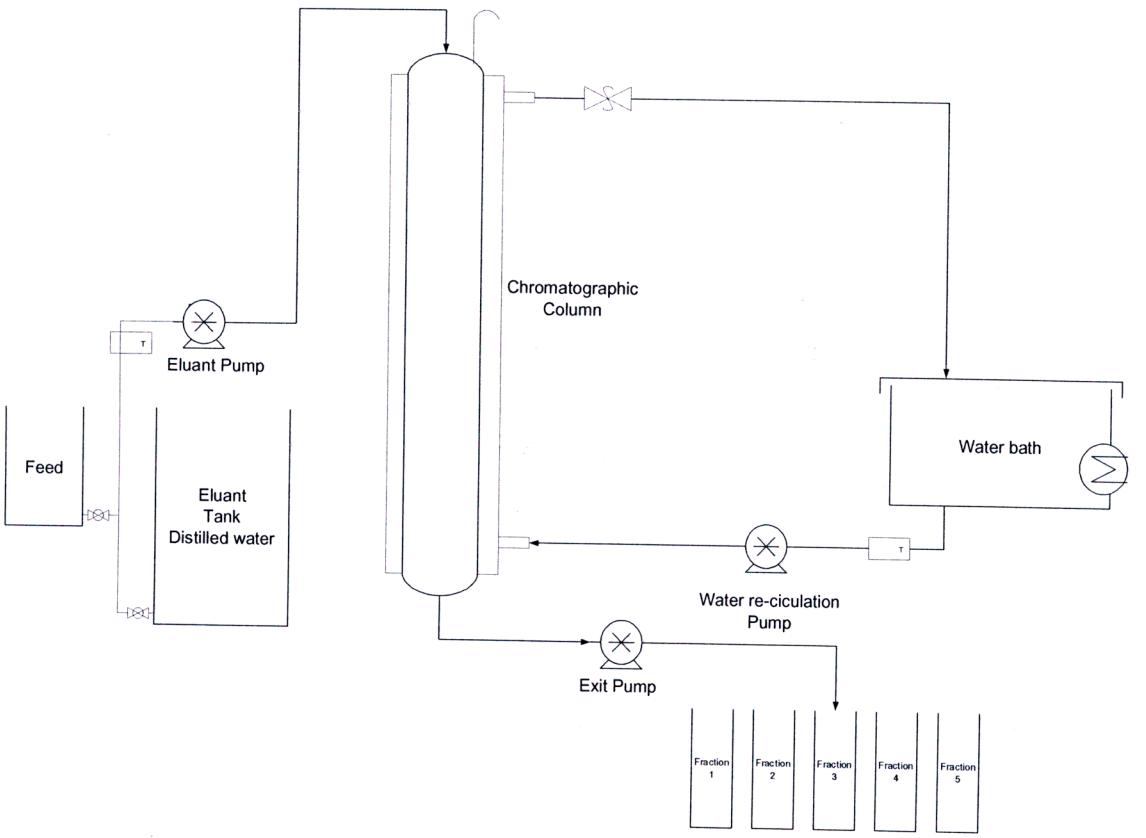
The pilot column used for the chromatographic separation described in this thesis is located in the development laboratory at SA Bioproducts (Pty) LTD, Umbogintwini. The company was in close contact with a German company (Amino GmbH) when designing and setting up the column. The column is a double walled 2m high glass column which uses re-circulating water in the jacket to keep the temperature of the resin and liquid constant. Internal diameter of the column is 51mm and the volume of the resin is 3.11*l* (1BV). Approximately 40% freeboard has been allowed. Since the column is made of glass there could be no pressure build up in the column. This was done by fitting an open vent to the top of the column.

Peristaltic laboratory pumps with variable speed drives were used to transfer the feed and exit streams. Silicon tubing was used at a diameter of 5mm. Another peristaltic pump was used to re-circulate water from a thermostat water bath through the jacket. This enabled the separation to be performed at a constant temperature. Tube lengths were kept as short as possible and insulation was used where possible. All pumps were calibrated and checked routinely.

The outer jacket chamber was 4mm wide and allowed for adequate heating of the resin. In order to ensure no pressure on the glass jacket a vent valve was incorporated into the design. Distilled water was used as the column eluant and this was heated by means of an element heater. It was important to ensure proper distribution of liquid through the cross section of resin. Poor distribution of liquid at the top of the column would promote channelling and this would interfere with the resolution of the separation. To avoid channelling a metal disk with 2mm holes was placed above the resin to act as a distributor and glass balls we placed over the disk. This ensured no splashing directly in the resin and the level of water was monitored constantly. In order to get the best distribution a level indicator was incorporated into the design which ensured that the column remained perfectly perpendicular to the ground.

Flowrates were measured by collecting a specific volume over a time period. Temperature of the feed and the water bath were closely monitored by means of a thermometer. Figure 9 below shows the setup of the chromatographic separation system. The equipment was set up so that the temperature gradient over the system was kept below 3°C.

Fig. 9a A schematic representation of the chromatographic system set up at SA Bioproducts.



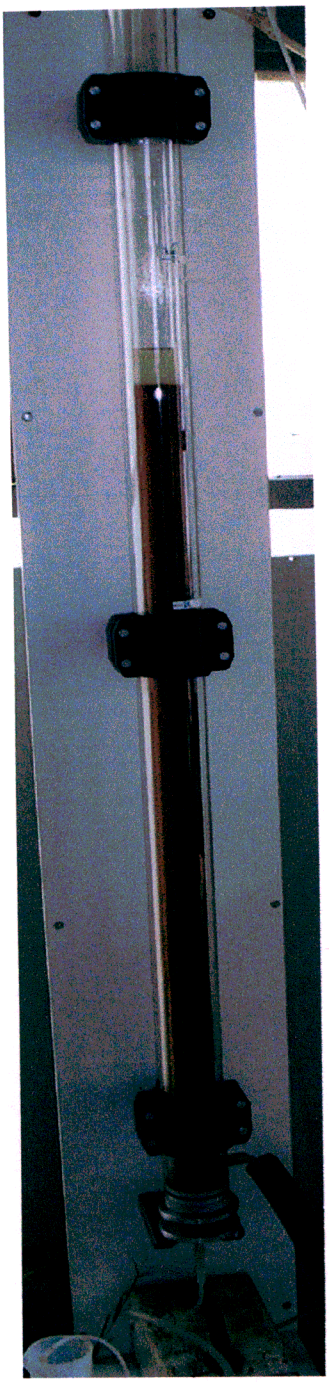


Fig. 9b above is a picture of the column used for the experimental work.

3.2. Pulse Testing

The pulse test is always the first step in determining the viability of a process. A column is loaded with resin in the ionic form of interest (as a starting point the resin would be in the sodium form as it is supplied). Several feed and rinse cycles are run to settle the media and to equilibrate the resin if the ionic makeup of the feed solution is different from the ionic form of the resin (this would be the case when using liquor from a fermentation plant as it would contain trace amounts of Ca^{2+}, Mg^{2+},K^{+} which would effect the separation. The feed solution is injected at the top of the resin bed as a finite slug, followed by the eluant, which is deionised water.

Fractions are collected after exiting the column, which are then analysed to show the separation profile. The degree of separation which can be achieved during these pulse tests is usually the criterion which determines whether further investigation in scale up should be attempted.

3.3. Test Procedure

Resin loading and column packing

For reproducible test results on different resins, the resin was loaded to consistent bed depths. Resins were loaded to the specified backwashed, settled and drained (BS&D) level which in our experiments was 1.5m and the height was marked to ensure no errors.

Prior to data collection three feed/rinse cycles were completed to equilibrate the resin for reproducible test results.

It is important to avoid turbulence and backmixing of the feed pulse and liquid in the free space above the packed resin bed. If there is any mixing of the feed with the eluant this would cause feed to move at different times down the column, thereby decreasing the separation efficiency. The feed pulse was introduced very close to the top of the packed resin bed so as to avoid density inversion mixing. The flow velocity of the feed pulse was also kept low so as to avoid substantial turbulence and mixing.

3.4. Filling of the Column

The resin which was used in the column was a strong cationic resin specially designed for use in chromatographic separations. The resin has an exceptionally uniform particle size distribution which produces a sharper separation. It is Dowex monosphere 99/320. Filling the column correctly with resin is an extremely important operation, or mechanical damage to the column itself or channelling of the liquor through a non-uniform resin which would give incorrect conclusions.

The resin as delivered in the Na^+ form was first treated in an open vessel with distilled water, to expand the resin to its fully hydrated state. This pre-swelling is carried out to prevent rupture of the column during later operation. The resin was left in the distilled water overnight. The resin was then poured rapidly into the column, with care being taken that there was a uniform packing of the resin particles and that the resin was always covered with liquid to prevent the inclusion of air bubbles. In order to avoid multistage bed packing the resin was added to the column faster than the settling rate of the beads. This was very important for achieving a uniform bed. As a final aid to removing any entrained air, the column was backwashed with distilled water to give a 40% bed expansion. Finally 3 bed volumes of distilled water were passed over the resin as a rinse. The column was now ready for use.

3.5. Choosing Parameters for Base case/Experiment

Both resin properties and process parameters affect the number of theoretical plates and the separation effeciency. The mean size, the size uniformity and the moisture content of the ion exchange resin are the critical resin parameters.

The flow velocity, the solute concentrations, the cycle frequency, the pressure drop and the temperature are the process parameters which are controlled to achieve a desired separation.

3.5.1. Resin Selection and Bead size

The mean bead size is chosen so that diffusion into and out of the resin beads is not the rate controlling step, and pressure drop in the column does not become excess. Small beads adsorb and release the solute quickly for sharp, distinct elution profiles. Larger beads take longer to adsorb and release the solute so that the elution profiles are wider and less distinct. The column with the smallest mean bead size has the greatest number of theoretical plates. Dowex and Finex were approached for test quantities of chromatographic resin. Dowex supplied the Dowex 99/320, and Finex supplied the Finex CS 16G. Both supplied resin in the Na^+ form, with the Dowex resin having a particle size distribution of between 310-330μm and Finex 210-230μm. Since Dowex resin was available first, it was used as the base case resin, with Finex tested at lower particle size.

3.5.2. Particle size Distribution

When there is a large distribution of bead sizes for a resin of a given mean bead size, it becomes difficult to obtain proper elution profiles. The beads smaller than the mean will elute the solute more rapidly and those larger less rapidly, to cause additional spreading or tailing of elution profiles. The only difference between normal ion exchange resin and chromatographic resin is the strict particle size distribution, and smaller bead size. The Dowex resin which has been used has 90% of the beads within 310-330μm.

3.5.3. Moisture Content

The moisture content of the resin affects the position of the elution profile. The shift occurs because resins in the monovalent form occupies only a single resin site while di-valent forms occupy two sites. Likewise, moisture content increases the rate of diffusion within the resin for non-ionic solutes. Moisture content on the Na^+ form of the resin was 50-55% while on the Ca^{2+} from was 48-52%. Moisture content is directly related to cross-linkage of the resin bead which gives the resin its strength.

3.5.4. Flow Velocities

The elution profile becomes sharper as the flowrate is decreased. The flowrate can be decreased to a minimum critical velocity (v_c) to ensure minimal profile tailing due to density and viscosity differences in the solution moving through the column. The critical velocity is defined by equation 12 below (Dorfer 1990).

$$v_C = \frac{g(\rho_2 - \rho_1)}{k(\mu_2 - \mu_1)} \qquad (12)$$

Where

k = permeability co-efficient of the bed (defined by the resin manufacturer)

Subscripts 1 and 2 in the above equation refer to feed and solvent respectively. It was calculated that a minimum velocity of 4.25×10^{-5}m/s could be used to avoid poor resolution. For the basecase a value of close to 8.5 times this value was used. A flowrate of 1BV/h was used. The above calculations can be referenced in Appendix 1.

3.5.5. Concentration

As the concentration of the feed increases it shifts the point of maximum component concentration to the right in the chromatogram, but does not influence the point at which the component initially emerges. For industrial chromatography to be effective and economically justifiable, the feed concentration should be greater than 3% of the dissolved solids. Isoleucine liquor from the commercial process consisted of approx. 3%

AABA, based on the dissolved solids. For the base case a 2% solution of AABA and 2% isoleucine were used.

3.5.6. Feed/Eluant Cycle

If the feed eluant cycle occurs too rapidly, the rapid expansion and contraction can cause the resin beads to fracture. Typical cycle times for industrial chromatography are between 1 and 1.5 hours, which result in expected resin lifetimes of several years. The cycle time was fixed according to the eluant velocity which converted into a cycle time of 1.06 hours.

3.5.7. Temperature

There are three reasons for working at high temperatures above 60°C:

- First, elevating the temperature will decrease the viscosity of the mixture.
- Secondly, in amino acid chromatography elevated fluid temperature is often necessary to prevent microbial growth on the ion exchange beads and in the column.
- Thirdly higher temperatures give higher diffusion rates which results in narrower peaks, and better separation.

A temperature of 75°C was chosen for the base case, which would be high enough to reduce the viscosity, prevent microbial growth, but also low enough to accommodate hand ability.

3.6. Optimisation of Overall Column Hydraulic Efficiency

With a resin in the Na^+ form the efficiency of the distribution system and physical properties of the column can be tested by doing a pulse test with a NaCl solution. Since the column is in the Na^+ form, the NaCl should proceed down the column with no resistance from the resin. Therefore band broadening or tailing would indicate that there were hydraulic problems with the column.

A 2% NaCl feed solution was used. A feed pulse of 0.1BV was used and flowrate of the eluant was adjusted to 1BV/h. Samples were collected in aliquots, and the salt concentration was analysed by electrical conductivity, and then converted to NaCl concentration to plot a distribution profile. Instead of using time on the x-axis BV was used, as the flowrate was kept constant

The distribution profile is shown in figure 10, and has no tailing or finger formation. This indicates uniform packing, radial distribution and overall operating control.

The next step was to perform experiments using different concentrations of NaCl in order to see the effect of concentration. From the profiles which can be seen in figure 11 it is evident that changing the NaCl concentration shifts the peak concentration to the right. This happens because even though the resin in the sodium form does not show affinity for the NaCl in solution, as the concentration in the feed is increased the concentration gradient between the resin and solution is increased and this in turn allows for equilibrium to be reached where Na^+ ions are exchanged. This takes time and therefore the higher the concentration, the later the peak emerges for NaCl.

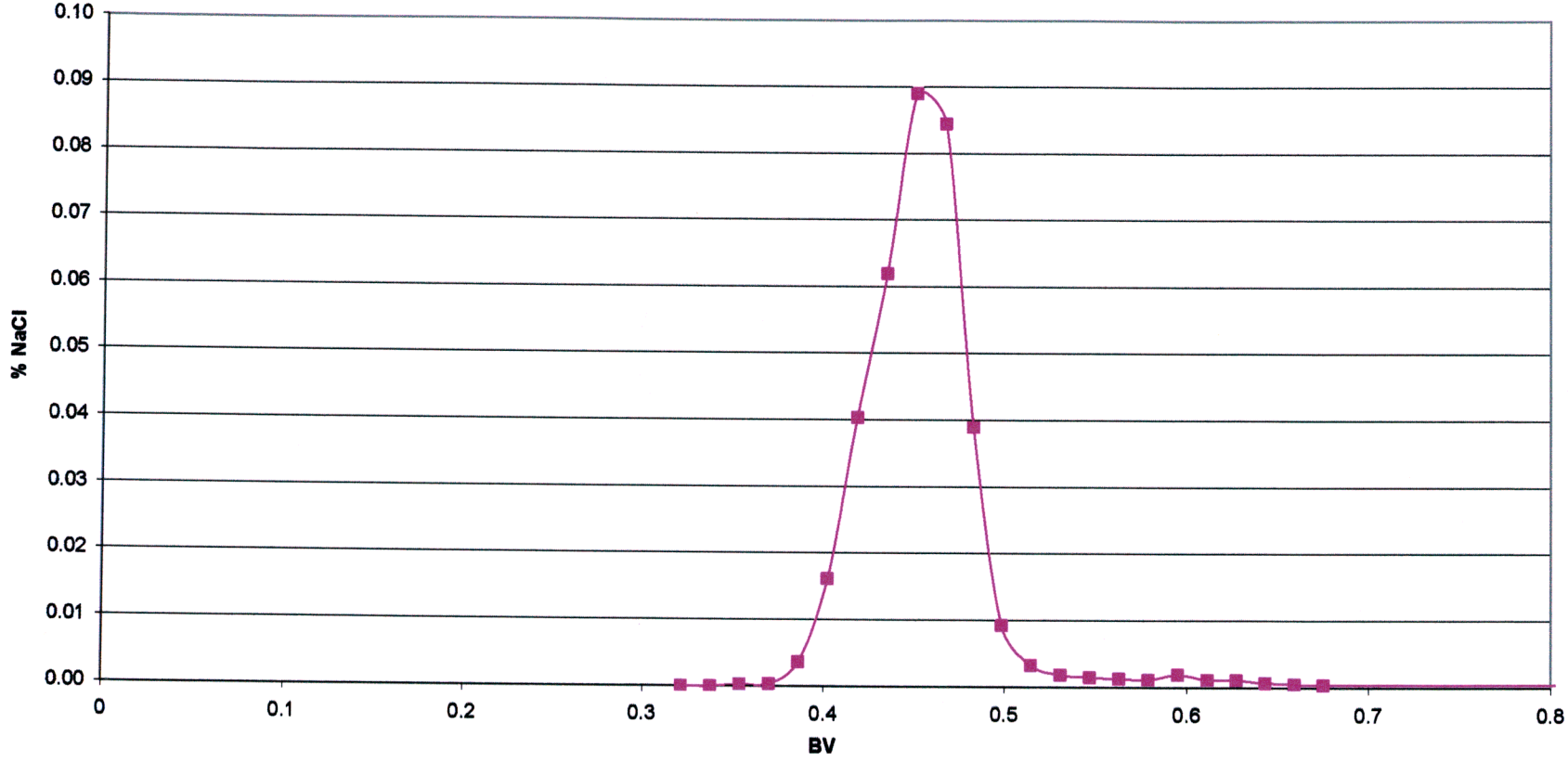


Figure 10. Distribution profile of 2% NaCl solution using Dowex monosphere 99/320 in the sodium form.

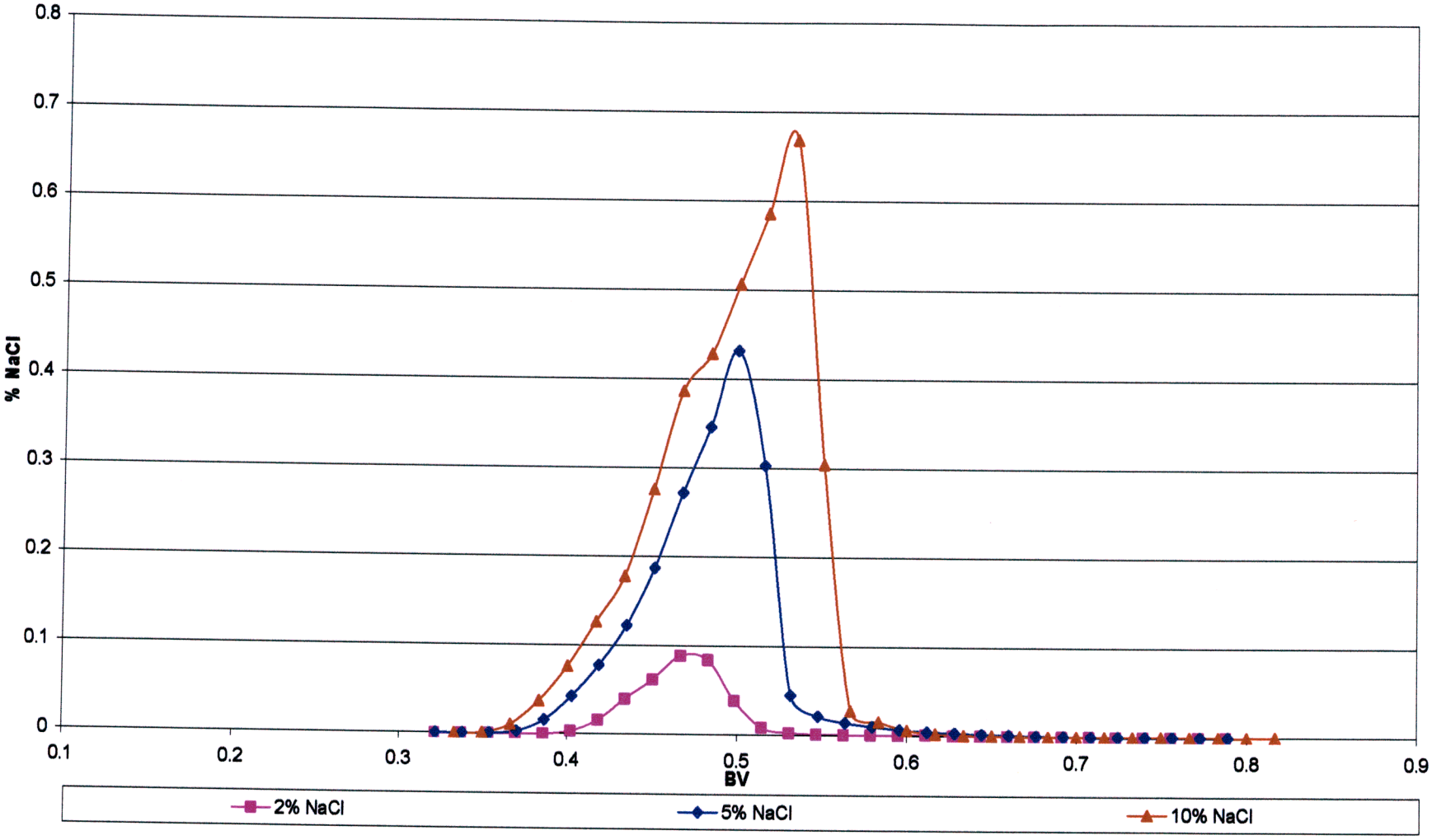


Figure 11. Distribution at various NaCl concentrations using Dowex monosphere 99/320 in the sodium form.

3.7. Introduction to Isoleucine Distribution Profile

In order to initially simplify the chromatographic separation results, test work was performed using pure isoleucine-water solutions.

2% isoleucine solution was used for the feed and the volume of feed exposed to the resin was 0.1BV. The flowrate of eluant was set at 1BV/h. The temperature of the system was set and controlled at 75°C, during the run.

Since pure isoleucine solutions were used for the distribution profile the chromatogram could be analysed by means of electrical conductivity which also reduced analytical time since analysis by HPLC is time consuming. From the conductivity data a calibration chart of conductivity vs. isoleucine concentration was constructed. The calibration chart can be found in appendix 2. From the distribution profile, figure 12 below, isoleucine is observed showing affinity for the Na^+ form of the resin by emerging later than NaCl. A new term is introduced in the figure which is C/C_f. C is the concentration of isoleucine in the analysed sample and C_f is the concentration of the feed. In this way it is possible to compare streams of different conditions.

This experiment had the advantage that there would be no interference with other solutes and the distribution would only be dependant on the affinity of the isoleucine to the resin and the physical properties of the chromatographic system. The maximum point on the distribution profile was at 0.85BV. The tests scheduled would indicate how the profile of isoleucine would change with changing conditions.

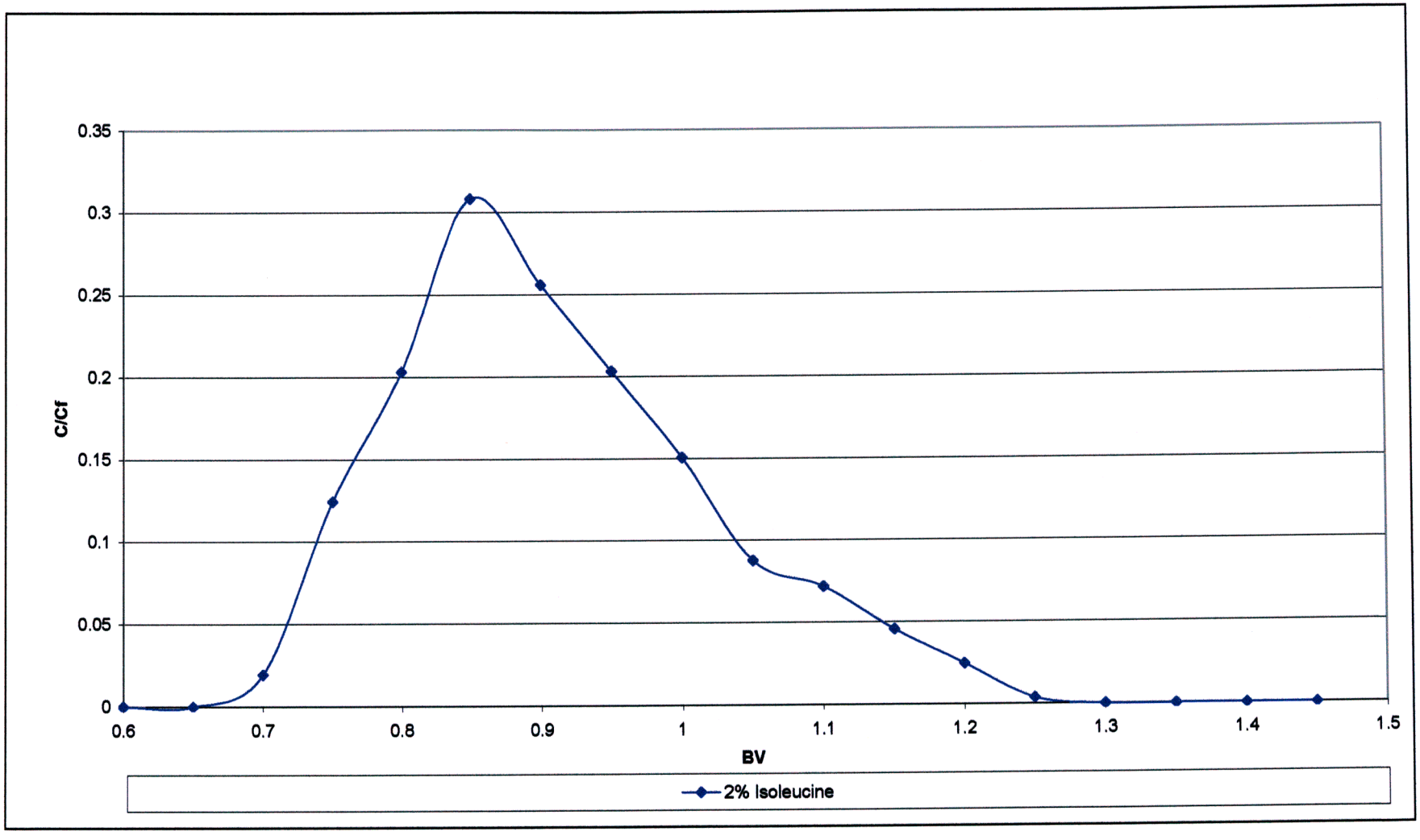


Figure 12. Distribution of pure isoleucine using base case conditions.

3.8. Model Isoleucine and AABA Tests

The aim of the project was to use SA Bioproducts isoleucine fermentation liquor rich with isoleucine and impurities namely alpha amino butyric acid (AABA), valine and alanine and separate this into two separate streams. The one should contain all the impurities which can be purged and the second stream contains pure isoleucine. For the project the purity of the isoleucine stream that could be collected was defined as 85%. Obviously for the process to be a success the overall recovery and concentration of isoleucine would have to be high enough to warrant a commercial process.

In order to evaluate the separation of isoleucine from AABA a solution of pure amino acids were used. Table 01 shows the tests planed to observe the separation of the mono-valent amino acids using the chromatographic system.

The base case was chosen and in all other experiments the variable being studied was changed, keeping all other variables constant with the base case.

In order to construct a chromatogram the exit stream, was analysed by "High Performance Liquid Chromatography (HPLC)". High performance liquid chromatography is the analytical scale of chromatographic separation which is used widely for analysing amino acids. The results of each experiment were compared using resolution, isoleucine purity, mass balance and overall recovery of isoleucine at the specified purity.

The feed was introduced as a pulse at the top of the resin bed. After complete adsorption of the sample on the top of the resin bed the two amino acids were eluted with deionised water. Prior to each experiment the resin column was backwashed and allowed to settle. This ensured a constant bed volume and prevented channelling. Table 1, shows the test conditions.

Run No.	Resin	Hydrated Particle Size mm	Temperature [°C]	Feed Conc. [ILE; AABA]	Eluant Flow	Feed Volume [BV]	Ionic Form of Resin	Height of Resin [m]	Description
1	Dowex 99/320	310-330	75	2%; 2%	1BV/h	0.10	Na^+	1.5	Basecase
2	Dowex 99/320	310-330	75	1%; 1%	1BV/h	0.10	Na^+	1.5	Low conc.
3	Dowex 99/320	310-330	75	4%; 4%	1BV/h	0.10	Na^+	1.5	high conc.
4	Dowex 99/320	310-330	75	2%; 2%	1BV/h	0.20	Na^+	1.5	High feed vol.
5	Dowex 99/320	310-330	75	2%; 2%	0.5BV/h	0.10	Na^+	1.5	low eluant flow
6	Dowex 99/320	310-330	75	2%; 2%	2BV/h	0.10	Na^+	1.5	High eluant flow
7	Dowex 99/320	310-330	30	2%; 2%	1BV/h	0.10	Na^+	1.5	low temperature
8	Dowex 99/320	310-330	90	2%; 2%	1BV/h	0.10	Na^+	1.5	high temperature
9	Dowex 99/320	310-330	90	2%; 2%	1BV/h	0.10	Ca^{2+}	1.5	Change ionic form
10	Finex CS 16GC	210-230	75	2%; 2%	1BV/h	0.10	Na^+	1.5	low particle size
11	Dow 99/320	310-330	75	2%; 2%	1BV/h	0.10	Na^+	1.8	high resin height

Table 01: Experimental plan for the project.

3.9. Base Case

In summary the base case chromatographic separation for isoleucine and AABA were set at the following:

Feed Volume	: 0.1BV
Eluant Flow	: 1 BV/h
Feed conc.	: 2% isoleucine, 2% AABA
Temperature	: 75°C
Resin ionic form	: Na^+
Resin	: Dowex 99/320 monosphere
Particle Size	: 310 – 330μm
Particle size dist.	: 90% within particle size

Samples were collected in 0.02BV aliquots in order to determine the separation profile and the samples were analysed by HPLC.

Results were expressed in terms of resolution as well as HETP. Most important to the separation was the mass balance over the amino acids as well as overall recovery. Purity is defined as (isoleucine/total solids)%. In the case of the following tests, the definition reduces to (isoleucine/(isoleucine + AABA))%.

4. Results/Discussion

4.1. Base case

Base case conditions resulted in peak profiles represented in figure 13 below. Observations from initial test work indicated that isoleucine showed greater affinity for the resin in the Na^+ form than alpha amino butyric acid. Alpha amino butyric acid occupies less space on the profile and this can be seen by the higher peak concentrations. The isoleucine, which shows greater affinity for the resin and is completely eluted at 1.45BV. Theory predicts that the greater the affinity for the resin, the broader the peak which can be clearly seen. In order to gain confidence in the results the basecase was run in triplicate as this would be used to compare all test work. All results were in a 5% error from each other. An overall recovery of 38.56% at 85% isoleucine purity was achieved.

HETP was calculated at 42mm with a theoretical number of plates being 35.2 (equation 6). The resolution was calculated at 22.7% (equation 7). To commercialise the separation process of ISOLEUCINE from AABA the highest recovery of pure isoleucine would be needed with the highest concentration of isoleucine to reduce the evaporation costs. The overall concentration of isoleucine collected from the basecase was calculated at 1.59g/l. The above calculations can be reviewed in appendix 3.

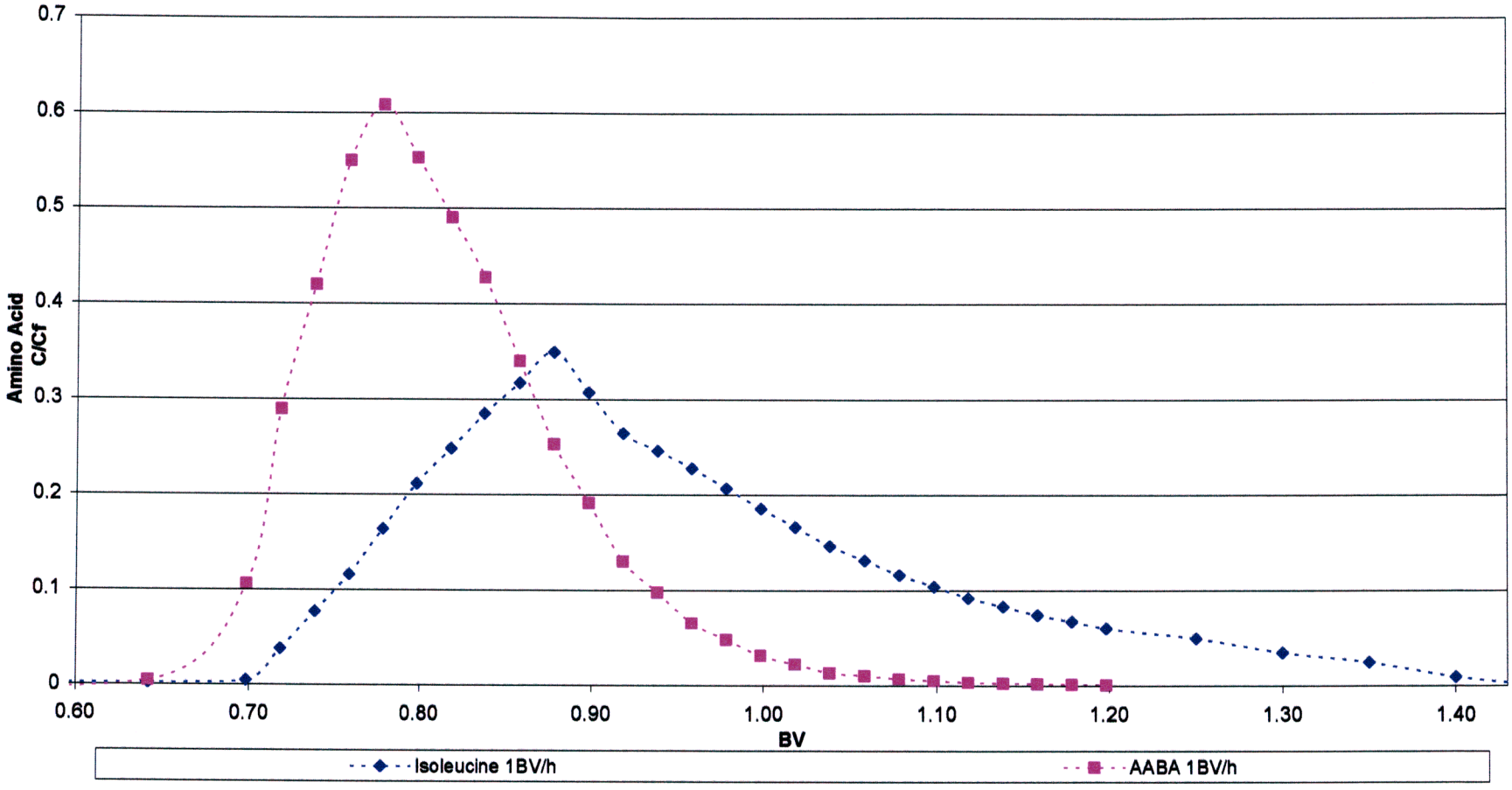


Figure 13. Distribution profile of alpha amino butyric acid and isoleucine using conditions chosen for basecase.

4.2. *Effects of Feed Concentration*

In large-scale chromatographic separation, the feed concentration is generally kept as high as possible in order to increase the throughput (yield per unit time) of the process. The separation of isoleucine-AABA mixture on a Dowex monosphere resin in the Na^+ form was tested at different concentrations. The following observations were made.

4.2.1. 4% Concentration

Increasing the feed concentration from the base case to 4% isoleucine and 4% AABA caused an increase in the retention volume (refer to figure 14 below). The isoleucine and alpha amino butyric acid peaks are broader and have lower peak concentration. The area of overlap increased and in turn decreased the overall recovery to 27.81%. The resolution which was calculated at 20.4%, HETP = 47.4mm with N= 31.6.

Even though the separation was similar to the basecase the broader peaks decreased the recovery but an increased averaged concentration was calculated at 3.43g/l isoleucine. This increase would reduce the costs of evaporation significantly. Confidences in the results were based on overall mass balance closure and repeatability of results. The test was run in triplicate and indicated good repeatability for all experimental work.

4.2.2. 1% Concentration

For the AABA-isoleucine pair, the concentration of the feed was decreased from the base case (2%) to 1%. The chromatogram is represented in figure 15. The retention volume of AABA was decreased marginally from 0.28 to 0.24BV, but isoleucine retention volume changed drastically from 0.6 to 0.4BV. The adsorption of the components was definitely dependent of concentration. At the lower concentration the isoleucine peak was sharper. The sharper peaks resulted in lower concentration collected and gave an average collectable concentration of 1.58g/l which was close to the base case collectable concentration. This was significant, given that it meant using a lower concentration feed will not incur higher evaporation costs.

Again confidence in the results was given by a good isoleucine balance, with the HETP being calculated at 309mm. The number of theoretical plates was also calculated at 4.8 stages. The resolution of the peaks was also calculated at 31.25%.

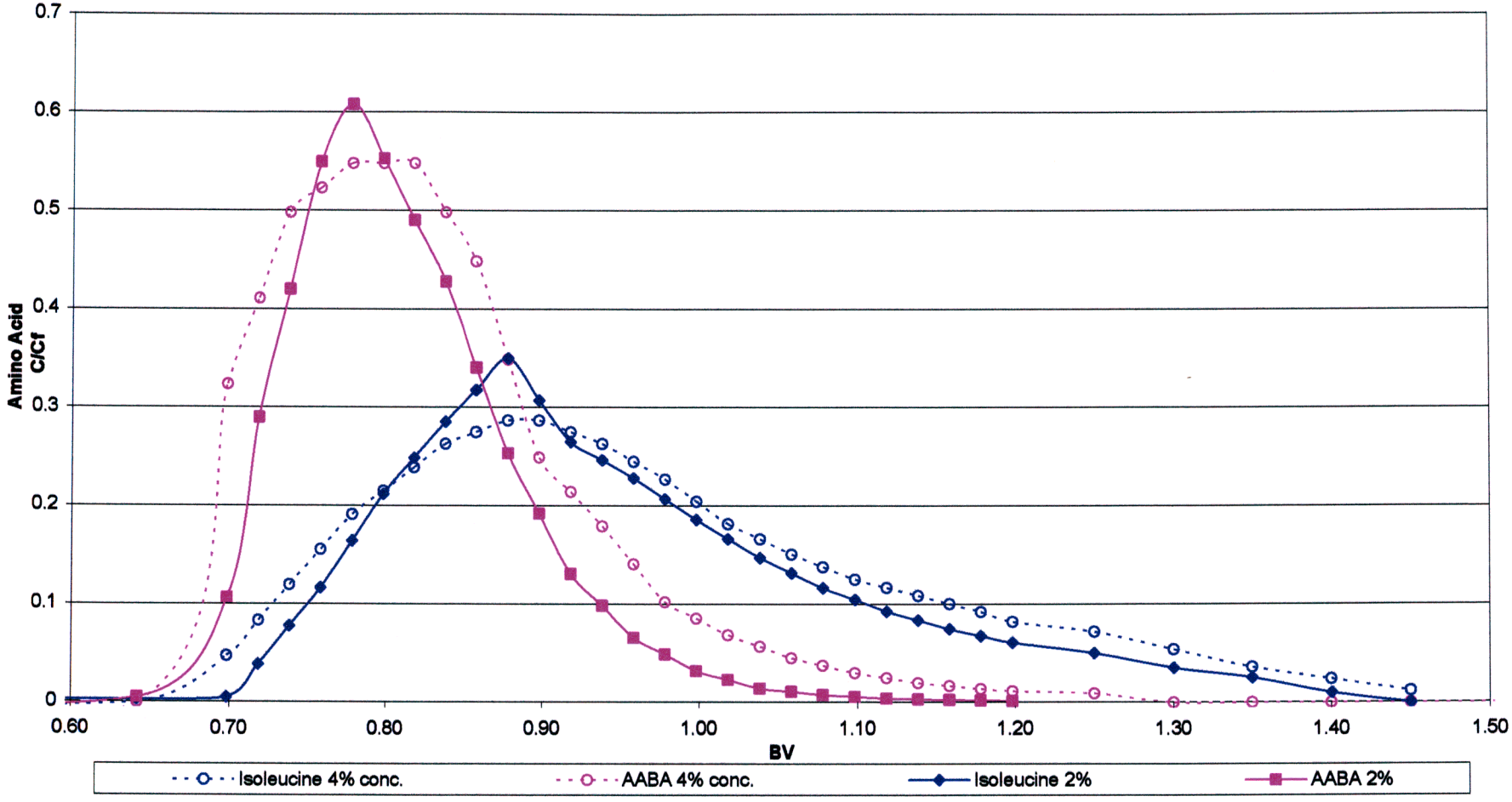


Figure 14. Comparison of 2%, amino acids with 4% represented on a chromatogram.

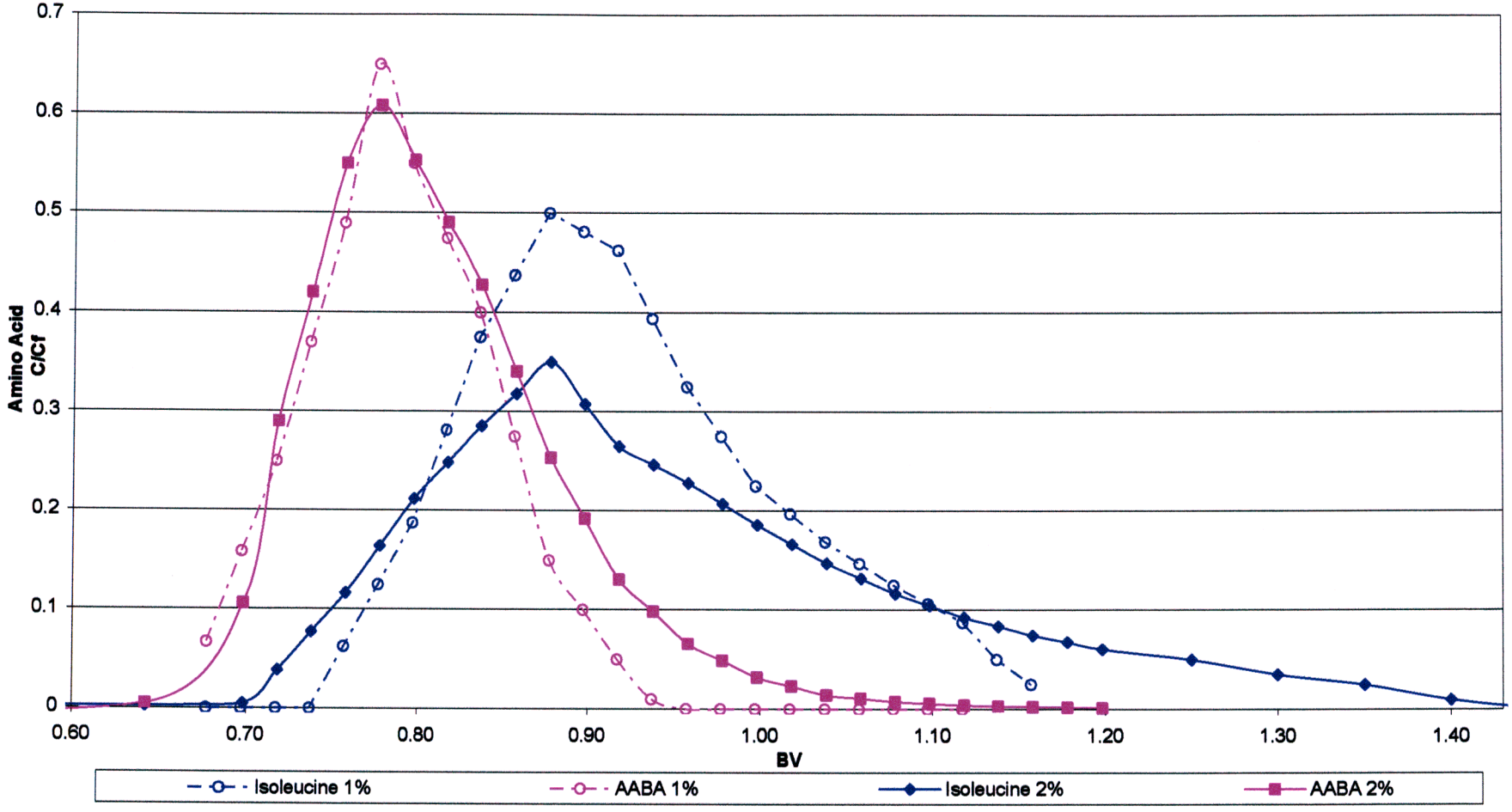


Figure 15. Comparison of 2%, amino acids with 1% represented on a chromatogram.

4.3. *Effect of Feed Pulse Volume*

In large-scale chromatographic separation, the feed volume is generally kept as high as possible in order to increase the throughput (yield per unit time) of the process. The maximum feed volume possible per cycle at equilibrium is equal to the occluded volume, which is the volume of liquid held between the beads and represents approximately one-third of the column volume. The separation of ISOLEUCINE-AABA mixture on a Dowex monosphere resin in the Na^+ form was tested at different feed pulse volumes of 2% amino acid concentration. The chromatogram is represented in figure 16 below. The following observations were made.

As the feed volume is increased from the base case 0.1 to 0.2BV, the on-column concentrations of AABA and ISOLEUCINE are increased. This increases the concentration of these materials in the eluant as can be seen from figure 16 below.

Additional overlapping of the peaks can be noticed immediately. The corresponding AABA drop of volumes (when the tail of the peak ends) as well as the break-through values remained the same. Increasing the volume increases the time available for solute to diffuse into the resin. The elution band then travels through the column at a slower rate than it would for a smaller feed volume. As the elution bands grew in size with increased feed volumes, the overlapping i.e. the area of cross contamination of the two curves increased.

HETP was calculated at 38.1mm and the number of plates was 39 plates. The resolution was calculated at 19.6%. The recoverable portion of the product was 18.38%. The average concentration of isoleucine collected was slightly higher at 1.78g/l.

An increase in feed volume results in higher concentrations of the isoleucine product being obtained. This will increase the productivity but lower the product purity. At the same time the percentage of the recycle would have to be increased, thereby decreasing the recovery. Thus low volumes of feed give good separation of the amino acids but at a

low productivity. Testing volumes lower than 0.1BV becomes uneconomic as the dilution becomes too great.

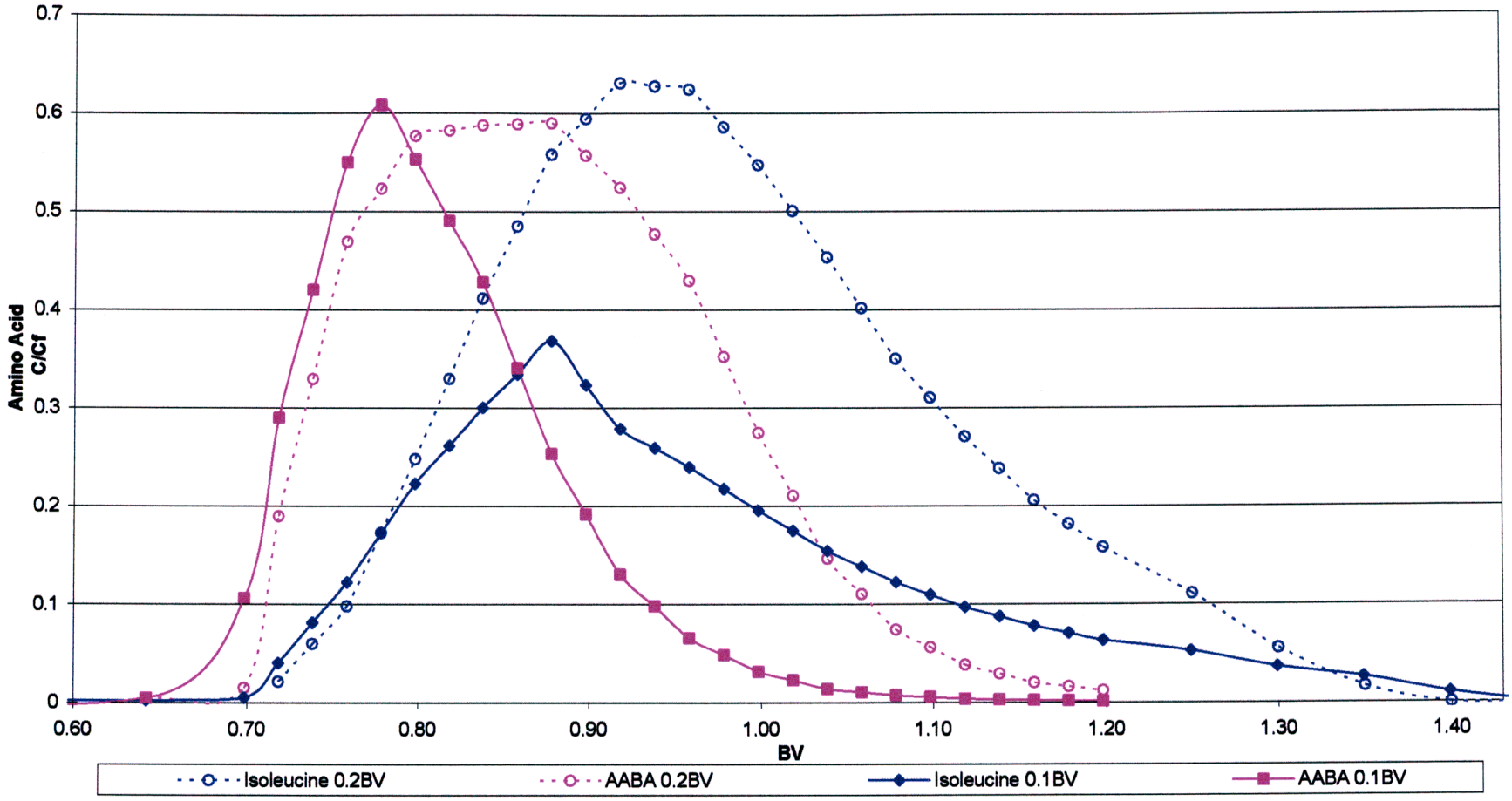


Figure 16. Representation of increased feed volume on chromatogram.

4.4. *Effects of Eluant flowrate*

Where possible, ion exchange processes in industry are carried out at maximum flowrates to increase the throughput. In order to view the effect of eluant flow on the separation the flowrate of eluant to the column was studied at 0.5, 1, 2BV/h. The chromatogram at 0.5BV/h is shown as figure 17 and 2BV/h as figure 18.

4.4.1. Decreasing the eluant flowrate

As the flowrate is reduced there is a sharpening of the peaks. The peak width for AABA was 0.3 and 0.25BV for flows of 1 and 0.5BV/h respectively. The peak widths for isoleucine were similar. The overlap of peaks became less pronounced and the separation efficiency increased which is in agreement with theory.

The narrowing of the component bands when decreasing the flowrate can be attributed to a decrease in HETP. The HETP is mainly determined and controlled by the mass transfer effect i.e. the intra-particle diffusion in the accessible internal pores of the resin. The HETP was calculated at 14.1mm and the theoretical number of plates was found to be 106 plates.

A recovery of 50.32% and a resolution of 40.20% were calculated. Even though the lower flowrate had a resolution of close to the base case trial, the overall recoveries were very different, with the higher recovery coming from the lower flowrate. The overall concentration of the collected product averaged 1.85g/l.

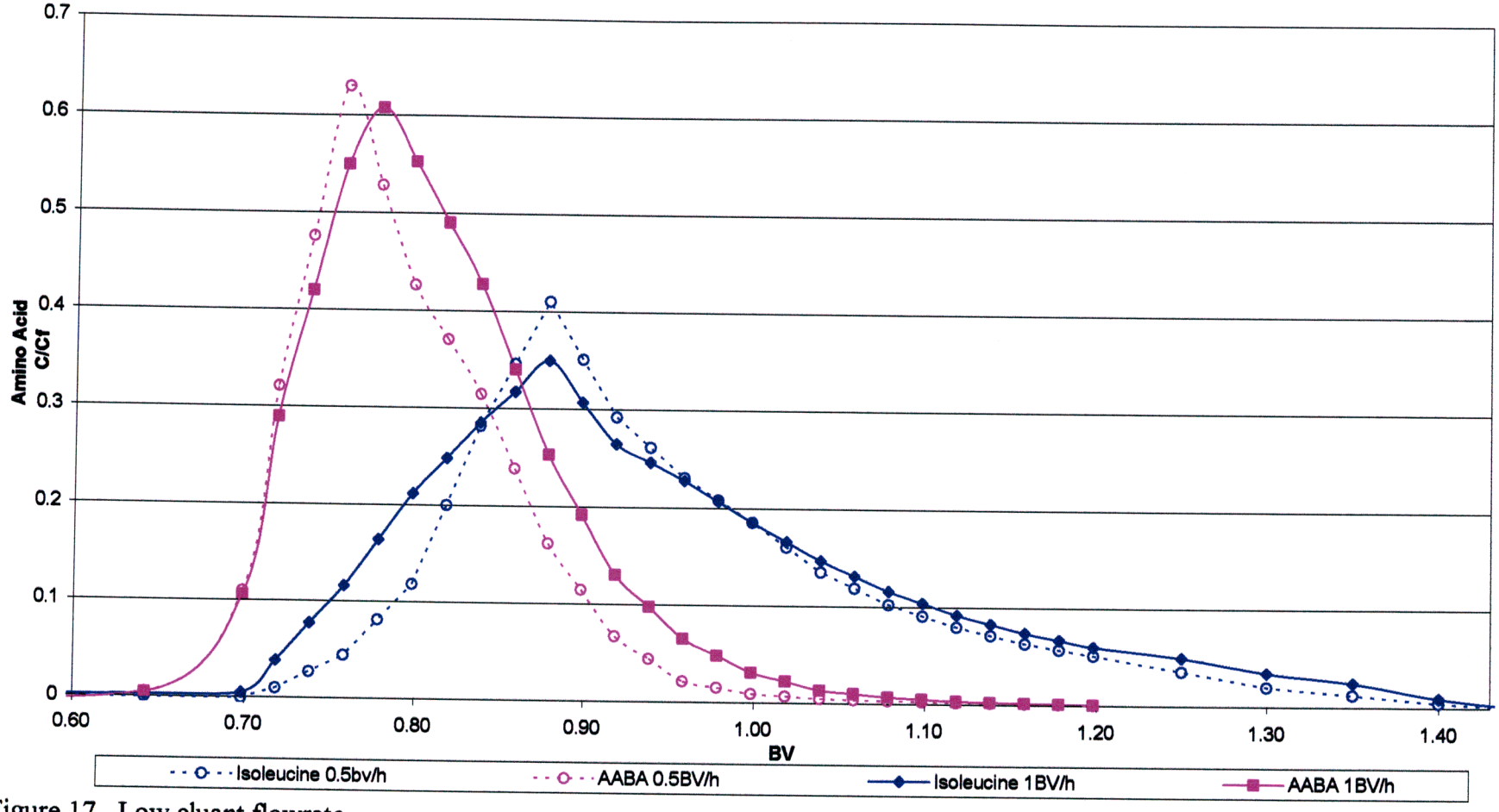


Figure 17. Low eluant flowrate.

4.4.2. Increasing the eluant flowrate

Increasing the eluant flowrate from 1BV/h to 2BV/h decreased the resolution to 17.5%. The HETP was calculated at 107mm N= 14. The emergence point of both AABA and isoleucine were brought forward from 0.7BV on the base case to 0.5BV with 2BV/h eluant flowrate. The drop of point of the peaks remained the same. This fits in very well with theory which states that if a column is overloaded the excess would proceed down the column and emerge first. 21.9% of the isoleucine was recovered. The average concentration of isoleucine collected was 1.3g/l.

It can be seen that an increase in the flow of eluate through the column increases the HETP which in turn causes early breakthrough and gives rise to the overlapping of curves and lower product purity. An overall balance between purity, recovery and flowrate needs to be struck in order to optimise the process.

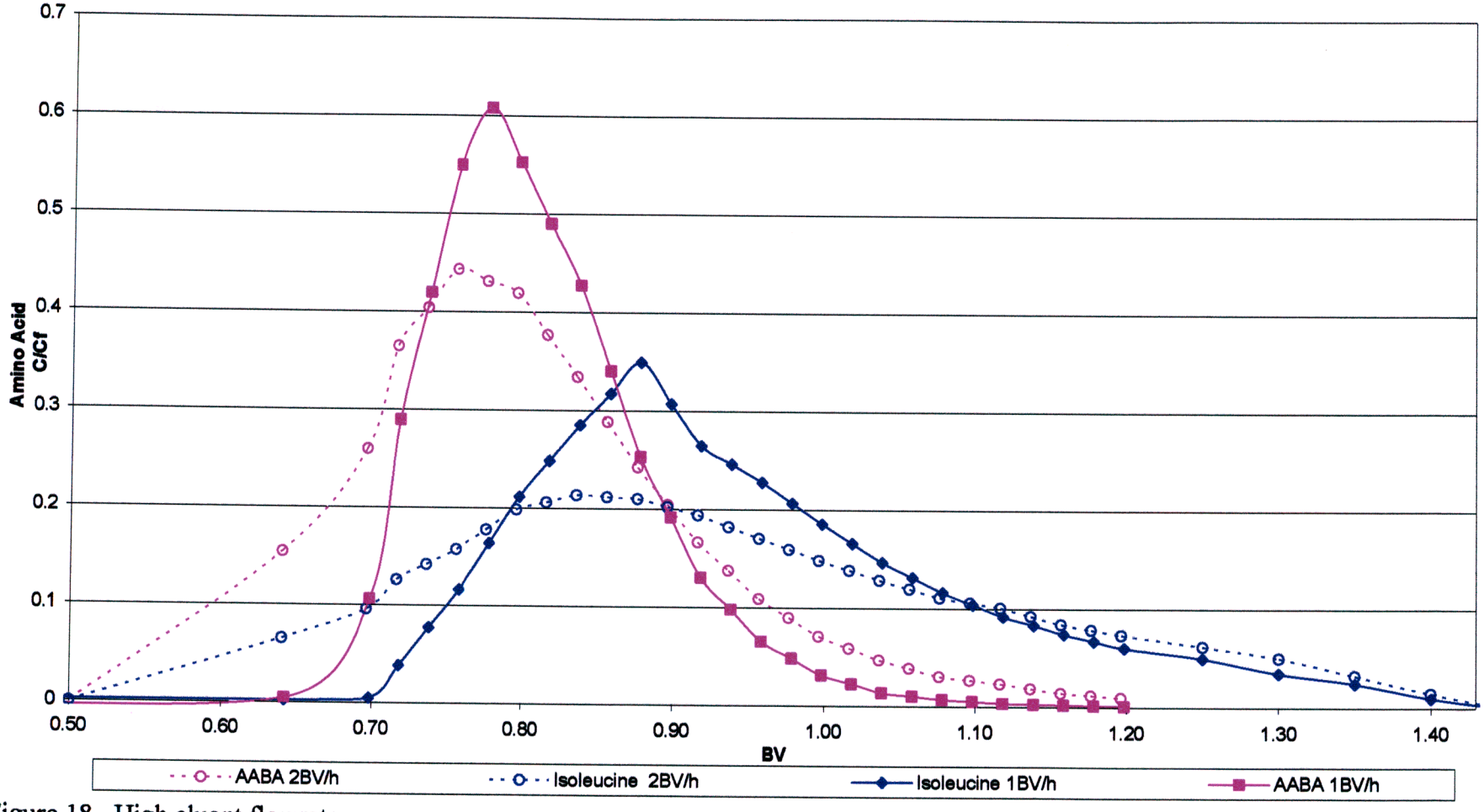


Figure 18. High eluant flowrate.

4.5. *Effects of Temperature*

Where possible, chromatographic separations are carried out at high temperatures in order to decrease the bacterial activity and reduce viscosities. The separation of AABA-isoleucine mixtures was studied at different temperatures to investigate the effect on separation.

4.5.1. Decreasing the Temperature below 75°C

The temperature chosen was 30°C and the separation was performed keeping all other parameters at base case conditions. The chromatogram is represented in figure 19. The highest concentrations achievable, running at 30°C were lower than those running at 75°C. The mass balance closed at 101.2. The amino acids are distributed more widely along the chromatogram, with the result being heavy overlapping. Decreasing the operating temperature decreases the rate of diffusion into and out of the resin and results in decreased separation efficiency. This reduces the separation forces and the breakthrough proceeds quickly down the column with more overlapping of peaks.

On overall recovery of 21.4% was achievable a resolution of 6.2% was calculated. A HETP of 87,6mm and 17 theoretical plates were also found. The average concentration of isoleucine that could be collected was 1.1g/l.

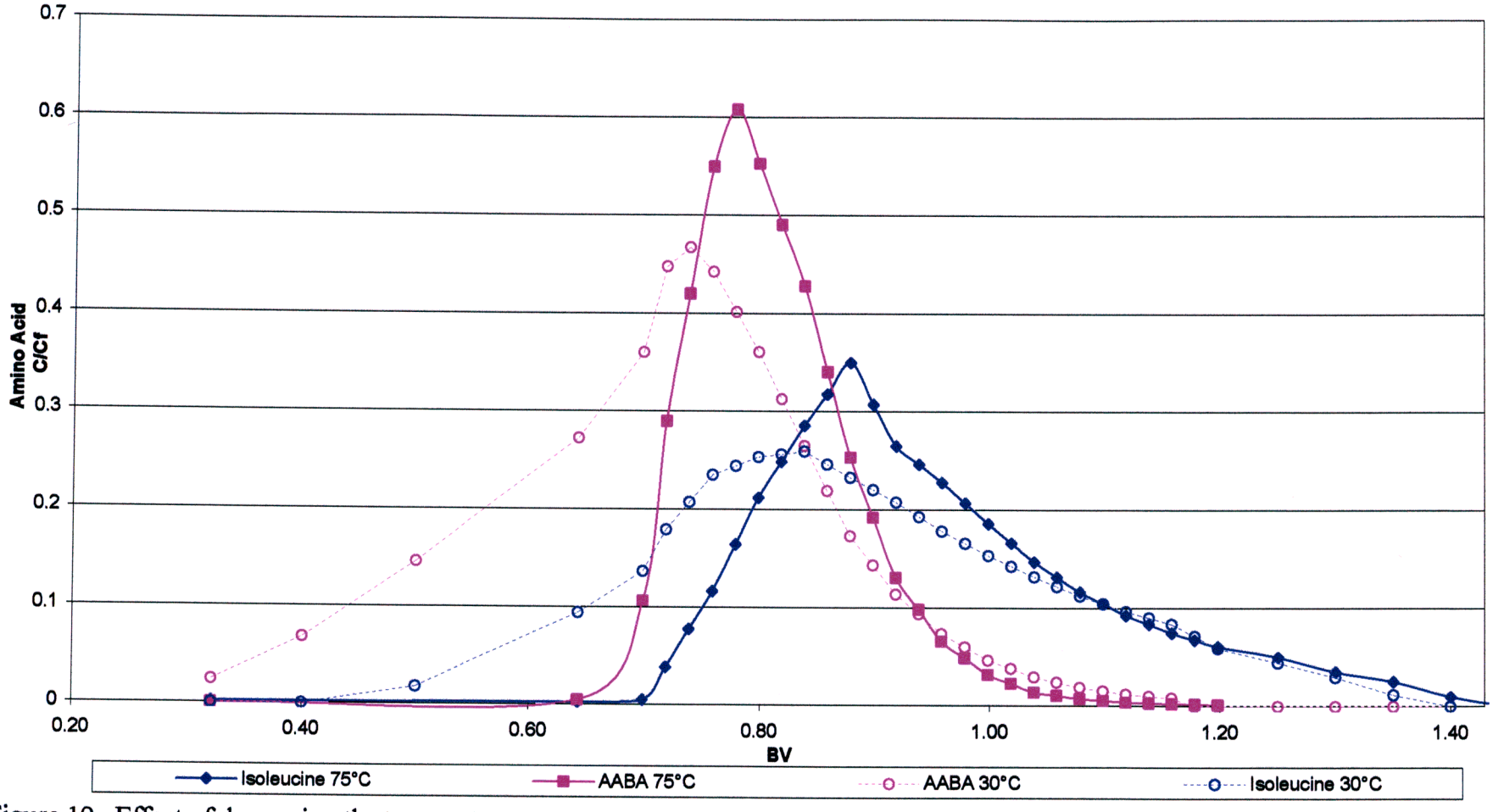


Figure 19. Effect of decreasing the temperature.

4.5.2. Increasing the Temperature above 75°C

The separation of AABA-ISOLEUCINE mixture at 90°C is shown in figure 20. The component peaks lie almost unchanged from those at 75°C with a calculated HETP of 20mm and 75 theoretical plates. The resolution was calculated at 40.19% which is very close to that at 75°C. The overall recovery of product was higher than the base case at 45.2%. The temperature could not be taken any higher than 95°C due to the system operating at atmospheric conditions.

It can be concluded that increasing the temperature of the system increases the rate of diffusion and results in improved mass transfer rates. Operating temperature does affect the distribution of solutes between the stationary and mobile phase resulting in poor resolution at lower temperatures. For the separation of isoleucine and AABA there is little incentive for operating above 75°C. Operating at high temperatures is more likely to degrade due to oxidation.

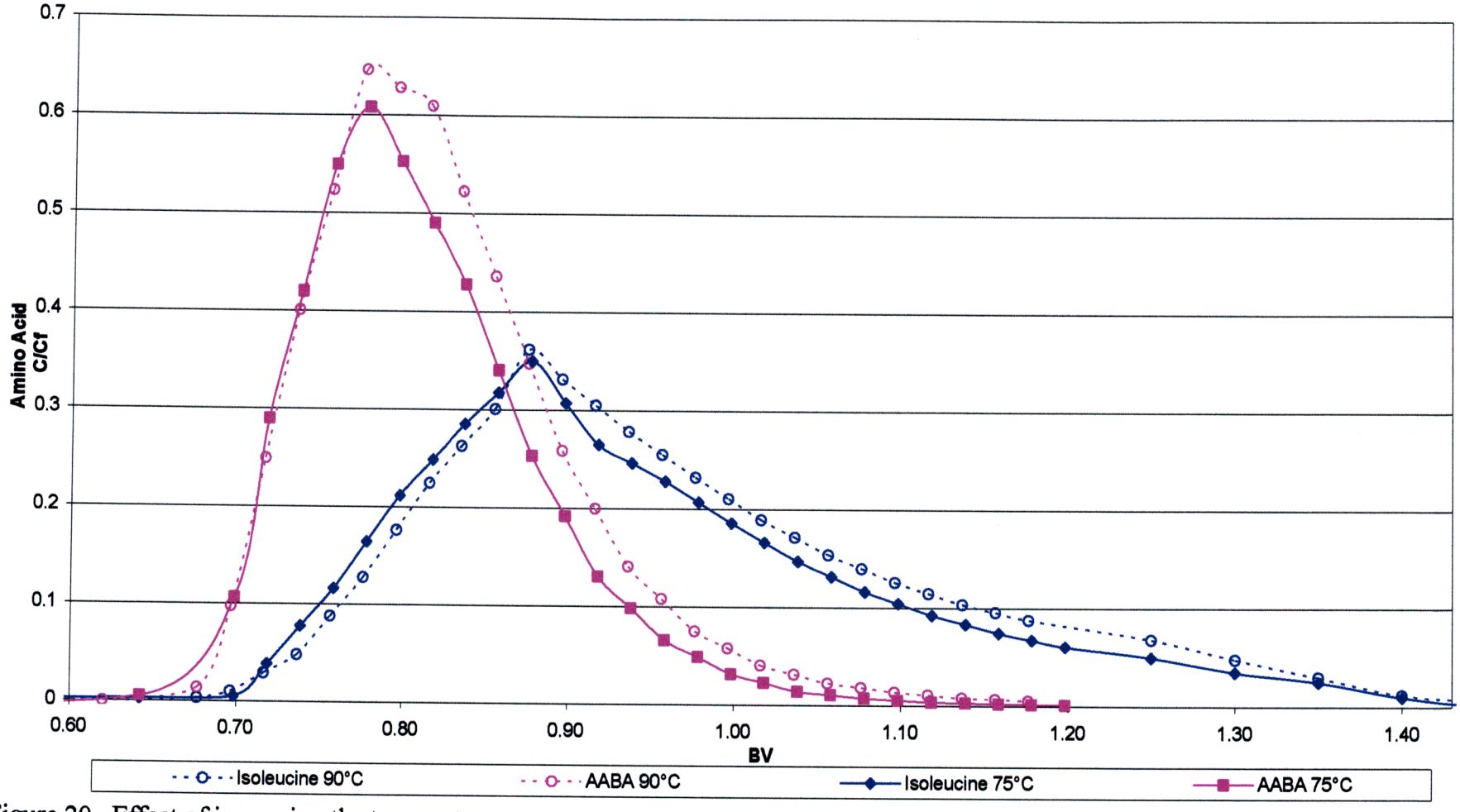


Figure 20. Effect of increasing the temperature.

4.6. *Effects of Ionic form of Resin*

Resin in the Na^+ form fell short on overall recovery of pure isoleucine. Changing the form of the resin to a di-valent was the following step. The resin was converted directly from the Na^+ to the Ca^{2+} using $CaCl_2$ at a pH of 6. A decrease in resin height was observed and the conversion of resin resulted in a loss of 10% resin hydrated volume. Resin height was made up to 1.5m by adding more resin in the Ca^{2+} form. Since calcium is a divalent molecule it attaches itself to two sites on the resin, and reduces the moisture content held in the bead. While there is a drop of the resin moisture content, the particle size of the resin is decreased slightly.

The chromatogram which is presented in figure 21 shows the distribution profile of AABA and isoleucine. Initially evident is that both acids are held back by the resin in the Ca^{2+} form and this delays the point of emergence. An increase of 0.42BV for AABA and 0.55BV increase for isoleucine is observed. By converting the resin to the Ca^{2+} form, enabled and increase in separation between the AABA and isoleucine. By doing this required more eluant but since the amino acids were slowed down most of the eluate could be recycled. The resolution was calculated at 57%, while the HETP equalled 22.8mm with an overall number of theoretical plates at 65. All of the isoleucine was accounted for, and the recovery of pure isoleucine was calculated at 80.71%. The average isoleucine concentration collected was 1.9g/l.

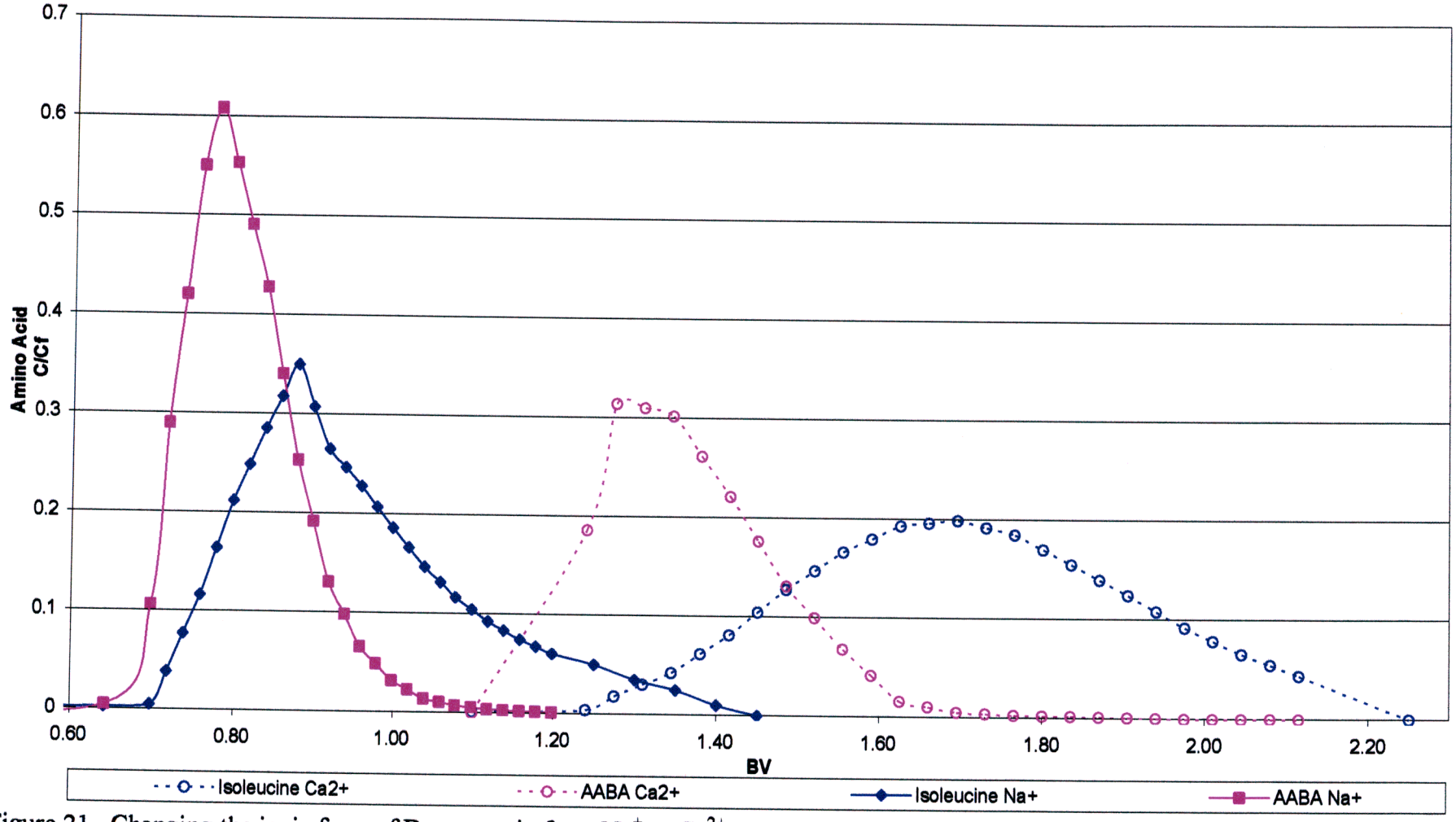


Figure 21. Changing the ionic form of Dowex resin from Na^{+} to Ca^{2+}

4.7. Particle Size of Resin

The efficiency of chromatographic separation is strongly related to the mean bead size of the resin. The mean particle size of the resin in large scale chromatographic applications is typically between 300 and 400μm. The range is a compromise between kinetic performance and pressure drop in the column.

Finex GS 16 with a particle size of 210-230μm in the Na^+ form was used, due to availability of the resin at a low particle size. From the chromatogram (figure 22) the peaks are sharper, which has a lower dilution effect. Since the beads are smaller the kinetic effects occur faster and this is indicative of the drop off point occurring sooner than the Dowex resin.

The resolution being measured at 27.3% and HETP = 26.2mm. The theoretical number of plates was also calculated at 56. By sharpening the curves the recovery was also increased to 41.7% isoleucine. The average concentration of isoleucine that could be collected averaged 2.68g/l.

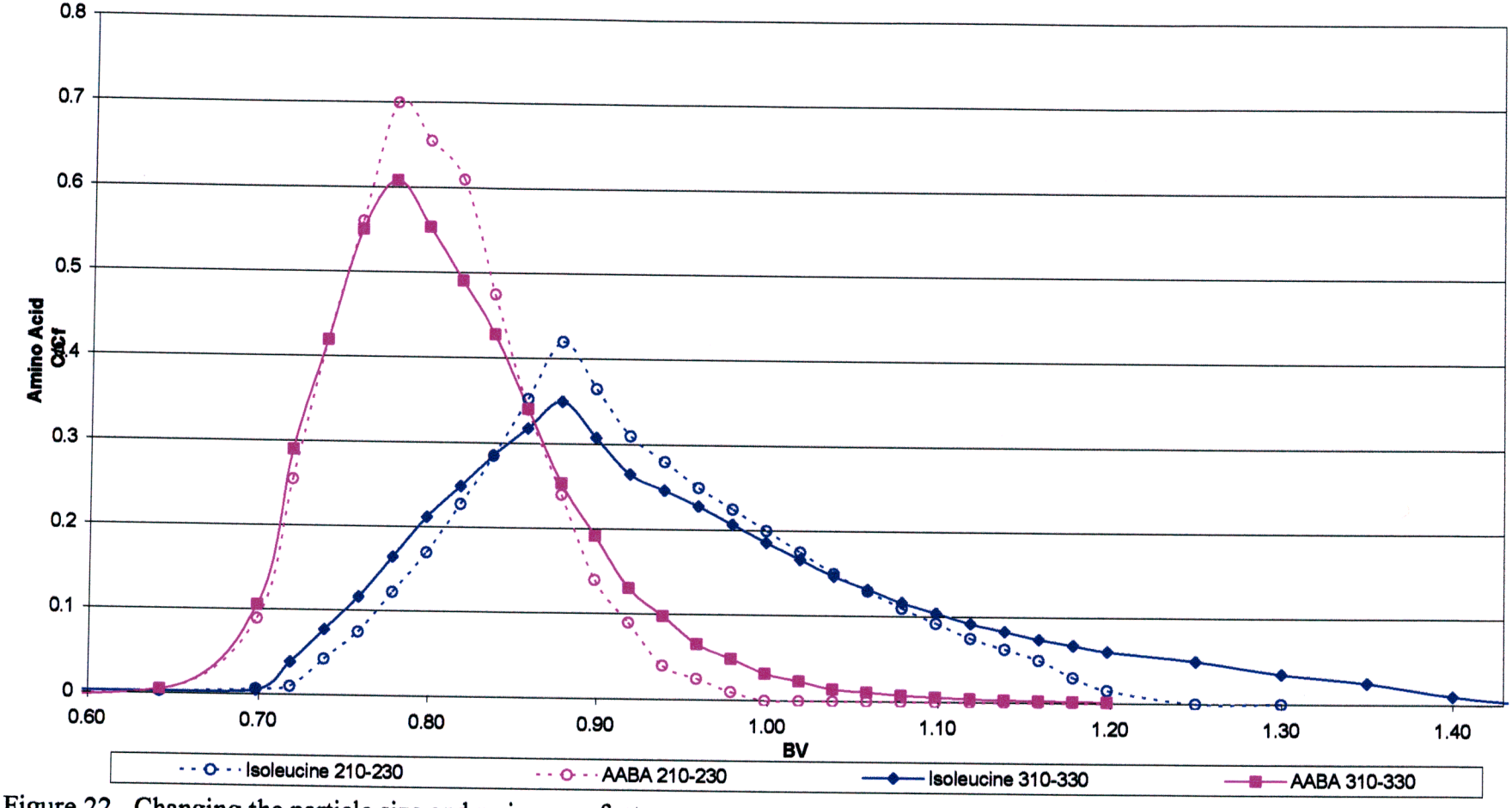


Figure 22. Changing the particle size and resin manufacturer.

4.8. *Increasing the height of the resin*

Increasing the mass transfer zone length can be important because this adds more time to the chromatographic separation medium and the distance between the two components which are required to be separated. The resin height in the column was increased from 1.5 to 1.8m by adding in 0.7*l* of resin in the Na^+ form. Since the resin volume was increased the actual volume of the feed which was added to the resin was also increased to keep the volume of the feed at 0.1BV. It is important that this is done so that the effect of resin height is seen and not the effects of lowering the feed volume.

From the chromatogram (figure 22) the peak separation of the AABA peak is identical to the basecase, although the isoleucine peak emerges slightly later than the basecase. This can be explained by the fact that there is a larger distance for separation, and the affinity difference between the individual amino acids for the resin becomes more pronounced.

By increasing the resin height the resolution was measured at 44%, with a HETP of 22.3mm and 80.7 theoretical plates. A recovery of 66.6% of isoleucine was calculated with the collectable portion of the product averaging 2.9g/l.

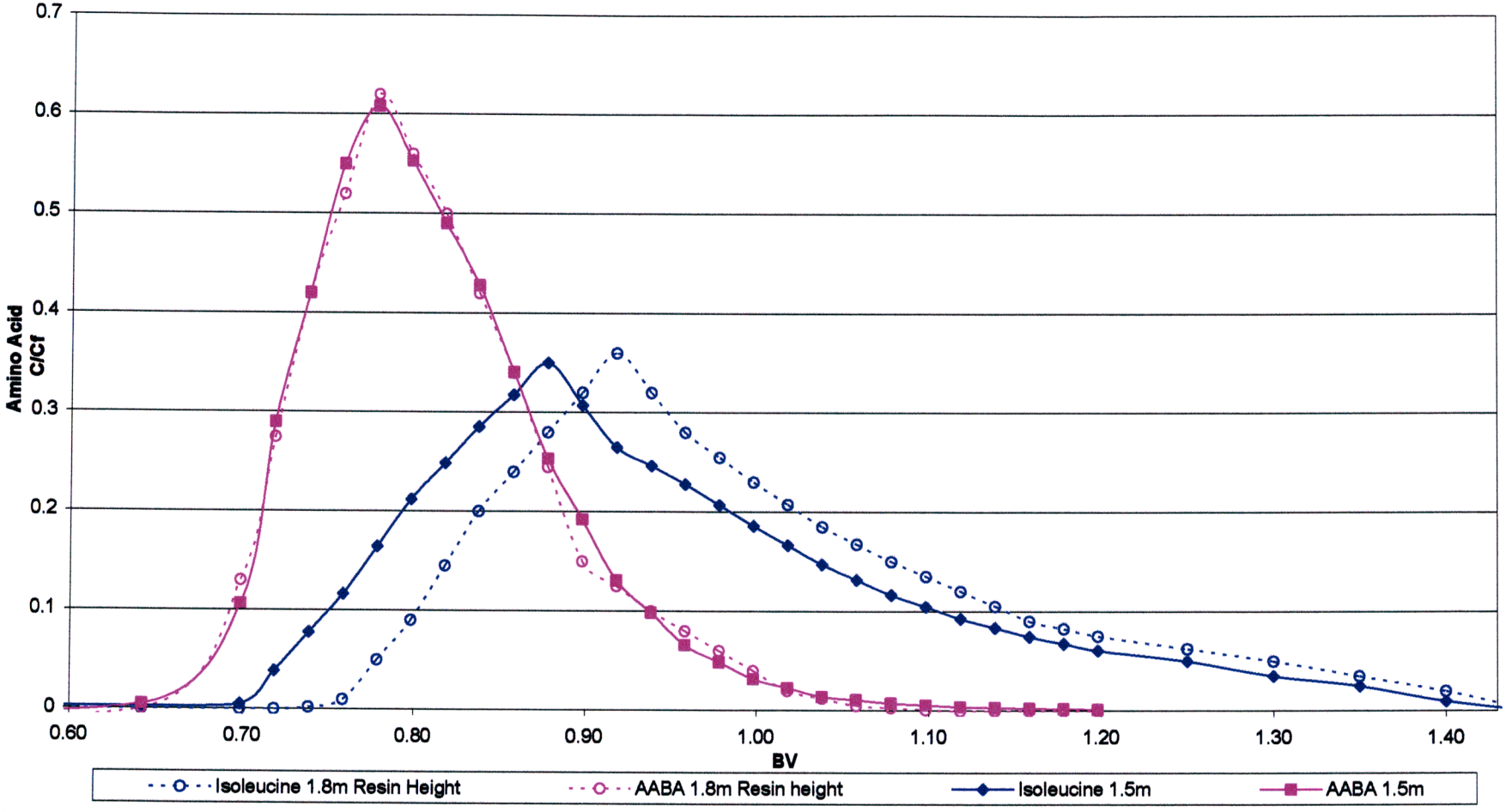


Figure 23. Increasing the height of resin bed.

4.9. Optimised Experiment

From the previous experiments a single optimised run was chosen, where the conditions were manipulated from the base case in order to give the optimum separation. The results from the experiments have been summarised in table 2. The following conditions were set:

- Concentration : Isoleucine (1%), alpha amino butyric acid (1%)
- Volume of feed : 0.1BV.
- Temperature : 95°C
- Eluate flow : 0.5BV/h
- Ionic form of resin : Ca^{2+}
- Particle size : 210-230μm
- Resin Type : Finex
- Resin Height : 1.8m

From the chromatogram represented in figure 23, a combination of the variables which were experimented with resulted in an efficient separation. A resolution of 80% was calculated together with a HETP of 22.7mm and a theoretical number of plates equalling 79. The recoverable portion of the product also increased to 86.8%.

Run No.	Description	Resolution [%]	HETP [mm]	Number of Plates	Concentration of collectable Product [g/l]	Recovery [%]	[Amino Acid Out/Amino acid In]*100 [%] (α)
1	Basecase	22.70%	42.00	35	1.59	38.56%	100.02%
2	High concentration	20.40%	47.40	32	3.43	27.81%	98.71%
3	Low concentration	31.25%	30.90	48	1.58	50.25%	102.00%
4	High feed vol.	19.60%	38.10	39	1.78	18.38%	97.00%
5	low eluant flow	40.20%	14.10	106	1.85	50.32%	98.50%
6	High eluant flow	17.50%	107.00	14	1.30	21.90%	103.20%
7	low temperature	6.20%	87.60	17	1.10	21.50%	99.20%
8	high temperature	40.19%	20.00	75	1.90	45.20%	91.00%
9	Change ionic form	57.00%	22.80	65	1.90	80.71%	98.90%
10	low particle size	27.30%	26.20	56	2.68	47.00%	101.50%
11	high resin height	44.00%	22.30	80	2.90	66.00%	97.68%
13	Optimised run	80.00%	22.70	79	2.92	86.80%	99.20%

Table 03, overall summary of experiments.

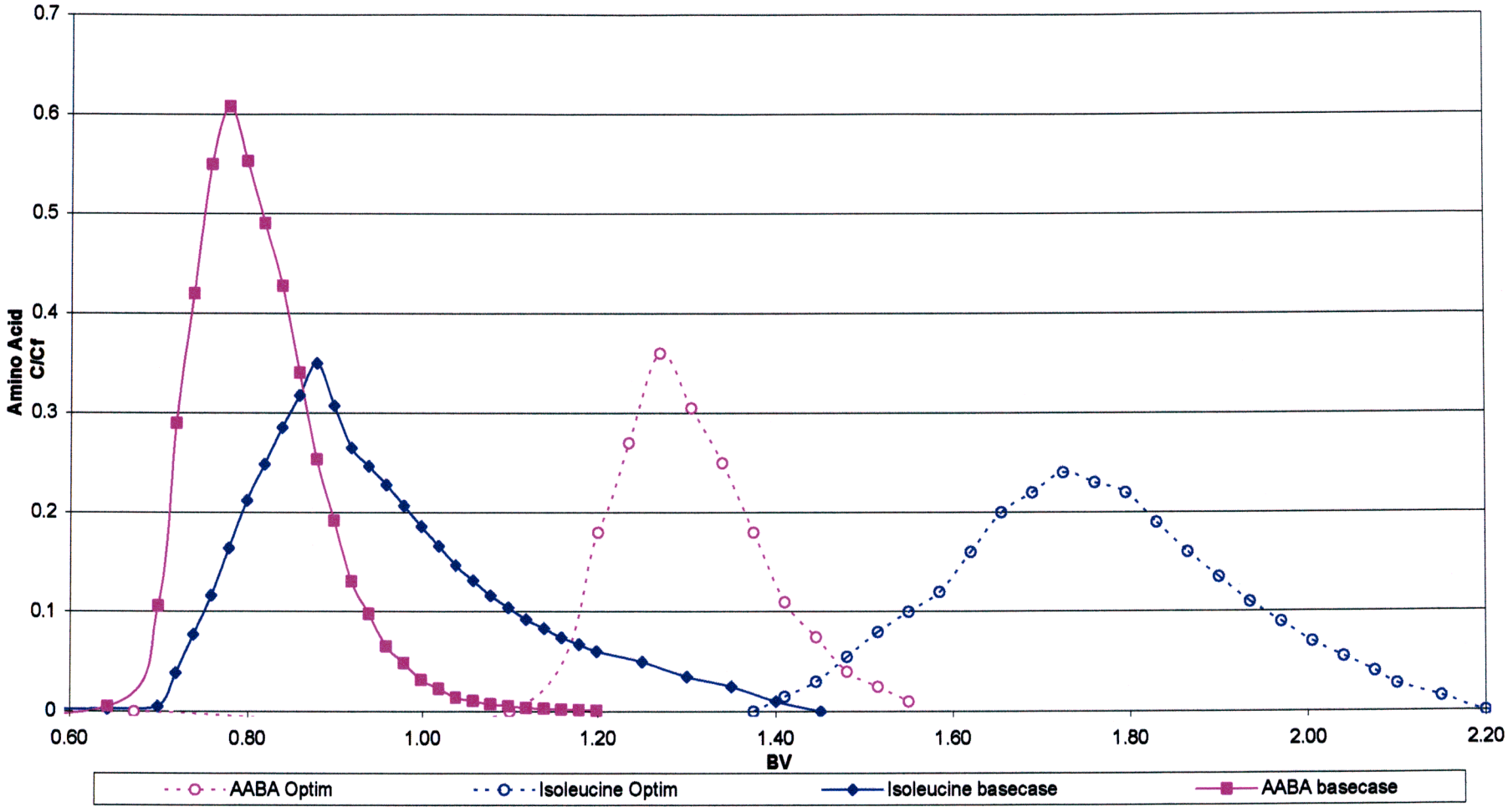


Figure 24. Optimum conditions from experiments

5. Conclusion

The separation of divalent amino acids from mono-valent amino acids has been proved successfully using ion exchange technology on commercial scale. It becomes more difficult when the amino acids that need to be separated are both mono-valent or divalent as their affinity for the resin is almost identical. The aim of the project was to separate two mono-valent amino acids (isoleucine and alpha amino butyric acid), and by changing operating and process variables whether a commercial application could be found.

A basecase was chosen and the chromatogram plotted to reveal a separation. The separation on the basecase indicated only a low recovery as most of the isoleucine was contaminated with AABA. The initial change in conditions was changing the feed concentrations. Increasing the feed concentrations to 4% moved the individual peaks closer and increased the overlapping regions thereby decreasing the recovery. Even though the feed concentrations were higher there was no indication of a more concentrated product. Decreasing the feed concentration increased the sharpness of the peaks and proved that resolution was concentration dependant.

Increasing the feed pulse volume from 0.1 to 0.2 BV resulted in additional overlapping of the peaks. The increase in feed pulse resulted in a decreased overall recovery.

Decreasing the eluant flow resulted in sharper peaks, with each peak taking up a smaller area on the chromatogram. By decreasing the eluant flow, ensured more contact time and the affinity difference between isoleucine and ABAA were enhanced. Products collected also had a higher concentration. A check was also done by increasing the eluant flow and this resulted in widening of the component bands, indicating poor resolution and products of lower concentration thereby concluding that decreasing the eluant flowrate increases separation.

Temperature is also an important process variable which can effect the separation on the resin and this was proved by operating at 30 and 90°C. Operating the separation at 30°C

showed very little separation and high dilution, while operating at 90°C showed increased resolution and higher concentrated products.

The biggest impact was seen when the resin was converted to the Ca^{2+} form. The chromatogram moved to the right by almost 1BV which increased the contact time. This is turn gave recoveries of 57%.

To complete the testing of process variables final tests were conducted on the resin. The initial test was to decrease the size of the resin bead. By decreasing the particle size and using a different resin (Finex) a sharper peak resulted with an increase in resolution and a more concentrated product.

Tests were done where the resin height was increased to evaluate the increase in contact time. Once the resin height was increased the amount of feed had to be increased as well in order to keep at 0.1BV. Increasing the resin height did have a very small effect on moving the amino acid with the greater affinity for the resin (isoleucine) to the right and this helped in increasing the resolution slightly. There was an increase in isoleucine concentration.

Finally a single optimised run was done where the temperature was kept at 90°C, the eluant flow was at 0.5BV/h, and resin was in the Ca^{2+} ionic form, low particle size (210-230μm) and 1.8m resin height. The overall optimisation resulted in recoveries of 86% with high isoleucine concentrations. The thesis proved that through optimisation a commercial application could be found and further research should be done on commercial scale.

Reference

1. Barker P.E., Barker S.A., Hatt B.W., and P.J. Somers, *Chem. Process Eng.*, (Jan. 1971). pg 64-66
2. Barker P.E. and Ganestos G., *Separation Scientific Technology*, (1987) Pg 255-275
3. Begovich J.M., A *high Capacity Pressurised Continuous Chromatogram*, (1998) Pg 104-107
4. Begovich J.M, Byers C.H. and Sisson W.G, Separation Science Technology, (1983) Pg 1167-1197
5. Burke D.J, *Adsorption Separation Technologies via Ion Exchange Resins*, (1983) Pg 102-153
6. Broughton D.B., *Adsorptive Separations-Liquids*, In Kirk-Orthmer Encyclopaedia of Chemical Technology Vol.1 3rd edition , (1978)Pg 213-216
7. Brown C.J. and Fletcher C.J., Ion Exchange for Industry, (1998) Pg 3-17, 129-148
8. Cores A.F, *Ion Exchangers: Properties and applications*, (1979) Pg 102-122
9. Dorfer K. *Ion exchangers*, Industrial Chromatography (1990) Pg 1030-1060
10. Dowex, *Ion exchange resin datasheet.* Distributed by Dow chemicals.(1999)
11. Ganetsos G., Barker P.E., *Preperative and Production Scale Chromatography* Vol. 61 , (1992)
12. Gross D., *Purification of Sugar Products by the Ion Exclusion process*, (1971) Pg 135-147
13. Gupta B., Bhatt S., Shukla R.P. *Donnan equil. Desugarisation,* (1997) Pg,14-21.
14. Guyot A. and Bartholin M., Designs and properties of Polymers as Meterial for Fine Chemistry, (1982) Pg 8.
15. Horvath C.G, *Ion exchange and Solvent Extraction*, (1983) Pg 215-226
16. Jagsuchies G., *Process Scale chromatography.*, Pharmacia LKB GmbH Freiburg (1997) Pg 10.1-10.45
17. Kempe H., Axelsson A., Nilsson B. and Zaccju G., *Chromatographic process applied to the separation of proteins.* J. chromatography, (1999) pg1-12

18. Kristiansen T., Hoffman O., *Affinity Chromatography.*, Pergamon Press Oxford (1978) pg 191-206
19. Moore R.D, *Liquid chromatographic separation of Saccharides, Organic and amino acids.* (1992) Pg 52-75
20. Moore S. and Stein W.H., *Chromatographic Determination of Amino acids on Ion Exchange Resin*, (1984) Pg 21-103
21. Morelli B.and Lampungnani L., Ion exchange Resins, Pg 18-54 (1975)
22. Munir M, *Int.Sugar J.*, Vol. 78, (1976). Pg 100-106
23. Nicoud R.M and Colin H., *Liq. Chromatography Gas Chromatography.* (1991) Pg 557,137,
24. Paananen H., *Trends in the chromatographic separation of Molasses*, Workshop on separation process in the sugar industry, USA. (1996) pg 22-36
25. Paananen H. and Kuisma J., *Chromatographic separation of molasses components.*, Finnfeeds Finland (1995) pg 1-24
26. Pailat D., Cotillon M. and theoleyre M.A., *Tech. of chromatographic sep. in glucose sep. processes.*, Applexion SA France, (1999) pg1-12
27. Rodrigues A.E., Dias M.M, Lopes J.C.B, *Theory of linear and nonlinear chromatography*, Kluwer Acedemic Publishers., (1998) pg25-36
28. Rouset F., *New developments in Chromatographic Separation of Beet Molasses.* British sugar EuroTechLink ,(1997) Pg 44-49
29. Rosensweig R.E., Zahn M., Lee W.K. and Hagan P.S., *Theory of dispersed Multiphase Flow*, (1983) Pg 137- 144
30. Sayama K., Senba Y., *Chromatographic Separation of molasses* (1992) Pg 1058-1059
31. Schubert J. and Richter J.W., *The use of ion exchangers for the determination of physical –chemical properties of substrates* (1986) , Pg 87- 98
32. Siegell J.H., *Ind. Chem. Process Design and Development.* Vol. 21, (1982) Pg. 135-141
33. Small H., *Ion Chromatography*, , (1990) Pg 32-43
34. Small H.and J. Solc, Ion-Chromatography-Principles and Applications in the theory and Practice of Ion Exchange, (1976) Pg. 126-128

35. Swinton E.A., Bolto B.A, Eldridge R.J., Nadebaum P.R. and Coldrey C.P, *Ion Exchange Techology*, (1984) Pg 423-540

36. Yoritomi et Al. US Patent, *Method for the chromatographic separation of soluble components in feed solution*, (1981)

Nomenclature

Concentration (C)	-	[g/l]
Mass (M)	-	[g]
Volume (V)	-	[l]
Time (t)	-	[h]
Bed volume (BV)	-	[-]
Velocity (v)	-	[m/s]
Flowrate (F)	-	[ml/min]
Temperature (T)	-	[°C]
Density (ρ)	-	[kg/m^3]
Viscosity (μ)	-	[Pa.s]
Area (A)	-	[m^2]
Resolution (R_s)	-	[%]
Theoretical plates (N)	-	[-]
HETP	-	[mm]

Appendix

Appendix 1 Calculation for volumetric flowrate

$$v_C = \frac{g(\rho_2 - \rho_1)}{k(\mu_2 - \mu_1)}$$

k = permeability co-efficient of the bed (defined by manufacturer).

$$v_C = \frac{9.81(1300 - 974.9)}{1.5x10^{10}(0.006 - 0.001)}$$

ρ_1 @ 75°C = 974.9 kg/m³ (Perry's 1984 Fig. 3-76)

μ_1 @ 75°C = 0.001Pa.s (Perry's 1984 Fig. 3-43)

$$v_C = 4.25x10^{-5}\, m/s$$

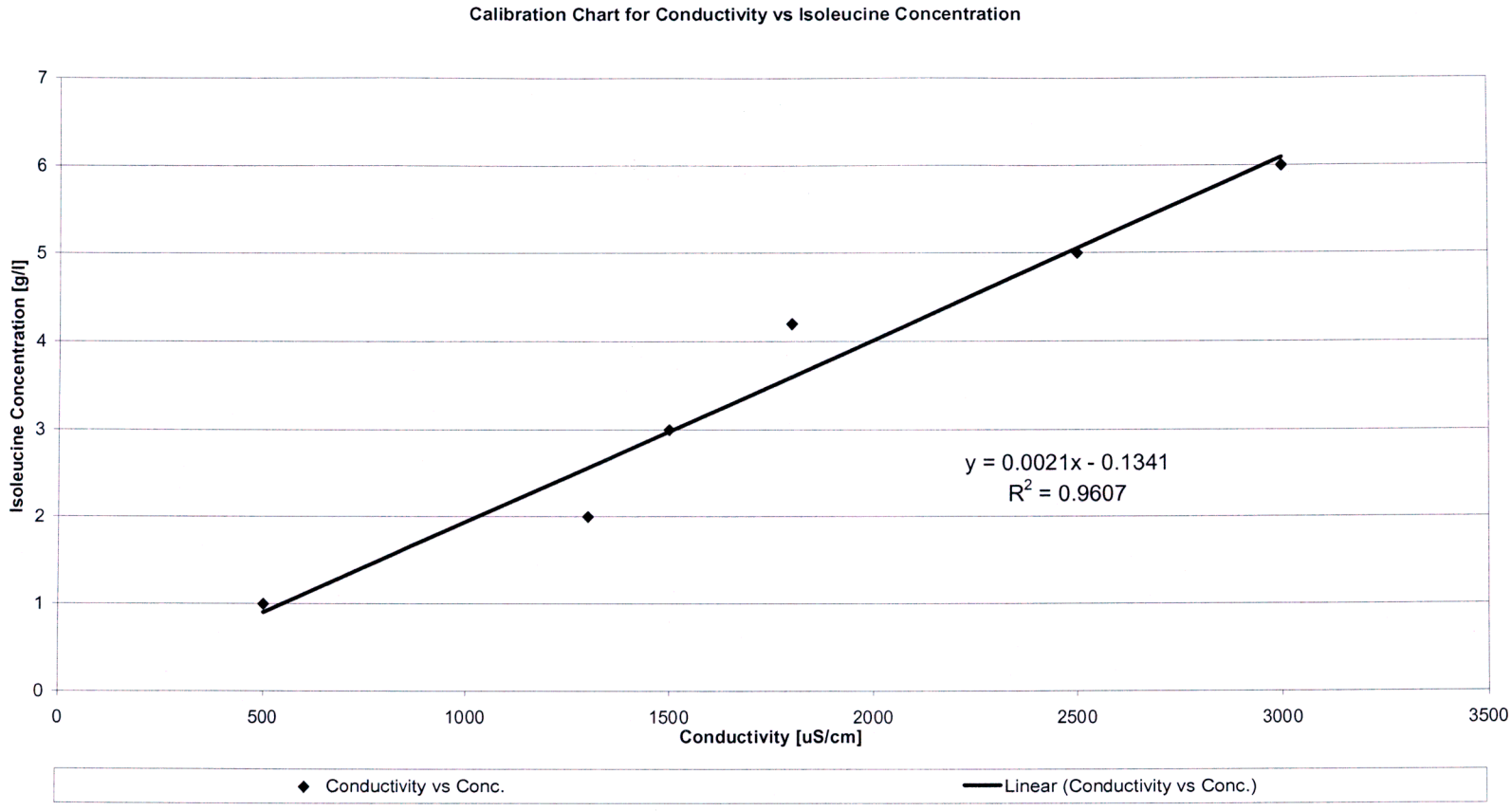
$$A = \frac{\Pi d^2}{4}$$

$$A = \frac{\Pi(0.055)^2}{4} = 2.37x10^{-3}\, m^2$$

$$\dot{V}_C = v_C * A = 4.25x10^{-5}\, m/s * 2.37x10^{-3}\, m^2$$

$$\dot{V}_C = 1x10^{-7}\, m^3/s = 3.62x10^{-4}\, m^3/h = 0.362l/h$$

As a starting point a volumetric flowrate of approx 8.5 times the critical flowrate was chosen. 1BV/h = 3.11l/h



Appendix 2 Calibration chart for Conductivity vs. pure Isoleucine concentration

Appendix 3

BV	Comm. BV	Isoleucine [g/l]	AABA [g/l]
0.32	0.32	0.01	0.01
0.32	0.64	0.05	0.10
0.06	0.70	0.10	2.10
0.02	0.72	0.77	5.72
0.02	0.74	1.55	8.29
0.02	0.76	2.33	10.85
0.02	0.78	3.29	12.00
0.02	0.80	4.24	10.91
0.02	0.82	4.98	9.68
0.02	0.84	5.71	8.44
0.02	0.86	6.36	6.72
0.02	0.88	7.00	5.00
0.02	0.90	6.15	3.79
0.02	0.92	5.30	2.58
0.02	0.94	4.93	1.94
0.02	0.96	4.56	1.30
0.02	0.98	4.14	0.97
0.02	1.00	3.72	0.63
0.02	1.02	3.33	0.46
0.02	1.04	2.94	0.28
0.02	1.06	2.64	0.22
0.02	1.08	2.33	0.15
0.02	1.10	2.09	0.12
0.02	1.12	1.85	0.08
0.02	1.14	1.67	0.07
0.02	1.16	1.49	0.05
0.02	1.18	1.35	0.04
0.02	1.20	1.21	0.03
0.05	1.25	1.00	0.00
0.05	1.30	0.70	0.00
0.05	1.35	0.50	0.00
0.05	1.40	0.20	0.00
0.05	1.45	0.00	0.00

Table 04. Results from the basecase experiment.

Sample Calculation for Isoleucine purity

$C_T = C_I + C_A$ (8)

= 0.01 + 0.01

= 0.02g/l

Where C_T is the total Concentration (g/l)

C_I is the isoleucine concentration (g/l)

C_A is the AABA concentration (g/l)

$$P_I = C_I / C_T \quad (9)$$

= Isoleucine / total concentration
= 0.01 / 0.02
= 0.5 or 50%

Where P_I is the isoleucine purity

Similarly table 05 below shows the calculation for the rest of the samples.

BV	Comm. BV	Isoleucine [g/l]	AABA [g/l]	Total Concentration [g/l]	Isoleucine Purity [%]
0.32	0.32	0.01	0.01	0.02	50%
0.32	0.64	0.05	0.10	0.15	33%
0.06	0.70	0.10	2.10	2.20	5%
0.02	0.72	0.77	5.72	6.49	12%
0.02	0.74	1.55	8.29	9.84	16%
0.02	0.76	2.33	10.85	13.18	18%
0.02	0.78	3.29	12.00	15.29	21%
0.02	0.80	4.24	10.91	15.15	28%
0.02	0.82	4.98	9.68	14.65	34%
0.02	0.84	5.71	8.44	14.15	40%
0.02	0.86	6.36	6.72	13.08	49%
0.02	0.88	7.00	5.00	12.00	58%
0.02	0.90	6.15	3.79	9.94	62%
0.02	0.92	5.30	2.58	7.88	67%
0.02	0.94	4.93	1.94	6.87	72%
0.02	0.96	4.56	1.30	5.86	78%
0.02	0.98	4.14	0.97	5.11	81%
0.02	1.00	3.72	0.63	4.35	86%
0.02	1.02	3.33	0.46	3.79	88%
0.02	1.04	2.94	0.28	3.22	91%
0.02	1.06	2.64	0.22	2.85	92%
0.02	1.08	2.33	0.15	2.48	94%
0.02	1.10	2.09	0.12	2.21	95%
0.02	1.12	1.85	0.08	1.93	96%
0.02	1.14	1.67	0.07	1.74	96%
0.02	1.16	1.49	0.05	1.54	97%
0.02	1.18	1.35	0.04	1.39	97%
0.02	1.20	1.21	0.03	1.24	98%
0.05	1.25	1.00	0.00	1.00	100%
0.05	1.30	0.70	0.00	0.70	100%
0.05	1.35	0.50	0.00	0.50	100%
0.05	1.40	0.20	0.00	0.20	100%
0.05	1.45	0.00	0.00	0.00	0%

Sample calculation for mass of Isoleucine

$$M_T = BV * V_R * C_T \quad (10)$$

= 0.32 * 3.11 * 0.02
= 0.0199 g amino acids.
Where M_T = Mass of total amino acids in sample (g)
BV = Fractional volume of resin
V_R = Volume of resin (l)

Simarly
$M_I = BV * V_R * C_I$
= 0.32 * 3.11 * 0.01
= 0.0099g isoleucine.
Where M_I = Mass of isoleucine in sample (g)

Table 06 below shows the total mass for all samples.

BV	Comm. BV	Isoleucine [g/l]	AABA [g/l]	Total Concentration [g/l]	Isoleucine Purity [%]	Mass of amino acids in sample [g]	Mass of Isoleucine in sample [g]
0.32	0.32	0.01	0.01	0.02	50%	0.01997	0.00998
0.32	0.64	0.05	0.10	0.15	33%	0.14975	0.09983
0.06	0.70	0.10	2.10	2.20	5%	0.38500	0.36750
0.02	0.72	0.77	5.72	6.49	12%	0.40368	0.35578
0.02	0.74	1.55	8.29	9.84	16%	0.61174	0.51533
0.02	0.76	2.33	10.85	13.18	18%	0.81980	0.67487
0.02	0.78	3.29	12.00	15.29	21%	0.95073	0.74640
0.02	0.80	4.24	10.91	15.15	28%	0.94233	0.67860
0.02	0.82	4.98	9.68	14.65	34%	0.91123	0.60179
0.02	0.84	5.71	8.44	14.15	40%	0.88013	0.52497
0.02	0.86	6.36	6.72	13.08	49%	0.81327	0.41798
0.02	0.88	7.00	5.00	12.00	58%	0.74640	0.31100
0.02	0.90	6.15	3.79	9.94	62%	0.61827	0.23574
0.02	0.92	5.30	2.58	7.88	67%	0.49014	0.16048
0.02	0.94	4.93	1.94	6.87	72%	0.42731	0.12067
0.02	0.96	4.56	1.30	5.86	78%	0.36449	0.08086
0.02	0.98	4.14	0.97	5.11	81%	0.31753	0.06002
0.02	1.00	3.72	0.63	4.35	86%	0.27057	0.03919
0.02	1.02	3.33	0.46	3.79	88%	0.23543	0.02830
0.02	1.04	2.94	0.28	3.22	91%	0.20028	0.01742
0.02	1.06	2.64	0.22	2.85	92%	0.17727	0.01337
0.02	1.08	2.33	0.15	2.48	94%	0.15426	0.00933
0.02	1.10	2.09	0.12	2.21	95%	0.13715	0.00715
0.02	1.12	1.85	0.08	1.93	96%	0.12005	0.00498
0.02	1.14	1.67	0.07	1.74	96%	0.10792	0.00404
0.02	1.16	1.49	0.05	1.54	97%	0.09579	0.00311
0.02	1.18	1.35	0.04	1.39	97%	0.08646	0.00249
0.02	1.20	1.21	0.03	1.24	98%	0.07713	0.00187
0.05	1.25	1.00	0.00	1.00	100%	0.15868	0.00000
0.05	1.30	0.70	0.00	0.70	100%	0.10885	0.00000
0.05	1.35	0.50	0.00	0.50	100%	0.07775	0.00000
0.05	1.40	0.20	0.00	0.20	100%	0.03110	0.00000
0.05	1.45	0.00	0.00	0.00	0%	0.00000	0.00000

Sample calculation for Mass balance

$$C_{FT} = C_{FI} + C_{FA} \qquad (11)$$

Where C_{FT} is the total Concentration of amino acids fed to the column (g/l)
C_{FI} is the isoleucine concentration fed to the column (g/l)
C_{FA} is the AABA concentration fed to the column (g/l)

= 19g/l AABA + 19.72g/l Isoleucine
= 38.72g/l amino acids.

$$M_{FT} = BV * V_R * C_{FT}$$
= 38.72 * (0.1BV * 3.11)
= 12.04g
Where M_{FT} is mass of amino acids fed to the column

$$\alpha = \sum (M_T / M_{FT}) * 100$$
= (11.89/12.04)*100
= 0.987*100 = 98.7%
Where α = ratio of exit and feed isoleucine to the column.

$$C_A = \sum \frac{M_{IP}}{V_l}$$
Where C_A is the average concentration collected in the product (g/l)
M_{IP} is the total mass of isoleucine with purities above 85% isoleucine (g)
V_l is the volume of liquid collected (l)
= (0.27057+0.2353+0.200+0.177+……..+0.0311)/ (0.02+0.02+……..+0.05)
= 1.59g/l

Now Resolution, HETP and N.

$$R_S = \frac{2(t_{R2} - t_{R1})}{(t_{W1} + t_{W2})} \qquad (7)$$

$$R_S = \frac{2(0.89 - 0.79)}{(0.28 + 0.6)}$$

$R_s = 0.227 = 22.7\%$

$$HETP = \frac{L}{N} = \frac{L}{16\left(t_R / t_W\right)^2} \qquad (6)$$

$$HETP = \frac{1500}{16\left(0.89/0.6\right)^2}$$

HETP = 42mm

➔ N = 1500/42
= 35.2 theoretical plates.