MEng Final Year Research Project

Biosurfactant Refinery: High Performance Alternatives to Synthetic Products

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30th April 2010

Abstract

Foam fractionation is a separation technique used to recover proteins from solutions found in a fermentation process. The aim of this research was to investigate the effects of varying foam column operating parameters on the protein enrichment and recovery. Both batch and continuous foam fractionation experiments were carried out. Foam fractionation products were collected in the bottom and top reservoirs forming the bottom and top products respectively. The batch studies took place with and without reflux. The presence of reflux caused higher enrichment of the top product collected. The continuous foam fractionation column was operated using the stripping mode whereby feed was introduced from the top of the column. The air and feed flowrates were then varied at three different values. It was found out that the enrichment and recovery of the top product increased at a low feed flowrate and a high gas velocity. These outcomes may be useful in finding the most cost effective method for protein recovery using foam separation.

Acknowledgements

I would like to thank Dr Peter Martin and James Winterburn for their continuous support, guidance and good advice throughout this research project. Also thanks to Ann Beeyo for her loyal company in the laboratory through the year.

Table of Contents

Abstract……...2

Acknowledgements……....3

Contents……..4

Nomenclature……..6

1 Introduction…….....7

2 Project Objectives……8

3 Literature Review ……....9

3.1 Foam……..9

3.1.1 Foam Structure…….....9

3.1.2 Liquid Holdup……12

3.2 Surfactants……...12

3.2.1 Structure of Surfactants……12

3.2.2 CMC and Surface Tension……14

3.2.3 Adsorption of Surfactants ……15

3.3 Foam Fractionation……..17

3.3.1 Modes of Operation……17

3.4 Biosurfactant Recovery……24

4 Foam Fractionation Experiments…….....27

4.1 List of Equipments and Reagents Used…..…….....27

4.2 Equipment Set-up……...……..….29

4.2.1 Batch Foam Fractionation……...29

4.2.2 Continuous Foam Fractionation…….31

4.3 Experimental Methods……...33

4.3.1 CPC Solution Makeup and Calibration…….33

4.3.2 Buffered Casein Solution Makeup and Calibration……..35

4.3.3 Feed Pump Calibration…….35

4.3.4 Batch Foam Fractionation……36

4.3.5 Continuous Foam Fractionation……...37

4.4 CPC Concentration, Enrichment and Recovery……...38

5 Experimental Results, Observations and Discussion……..40

5.1 CPC Calibration……..40

5.2 Casein Calibration……41

5.3 Feed Pump Calibration……42

5.4 Batch Foam Fractionation……...44

5.4.1 CPC Feed without Reflux……..44

5.4.2 CPC Feed with Total Reflux……..46

5.5 Continuous Foam Fractionation……..50

5.5.1 CPC Feed with Varied Feed Flowrates…….50

5.5.2 CPC Feed with Varied Air Flowrates……...54

5.5.3 Mixture of CPC and Buffered Casein as Feed…….59

6 Further Work……....61

7 Conclusion…….....62

8 References…….....63

9 Appendix…...…….....66

Nomenclature

Symbol	Meaning
a_i	Activity coefficient of component i
C_S	Concentration of surfactant in the bulk liquid
n	Ionic charge of the surfactant
R	Gas constant
T	Absolute temperature
Vaqueous	Liquid volume in foam
Vtotal	Total liquid volume in foam fractionation column
ε	Liquid Holdup
γ	Surface tension
Γ_i	Surface excess of component i
Γ	Concentration of surfactant on the surface

1 Introduction

Foam fractionation is an established separation technique. There have been many increasingly important uses of foam fractionation for the past forty years. These include in the removal of biological proteins from waste material to reduce the cost of pollution control and also the purification of proteins from complex mixtures of components for further uses in food and pharmaceutical industries (Charm, 1972). There is still a growing demand in the use of microbial proteins as a food source and also as biopharmaceutical products (Charm, 1972). Foam fractionation may be the best technique to employ in purifying or recovering these proteins at a larger scale operation with reduced cost.

This project mainly concentrates on finding the optimum working parameters for the foam fractionation column to produce higher top product recovery. Other people's works in the foam separation field were reviewed in Chapter 3. This chapter also included in the current state of knowledge about foam and surfactants structures, the fundamental principles of foam fractionation and also the importance of biosurfactant recovery. Meanwhile Chapter 4 describes the types of reagents and equipments used, equipments layout and also the experimental techniques employed. The batch and continuous foam fractionation processes required different types of equipment set-up. The experimental methods used include the making and calibration of Cetylpryridinium Chloride (CPC) and buffered Casein solutions and also ways to operate the batch and continuous experiments. Chapter 5 describes the results of batch foam fractionation with and without reflux and also continuous foam fractionation without reflux at different air and feed flowrates.

2 Project objectives

This research project was carried out mainly to continue the foam fractionation studies done by Dutton (2007) in the University of Manchester. The main objectives of the project were developed throughout this research study.

Several experiments were conducted during the first semester aiming for familiarisation with the laboratory work and also to work effectively within the laboratory guidelines. At first, the CPC solution was made and calibrated before setting up and carrying out the batch foam fractionation experiments. The presence of external reflux in the batch process was also investigated.

Most of the major work was taking place in the second semester which focused on continuous stripping foam fractionation processes. These continuous studies were meant to replicate the integrated bioreactor and foam fractionation experiments conducted by Chen *et al.* (2006). The effects of varying the air and feed flowrates on the enrichment and recovery of the top product were investigated. In addition, the effects of changing these flowrates on the time taken to reach the steady state condition were also analysed. These experiments are useful for developing an effective separation technique that can result in higher top product recovery and more consistent product quality applicable for food, cosmetic and pharmaceutical industries (Chen *et al.*, 2006).

3 Literature Review

This section introduces the structures of foam and surfactants, the foam fractionation process and also its important application in recovering biological surfactants (biosurfactants).

3.1 Foam

3.1.1 Foam Structure

The foam consists of polyhedral bubbles and some entrained liquid. Lemlich (1968) mentioned that the dodecahedral bubbles are assumed to be of equal sized throughout the foam fractionation column and that every three bubbles in a foam pressed against each other, flattening their thin films causing the formation of channels or capillaries called Plateau borders in between these films.

Lemlich (1968) had developed a foam drainage model based on polyhedral foams as shown in Figure 1. The interstitial suction and also the low liquid content of polyhedral foams favours them for foam fractionation. Grassia *et al.* (2005) mentioned that the growth of foam is directly related to the rupture or collapse of bubbles thin film and also liquid drainage. Foam films are assumed to have a well defined tensile strength and this is proven by the foam drainage mathematical model solved by Grassia *et al*. (2005) in the University of Manchester.

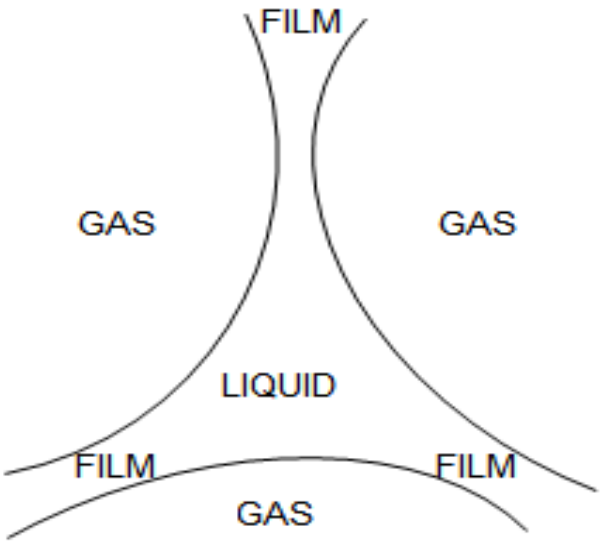

Figure 1: Cross section of a Plateau border in foam. The liquid is flowing through the Plateau border in this case. Picture adapted from Lemlich (1968)

The liquid from thin film drains into the Plateau border due to two forces; Plateau border suction and disjoining pressure whereas the liquid in the interconnected network of Plateau borders drains due to gravitational force (Uraizee and Narsimhan, 1992). Grassia *et al.* (2005) stated that the capillary suction forces on film depend on the cross-sectional area of Plateau borders. There is a limited Plateau border area at the top of the column.

Uraizee and Narsimhan (1992) believed that the rupture of thin films and liquid drainage caused the bubbles to coalesce and grow larger. Du *et al.* (2002) discovered that the bubble size distribution pattern remained constant when a continuous foam fractionation process reached its steady state condition. Du *et al.* (2002) also mentioned that the bubble size distribution was affected by the column wall because it approaches a flat profile across the column. Such distribution was measured using a capillary probe with photoelectric sensors (Du *et al.*, 2002). In reality, it is not possible to have perfectly equal size bubbles inside the foam fractionation column and some changes must always occur in the bubbles distribution size (Lemlich, 1968).

Lemlich (1968) stated that there are two reasons for internal bubble coalescence. The first one is the diffusion of gas from small bubbles to large bubbles which is not really significant in foam fractionation process. The other reason is the rupture of thin films that separates the bubbles. The unequal size of two spherical bubbles lead to a difference in pressure between the bubbles. This pressure difference causes gas to diffuse from the smaller to larger bubble. The larger bubble will grow bigger while the small bubble becomes even smaller until it completely disappears.

The elasticity of the thin film means that the film surface is easily stretched. Once the film is stretched, there was a reduction in the number of surfactants at the surface and this causes the surface tension to increase. Stretching the film can deplete the adsorbed surfactant due to two main reasons; the Marangoni and Gibbs effects. The Marangoni effect is defined as the inability of surfactant molecules to diffuse instantaneously from the interior of the film to the surface. Meanwhile, the Gibbs effect causes the bubbles surface to be incompletely covered with surfactants due to the limited supply of surfactants in the interior of the film. Since CPC is an ionic surfactant, there is an electrostatic repulsion between the charged bubble surfaces and this helps to oppose film thinning and rupture (Lemlich, 1968).

Lemlich (1968) also mentioned about external bubble coalescence which is related to the different methods for collapsing foam. Collapsed foam is more desirable than uncollapsed foam as the latter one occupies a larger volume which can be very messy in an experiment. Examples of collapsing foam methods include the use of mechanical device such as rotating foam breaker, sonic and ultrasonic vibration and also chemical additives.

3.1.2 Liquid Holdup ε

Liquid holdup is the ratio of liquid volume in the foam to the total liquid volume in the foam fractionation column. Lockwood *et al.* (2000) defined liquid holdup as the volume of the aqueous fluid in a defined region of interest along the length of the foam column at the same axis. In other words, it is the fractional amount of liquid contained in the column.

$$\varepsilon = \frac{V_{aqueous}}{V_{total}}$$ ---Equation 1

Lockwood *et al.* (2000) used a nuclear scintigraphic imaging technique to measure liquid holdup in a foam fractionation column and they discovered that the greatest changes in liquid holdup occurred just above the bubble-liquid interface especially when a high gas velocity was used to generate the foam. Their experiments showed that the volume of water in the foam did not change very much with an increase in the column height. Uraizee and Narsimhan (1992) also suggested that the liquid holdup decreases at higher air flowrates due to faster liquid drainage rate from the film into the Plateau border.

3.2 Surfactants

3.2.1 Structure of Surfactants

Surfactants or also known as surface active molecules are substances which exhibit remarkable behaviour at surfaces or interfaces as they possess both hydrophobic and hydrophilic groups. Surfactants are present in many gas-liquid systems and can cause a significant change in dispersion properties even when they are in small amount. At the bubble-liquid interface the hydrophobic part of the molecule will project towards the bubble while the hydrophilic group will orientate itself as much as possible within the aqueous phase (Lemlich, 1968). This is illustrated in Figure 2 below.

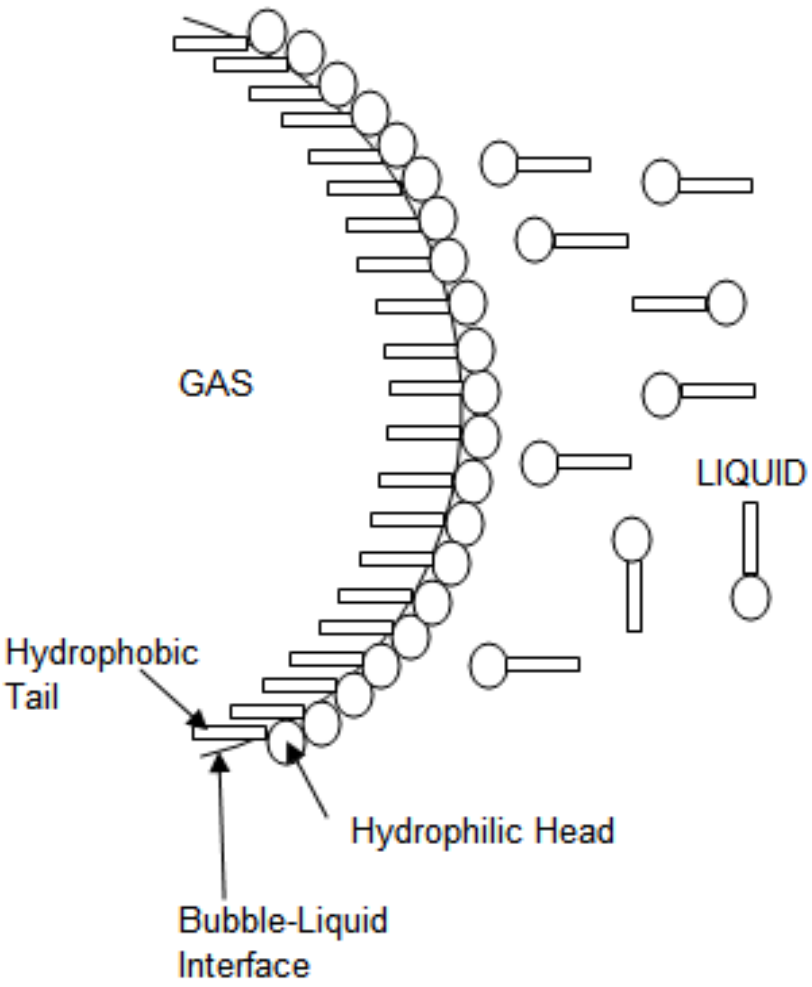

Figure 2: Cross section of part of the bubble-liquid interface. Picture adapted from Lemlich (1968)

The surfactant molecules are partitioned between solution in the bulk and adsorption at the interface in order to achieve a lower energy state (Darton and Sun, 1999). This will reduce the surface energy and also the surface tension at the interface. Chen *et al.* (2006) stated that surfactants can be chemically synthesised from fossil fuel derivatives or naturally produced by many living organisms during the fermentation process which are also called as biosurfactants often typically found at lower concentration.

3.2.2 Critical Micelle Concentration (CMC) and Surface Tension γ

Darton and Sun (1999) defined CMC as the maximum concentration of surfactant that forms a monolayer on the bubble-liquid interface and thus, giving the lowest value for the surface tension. Dutton (2007) established that CPC has a CMC of $1.0\times10^{-3}\,\text{mol}\,\text{L}^{-1}$. Above the CMC, any surfactant added will not lower down the surface tension and they will instead aggregate to form micelles. Micelles are collections of spherical molecules that have their hydrophobic parts pointing towards the centre of the group to achieve a minimum energy configuration (Darton and Sun, 1999).

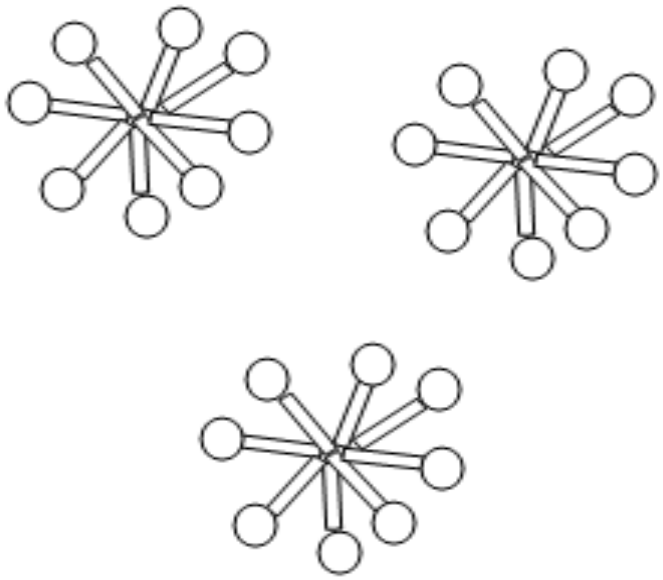

Figure 3: Collection of Micelles.

Below the CMC, the equilibrium process for the surface tension consists of diffusion of surfactant molecules between bulk liquid and sub-surface layer and also the transfer of molecules from the sub-surface layer to the surface. The creation of a fresh surface will cause the surface tension to become higher than its equilibrium value. However, the surface will age as surfactant molecules are adsorbed on it causing lower surface tension (Darton and Sun, 1999).

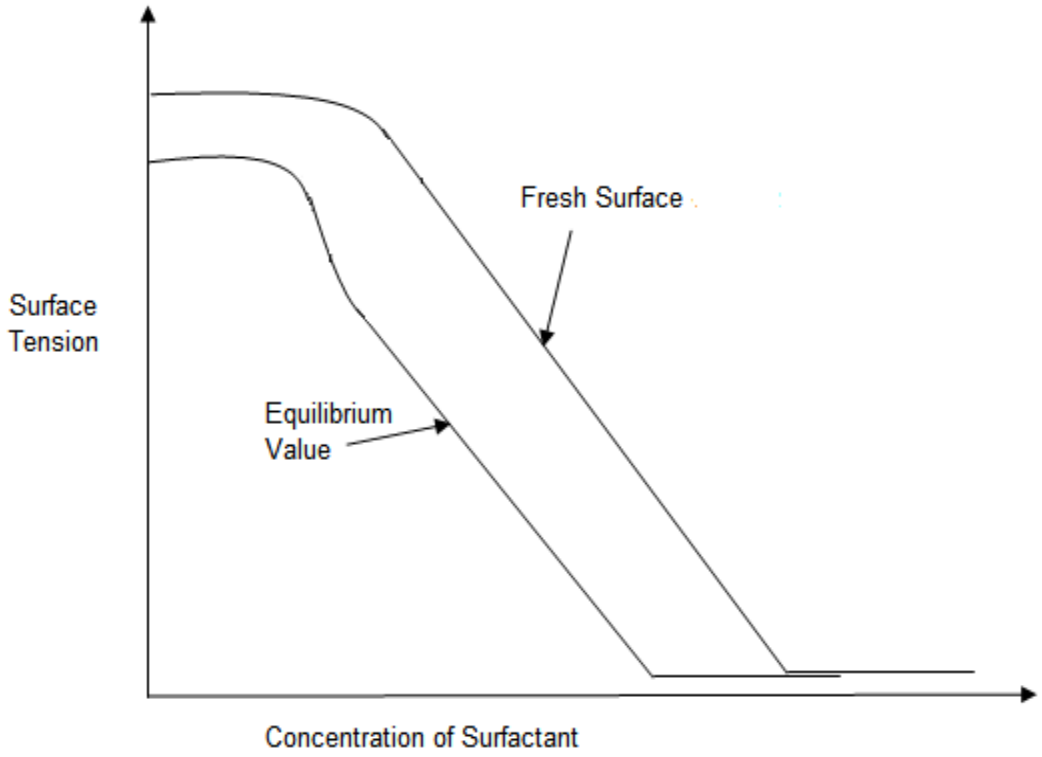

Figure 4: Surface Tension of Surfactants. Picture adapted from Brunner and Lemlich (1962)

3.2.3 Adsorption of Surfactants

Swain (2005) mentioned that the degree of adsorption of surfactants at the bubble surface can be measured by the surface excess Γ. The surface excess is defined as the concentration of surfactants at the bubble surface. The unit is in moles per area.

The Gibbs adsorption equation is used below to indicate the equilibrium adsorption at a gas-liquid interface and also to derive the surface excess (Lemlich, 1972).

$$d\gamma = -RT\sum \Gamma_i d \ln a_i \quad \text{--- Equation 2}$$

There have been limitations in the use of Equation 2 since it is difficult to measure small changes in the surface tension very precisely. Moreover, there are uncertainties in identifying the species component and finding its activity coefficient.

Wall (2007) mentioned that the activity coefficient of the surfactant is assumed to be constant and equivalent to its concentration in the bulk liquid, C_S below the Critical Micelle Concentration. Lemlich (1972) simplified Equation 1 to Equation 2.

$$\Gamma = -\frac{1}{nRT}\frac{d\gamma}{d \ln C_S} \quad \text{--- Equation 3}$$

Wall (2007) stated that n is equal to 2 for CPC. Weast and Selby (1967) mentioned that the surface excess for a monolayer can be roughly estimated from the size and packing of molecules which is determined by the interatomic distances and intermolecular forces respectively. Foam fractionation is one of the experimental methods available for finding the surface excess (Lemlich, 1968).

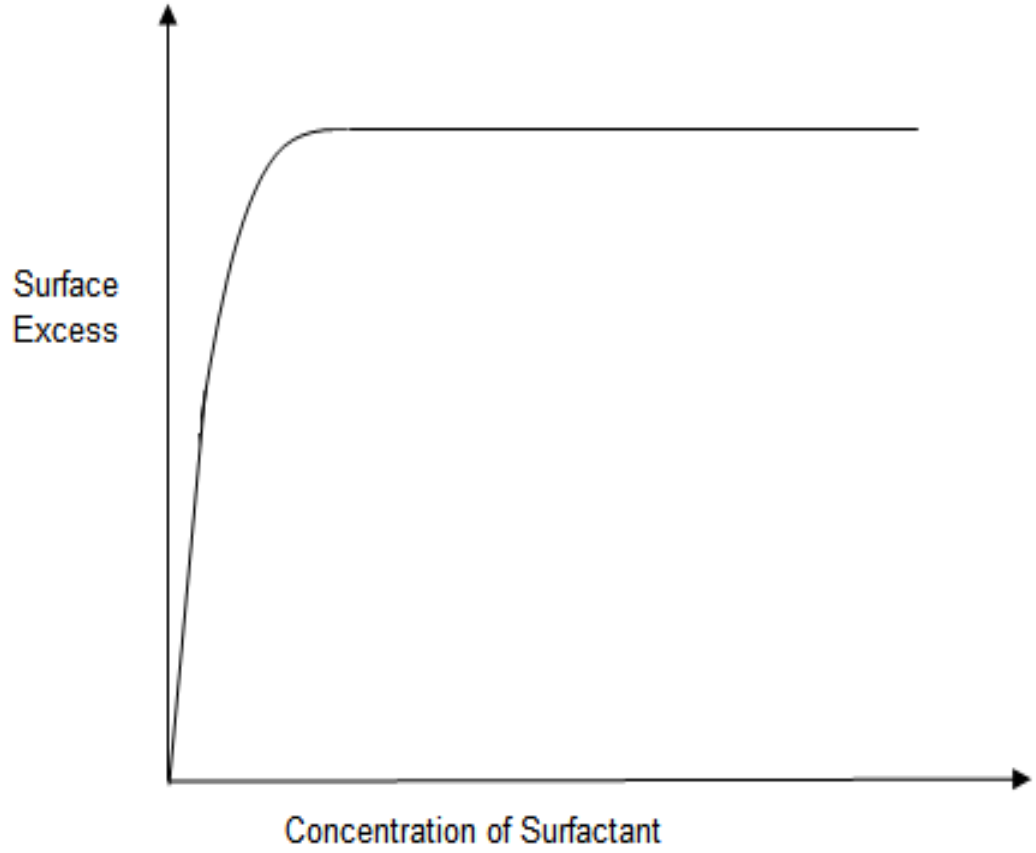

Figure 5: Surface Excess of Surfactants. Picture adapted from Lemlich (1972)

Figure 5 shows the changes in surfactant concentration at the interface with surfactant concentration in the bulk liquid at equilibrium. Lemlich (1972) stated that there is a linear relationship between these two at lower surfactant concentrations in the bulk liquid. However, such linear relationship can no longer hold at higher surfactant concentration in the bulk liquid causing the curve to flatten reaching its maximum surface excess value. This means that there is a saturation of surfactants on the surface at this maximum value.

3.3 Foam Fractionation

This section will explain on the fundamental mechanisms of foam fractionation including the modes of operation and also the effect of external reflux on the separation process. According to Lemlich (1968), foam fractionation is a method of concentrating surface active molecules based on their adsorption at the surfaces of bubbles in the bulk liquid which then rise up through the column forming controlled foam that can be collapsed and collected.

Rubin (1972) mentioned that foam fractionation utilises the formation of foam which act as a medium of large interfacial area for partial separation of surface active molecules from the bulk liquid. Darton and Sun (1999) established that there is a deformation in the bubbles shape when the foam is formed due to faster liquid drainage in the Plateau border.

3.3.1 Modes of Operation

Lemlich (1972) stated that foam fractionation experiments can be conducted as batch and continuous operations as shown in Figures 6 and 7. The bulk liquid is continuously being fed into the column in Figure 7. Chen *et al.* (2006) mentioned that this has many advantageous including a consistency in the product quality, higher concentrated product as well as larger values of yield recovery compared to the batch operation in Figure 6.

It is preferable to operate a continuous rather than a batch foam fractionation process for larger scale production of products.

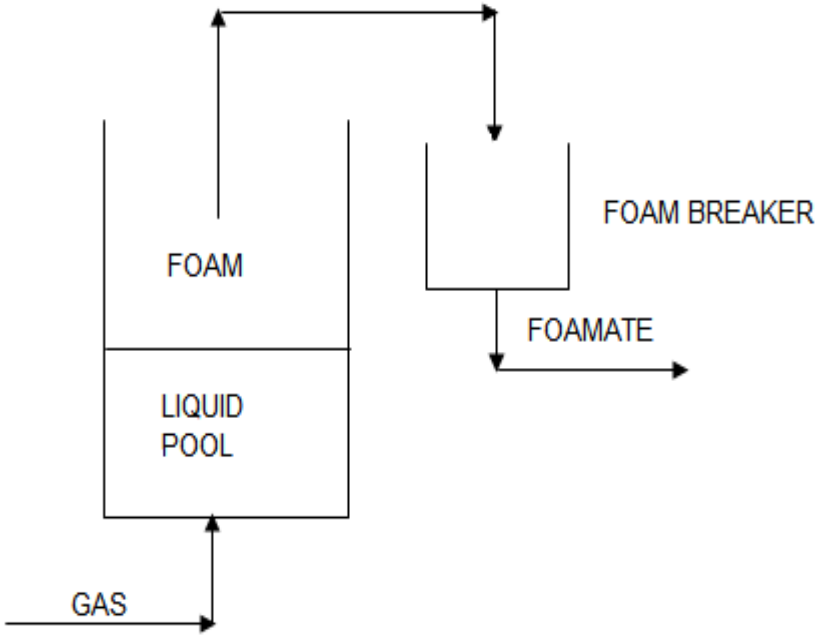

Figure 6: Batch operation of a foam fractionation column. Picture adapted from Lemlich (1972)

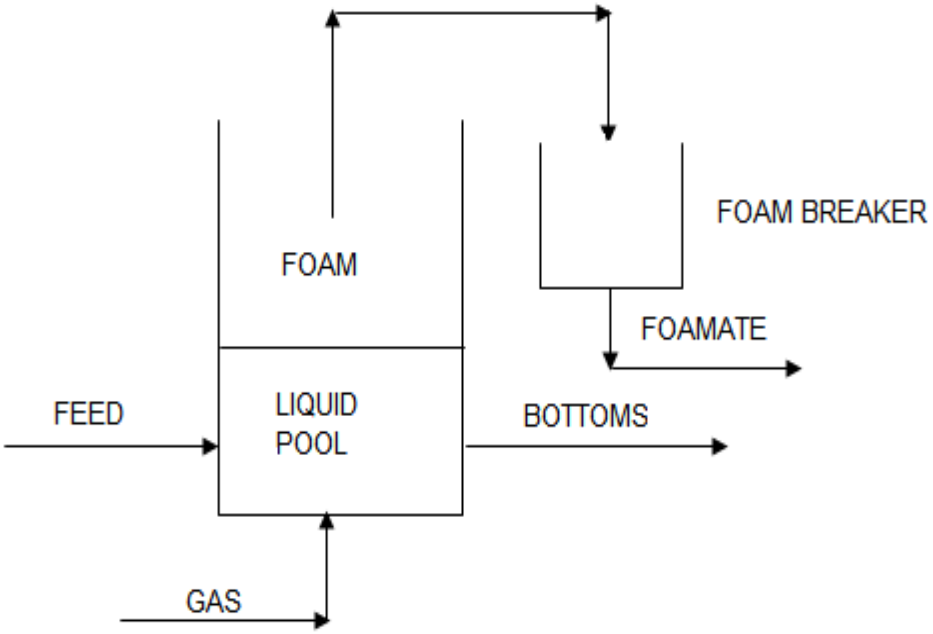

Figure 7: Continuous operation of a foam fractionation column. Picture adapted from Lemlich (1972)

Lemlich (1972) also mentioned that the arrangement of a continuous foam fractionation column can be divided into three ways namely; enriching, stripping and combined. Both of the enriching and combined methods involve in addition of reflux that returned the top product back to the column. Addition of reflux allows higher enrichments of the top product to be obtained.

It is also possible to combine both enriching and stripping methods to benefit from the purification of bottom and top products at the same time. This is illustrated in Figure 11. Hence, there is a similarity between the operations of foam fractionation and distillation columns which involved in enriching, stripping and combined methods.

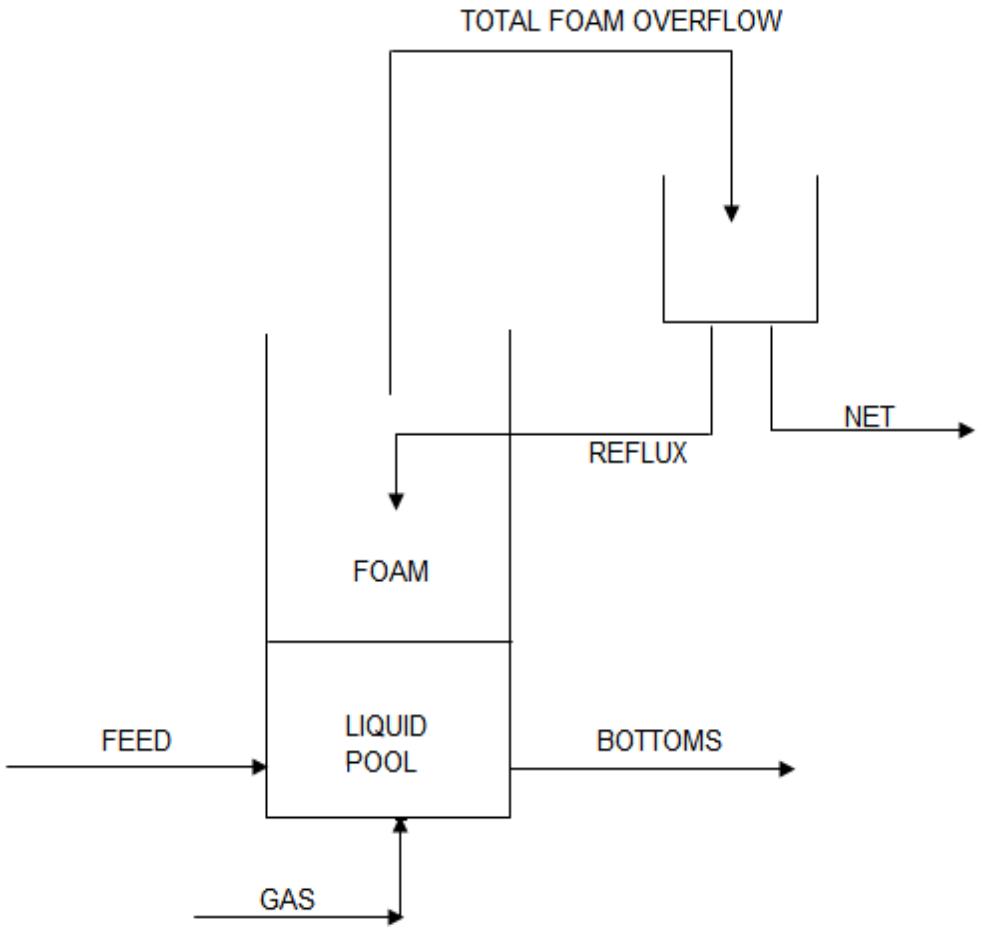

Figure 8: Continuous, enriching operation of foam fractionation involving external reflux. Picture adapted from Lemlich (1972)

The feed must enter from the bottom of the column for the enrichment method. Some of the collapsed foam collected will be purposely returned to the top of the column using a pump and this process is known as external reflux. Lemlich (1968) defined reflux as the ratio of returning foam's flowrate to the flowrate of total foam overflow. The value of reflux ratio ranges from zero (no reflux) to infinity (total reflux).

The returning liquid will trickle down the column through the rising foam. Lemlich (1968) established that the two countercurrent streams will result in mass transfer of surfactant taking place. This can cascade the enrichment of net top product since the external reflux is more concentrated in surfactant than before. If reflux is added, the collapsed foam collected is expected to be more enriched in surfactant compared to a column operation without reflux.

Rubin (1972) stated that it is difficult for the continuous column to reach steady state if it is operated under total reflux. In theory, the surfactant concentration in the collapsed foam should reach infinity but this cannot take place in a real experiment. The reason for this is that there will be a point of time when the foam could no longer reach the top of the column causing the enrichment process to stop. The surfactant concentration in the foamate is expected to increase with time while its concentration in the liquid pool should be decreasing. Rubin (1972) also suggested that the total reflux foam fractionation column should be used to concentrate surfactants that are initially present in very low amount in the bulk solution.[8]

Lemlich (1968) mentioned that the internal reflux can also occur in the foam fractionation column at the same time. This internal reflux originates from bubbles coalescence and collapse in the rising foam causing some surfactants and also liquid to be released down the column. Lemlich (1968) explained that the internal reflux can be purposely increased by lengthening the whole column or just the top part of the column. This will slow down the rate of rising foam allowing larger contact between downflowing liquid feed and the foam. As a result, there is an increase in the concentration of surfactants in the top product allowing higher overall product enrichment.

The operation of a continuous stripping column is not so straight forward as the feed must enter from the top of the column and flows down to the bottom passing through the rising foam inside the column. Rubin (1972) mentioned that the countercurrent movement of the feed and the foam can result in continuous mass transfer of solutes within the foam. Lemlich (1968) stated that the interstitial liquid of pool composition in the Plateau border is likely to be replaced with interstitial liquid of feed composition causing the foam to contain more surfactant. The foam can then be collapsed and collected

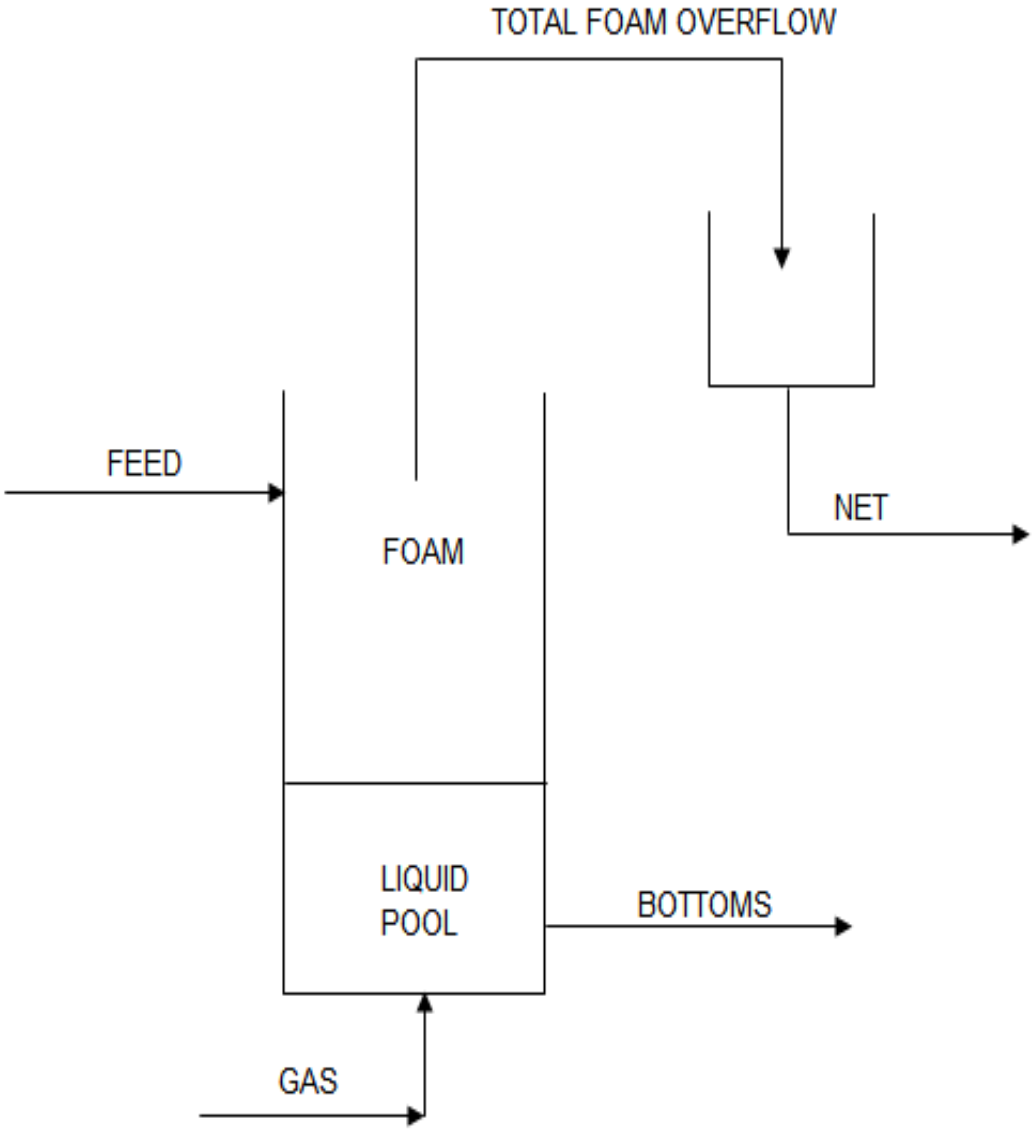

Figure 9: Continuous, stripping operation of foam fractionation without external reflux. Picture adapted from Lemlich (1972)

Goldberg (1968) has developed two models for short stripping foam fractionation columns. For both models the stripping column length is said to be divided into three regions namely; the mixing region around the feed entry point, the mixing region at the bottom liquid pool and also a countercurrent region in between these two. The first model assumed continuous solute transfer between countercurrent streams within the foam while the other model proposed that most of the stripping is due to end effects originating from good mixing contact between falling liquid stream and rising foam.

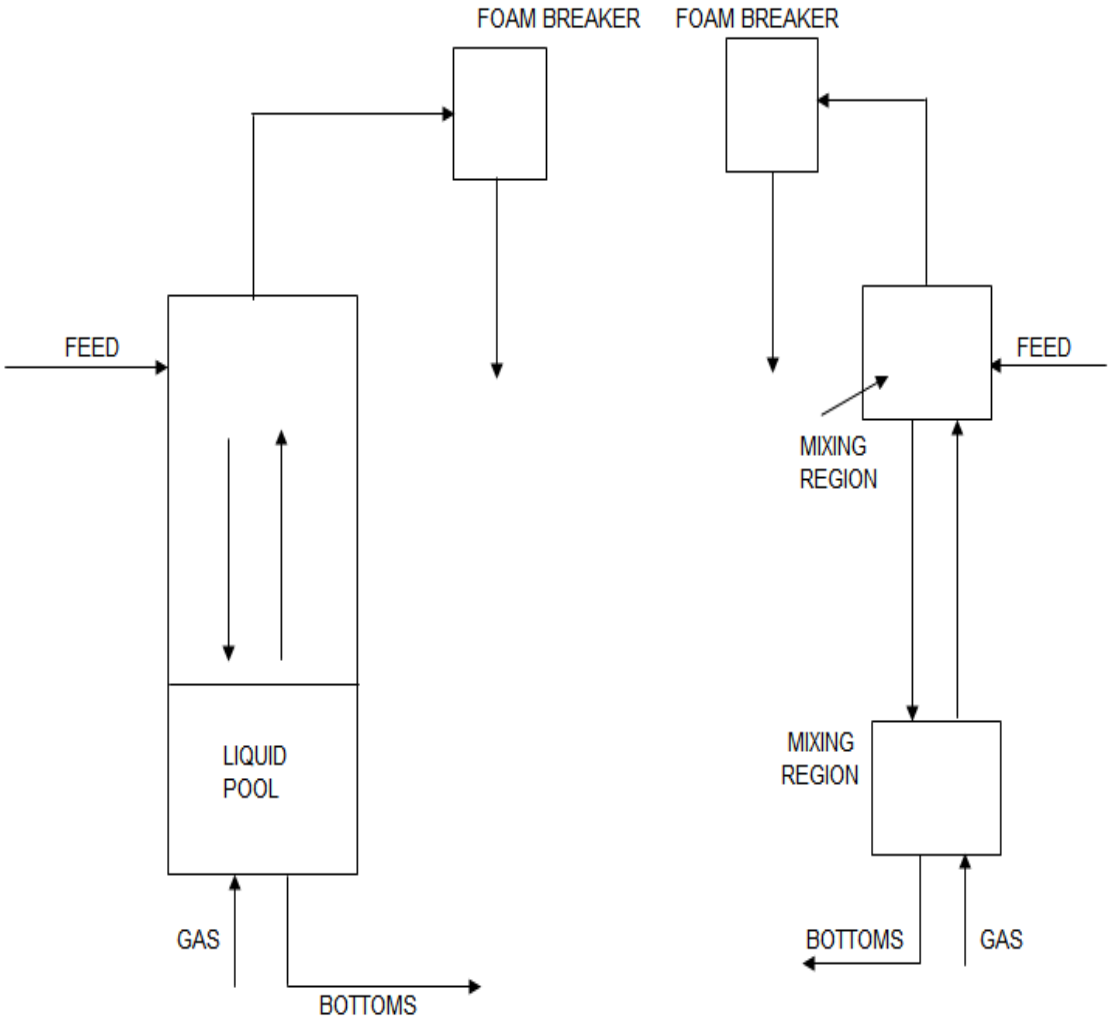

Figure 10: Two models for stripping columns whereby. The left diagram shows that there are solutes being transferred in the countercurrent region while on the right, it is assumed that no solutes are transferred in the countercurrent region. Picture adapted from Goldberg and Rubin (1970)

Goldberg (1968) varied the column operating parameters including feed concentration, the height of stripping section, air flowrate, column diameter and feed flowrate in order to test the more recent model with no solutes transferred in the countercurrent region. The air flowrate was varied between 1.7 and 3.3 $L\,min^{-1}$ while the feed flowrate was changed from 35 to 155 $mL\,min^{-1}$. These data can help to provide the starting points for varying air and feed flowrates in this research project.

Goldberg (1968) concluded that the column height does not affect the degree of separation as long as the countercurrent region still exists. The countercurrent region is said to be disappearing when the height of stripping section is below 10 cm.

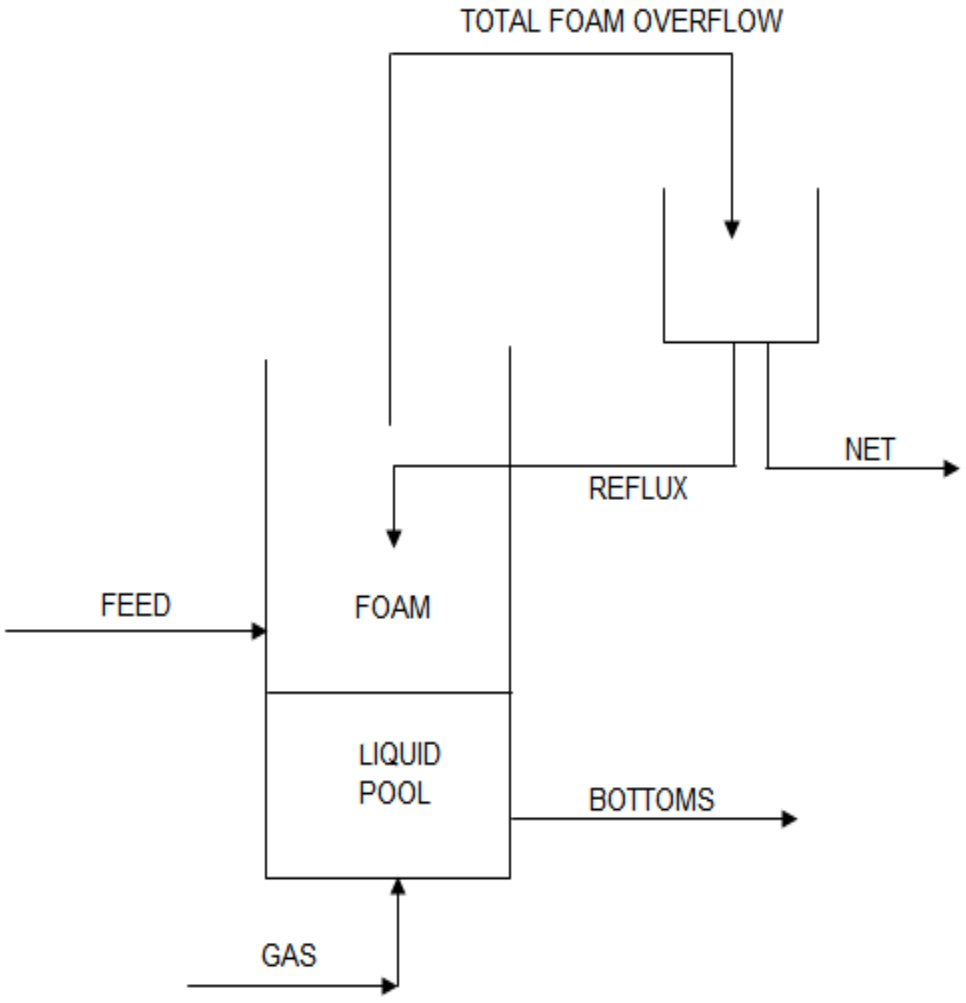

Figure 11: Continuous, combined operation of foam fractionation with external reflux. Picture adapted from Lemlich (1972)

3.4 Biosurfactant Recovery

Biosurfactants offer many advantages in their uses and potential applications and thus, they have become increasingly important in the commercial industries. Chen *et al.* (2006) mentioned that biosurfactants are used as therapeutic agents in the pharmaceutical industry, moisturisers in the cosmetic industry, emulsifiers in the food industry and also to facilitate in the removal of underground oil in the oil industry. They can also be used as an alternative to the chemically synthesised surfactants and this will help to protect the environment (Chen *et al.*, 2006).

The application of biosurfactants has been limited by their expensive production costs and also their current inability to compete with chemically synthesised surfactants based on performance (Dutton, 2007). Dutton (2007) stated that the costs of large scale production and purification of biosurfactants must be cut down by a magnitude order of two before biosurfactants can penetrate the market and sold as biopharmaceutical products, beauty products or even household detergents.

Chen *et al.* (2006) has designed an integrated bioreactor with foam fractionation column which is used for culturing a bacterium species called *Bacillus subtilis* (BBK006) and recovering a product called surfactin. The main function of integrating this equipment is for the simultaneous production and also recovery of surfactin. The upstream and downstream unit operations are combined as a single process. This can help to minimise the equipment cost, increase the biosurfactant productivity, maximise the fermentation efficiency and reduce the waste products.

Surfactin is known to be one of the most highly surface active molecules even at lower concentrations as it possesses the ability to reduce the water surface tension from 72 to 27.9 $\mathrm{mN\,m^{-1}}$ (Chen *et al.*, 2006). Surfactin also displays other characteristics. It has antiviral, antimicrobial and anti-inflammatory properties (Dutton, 2007). The primary chemical structure of surfactin consists of a ring of amino acids (only one peptide) and also a $C_{13} - C_{15}$ side chain as shown in Figure 12(Chen *et al.*, 2006).

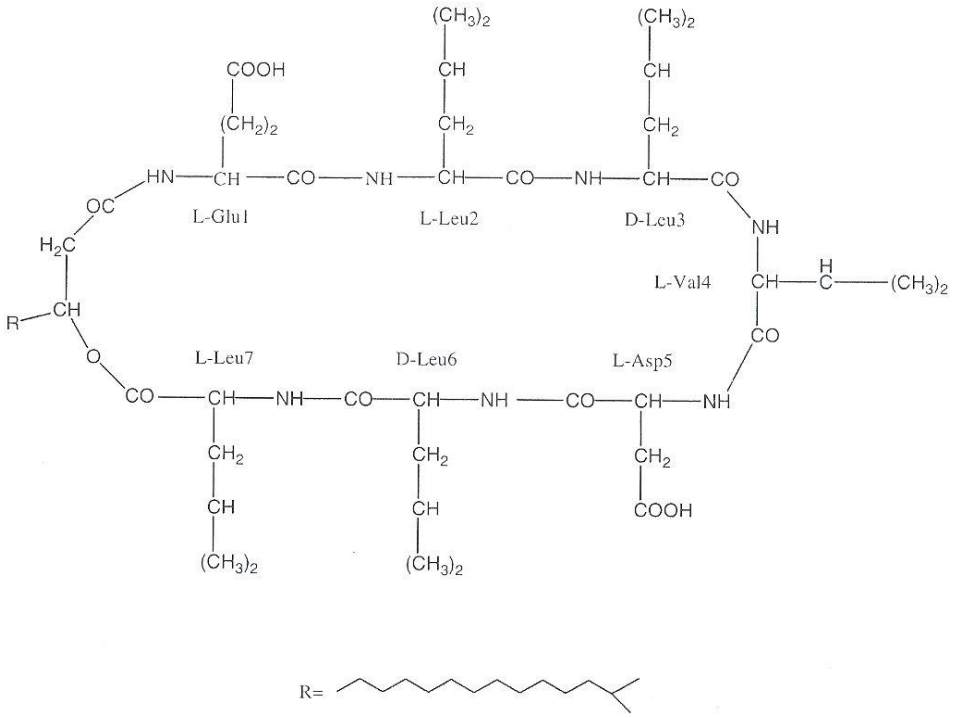

Figure 12: The primary chemical structure of surfactin. Picture courtesy of Chen *et al.* (2006)

Glucose is the substrate used during the fermentation process of BBK006. The study of surfactin production is more attractive than other biosurfactants production as surfactin only shows variation in lipid chain length while other biosurfactants varied in both peptides and lipid chain length. The production process of surfactin can be carried out in batch, semi-batch or continuous mode.

The continuous cultivation of BBK006 is chosen over its batch cultivation as the former provides a stable environment of biomass, substrate and product concentrations to facilitate optimum performance (high productivity). This can lead to more consistent product quality and also for easier process control. In the continuous culture, the rate of surfactin production varied with the dilution rate and also the original concentration of glucose in the feed.

Major foaming can occur during the fermentation process as time increases. The formation of foam can fill and block the reactor. Thus, there is the need to remove this foam using the foam fractionation column. The column contains a rotating mechanical device to break the bulky foams resulting in the collection of a concentrated surfactin foamate. Sometimes anti-foam chemicals are added to the reactor in order to prevent bubbles overproduction which can temporarily stop the reactor.

The foam fractionation column can be operated as a single or multi-staged enriching, stripping or combined process. During the fermentation of bacteria BBK006, the bulk liquid produced often contains a mixture of liquid and dissolved surfactin molecules which forms the column feed. The main goal is to recover as much biosurfactant surfactin as possible. This can be achieved by stripping the surfactin molecules from the liquid mixture (Chen *et al.*, 2006).

Culturing the bacteria is not an option for this research project due to the limited amount of time available. Therefore, CPC is used as the test surfactant for convenience. CPC properties and behaviour at interfaces are made available due to the extensive research done by Dutton and Winterburn in the University of Manchester. The protein casein is also being considered in this project since CPC is not a biological surfactant. Casein is chosen because it is mainly cheap and does not react with CPC molecules when they are mixed together forming the feed solution. The continuous, stripping foam fractionation process is to be investigated in detail since Chen et al. (2006) believed that the stripping mode can produce higher top product recovery.

4 Foam Fractionation Experiments

This section introduces the reagents and equipments used for foam fractionating, the layout of equipments for batch and continuous foam fractionation and also the methods adapted to conduct these experiments.

4.1 List of Reagents and Equipments Used

Reagents
1-Hexadecylpyridinium chloride monohydrate (CPC)
Coomassie (Bradford) Protein Assay Reagent
Distilled Water
Ethanoic Acid
General Purpose Grade Casein
Sodium Hydroxide

Equipments

2-20 µL Pipette
50-200 µL Pipette
200-1000 µL Pipette
1000-5000 µL Pipette
3 L Glass Vessel
5 L Glass Vessel
10 L Glass Vessel
Air Pump
Air Rotameter
Air Spargers
Air Valve
Beakers
Bottom Reservoir
Conical Flask
Cuvette Rack
Data Logger for Mass Balances
Foam Breakers
Glass Funnel
Glass Tube
Green Feed Pump
Inverted J-Shaped Columns
Lab Jack
Mass Balances
Magnetic Stirrer
Motor Controller Unit Operating Foam Breaker
Plastic Tubing
pH Meter
Reflux Pump Control
Rotamixer
Rubber Stopper
Spatula
Top Reservoir
UV-Glass Cuvette
UV-Plastic Cuvette
UV-VIS Spectrophotometer
Vials
Volumetric Flask

4.2 Equipment Set-up

The equipments used for both batch and continuous foam fractionation experiments are described below.

4.2.1 Batch Foam Fractionation

In the batch process, the CPC feed was placed inside the bottom collector. The feed was sparged with air using a metal sparger to generate bubbles with larger surface that rose to the top of the column. CPC molecules selectively adsorbed onto the surface of these bubbles. The system foams and the foam broke up into smaller sizes with the help of the foam breaker to increase the enrichment of adsorbed CPC molecules. The batch foam fractionation process is carried out with and without reflux.

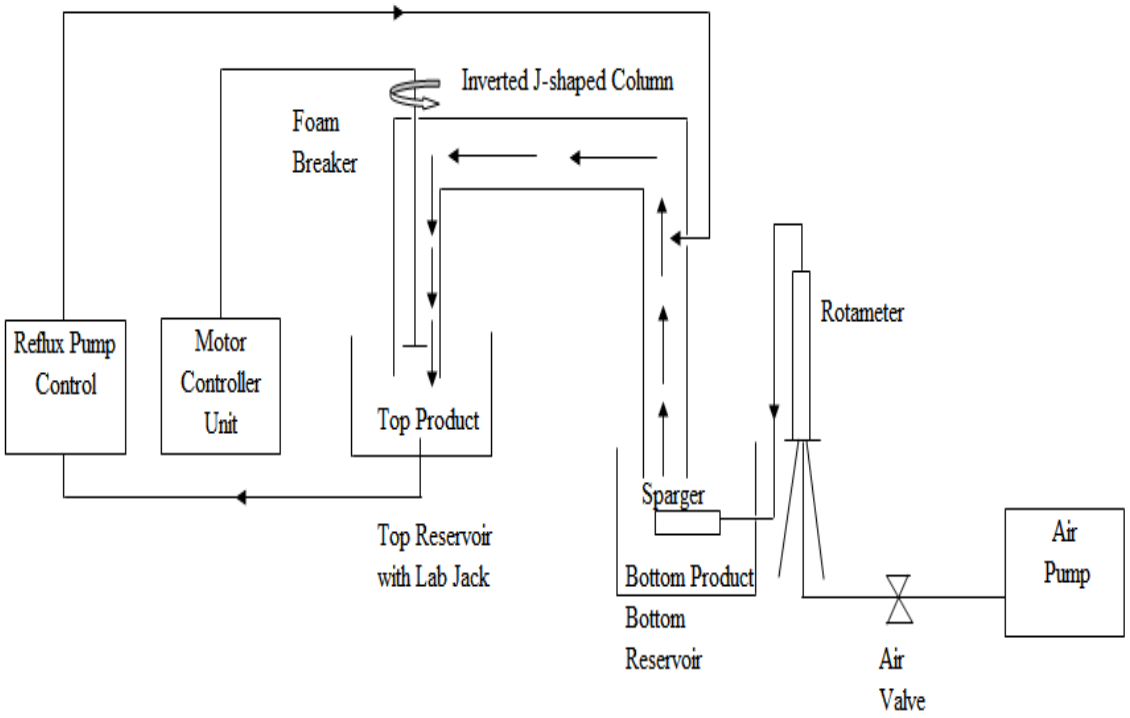

Figure 13: Schematic diagram of batch foam fractionation with reflux. In the absence of reflux, there was no pump control being used to partially return some of the top product collected to the top of the column.

The layout of equipments in the laboratory is shown below.

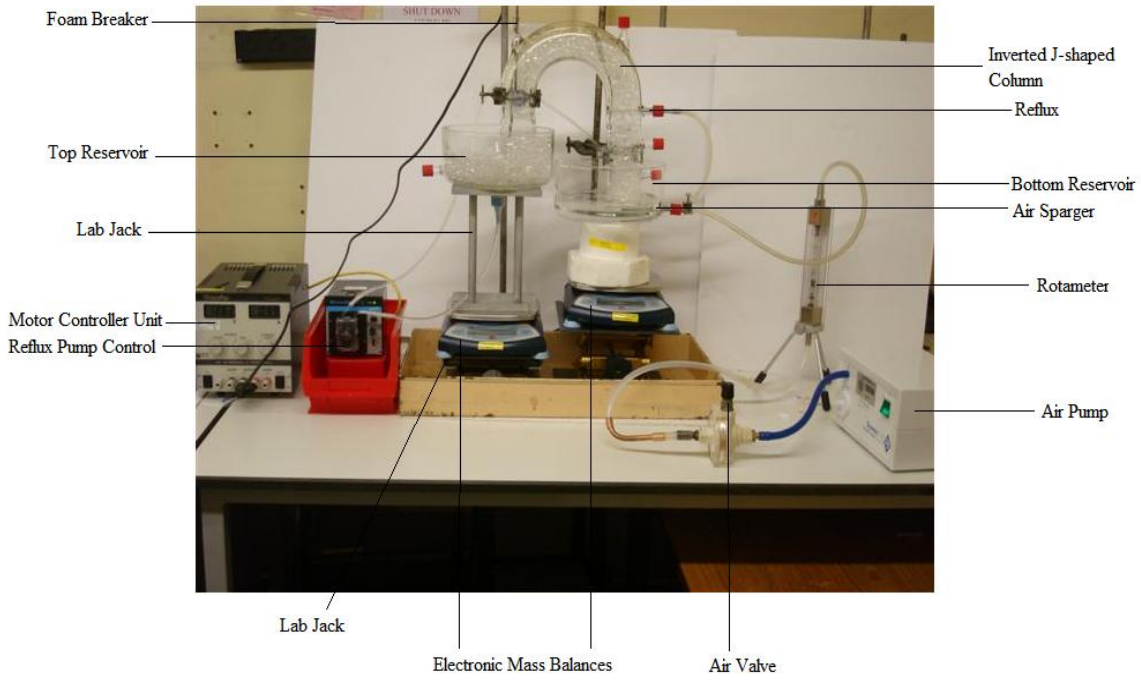

Figure 14: Batch foam fractionation with external reflux.

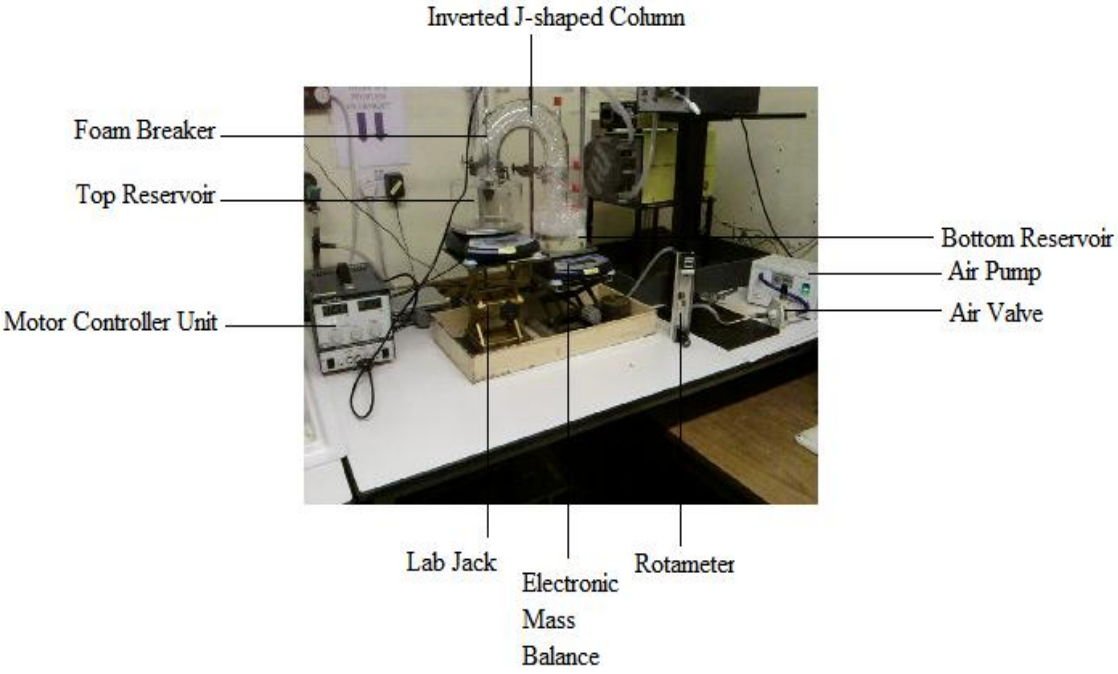

Figure 15: Batch foam fractionation without reflux.

4.2.2 Continuous Foam Fractionation

The continuous stripping foam fractionation was meant to mimic the integrated fermentation and foam fractionation and also to investigate the operating conditions that could maximise the enrichment and recovery of the top product.

The schematic diagram for continuous foam fractionation is shown below.

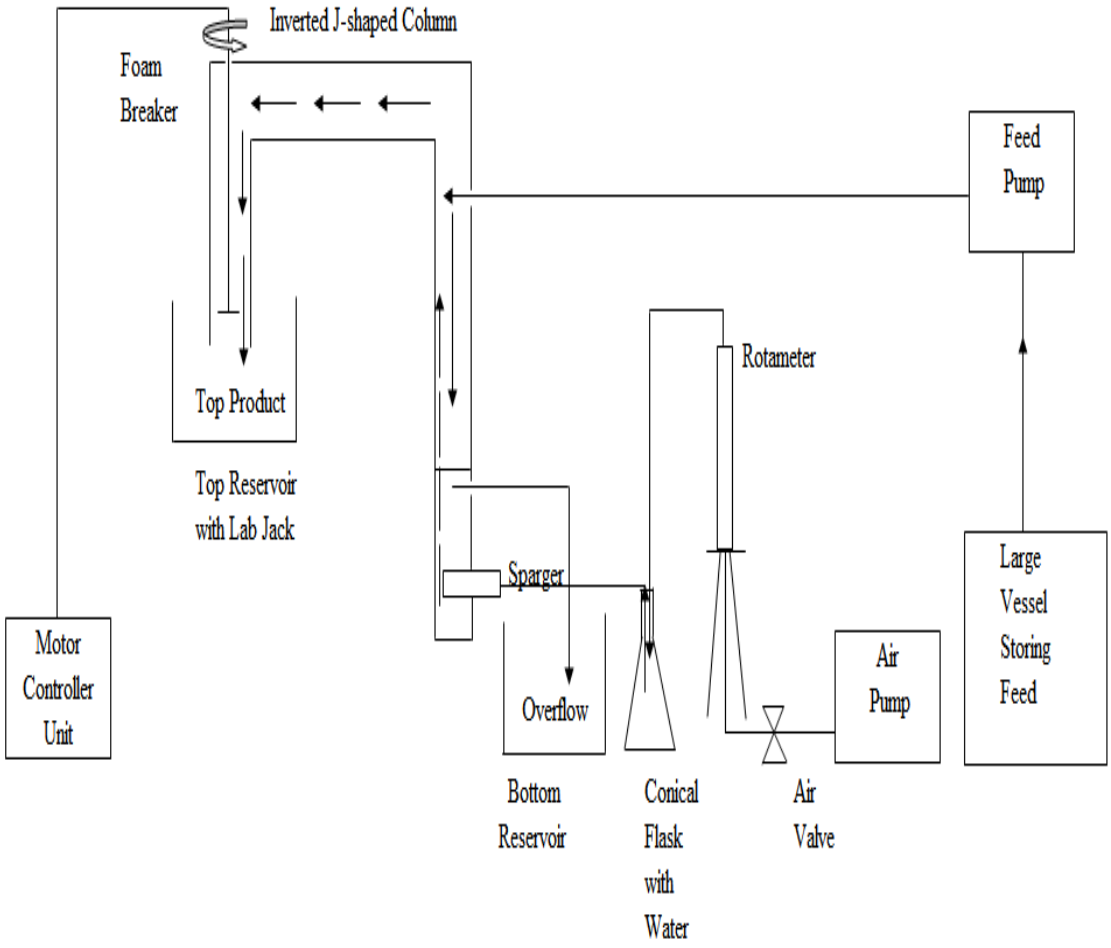

Figure 16: Proposed layout of laboratory apparatus for continuous stripping foam fractionation.

The picture below demonstrates the actual set-up of apparatus in the laboratory.

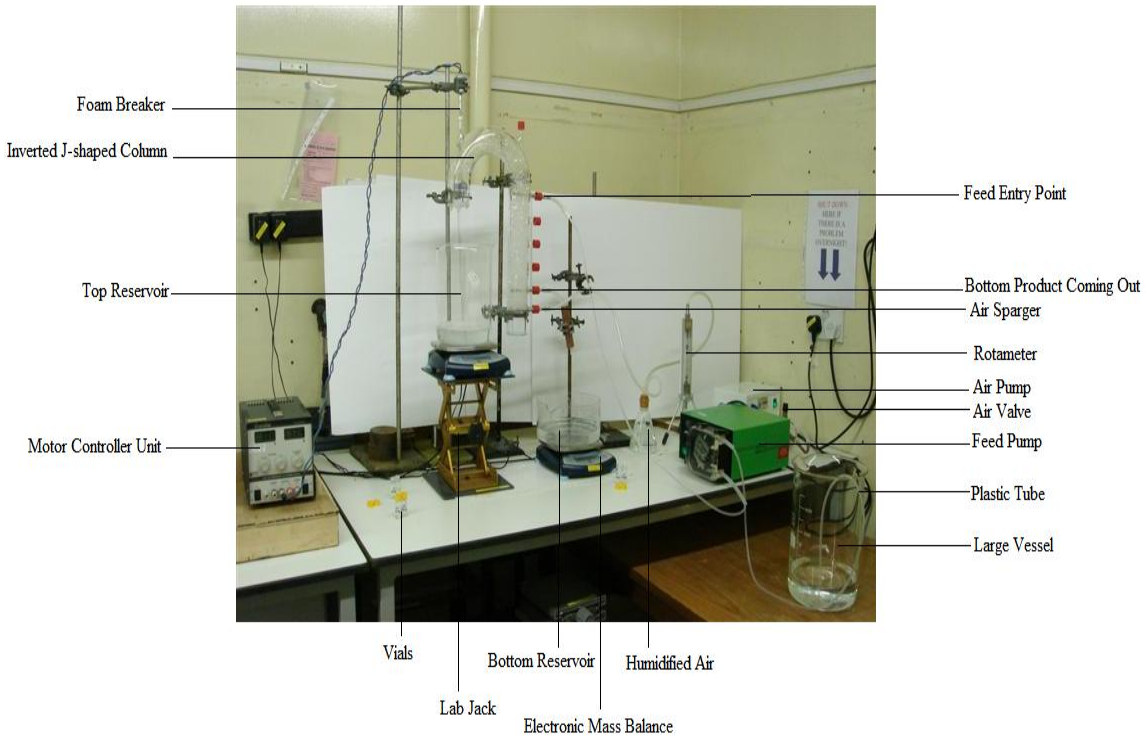

Figure 17: Continuous stripping foam fractionation using a mixture of CPC and protein Casein as the feed in a 5 L vessel.

An inverted J-shaped column sealed at one end was used to store a small pool of liquid feed inside the column. This pool of liquid was sparged with humidified air through a metal sparger to foam the bottom of the column. A large vessel was also used to store higher amount of liquid feed needed in a continuous operation. The feed in the large vessel was continuously being pumped into the top of the column using a green-coloured pump.

The continuous column was designed to be longer than the batch column so that feed entered from the top of the column at some distance above the foaming liquid pool. This allowed the countercurrent movement of feed and rising foam and hence, stripping to take place. The bottom and top reservoirs were collecting the overflow and top product respectively. The overflow represents the waste product in a fermentation process while the top product represents the wanted proteins.

A continuous operation aims to reach the steady state condition and thus, it often lasts longer than a batch operation. This research project concentrates more on using CPC rather than a mixture of CPC and buffered Casein as the feed. This is because there are few problems being encountered with the protein Casein.

4.3 Experimental Methods

The CPC and buffered Casein solutions were made and calibrated before performing the batch and continuous foam fractionation experiments.

4.3.1 CPC Solution Makeup and Calibration

CPC has a critical micelle concentration of 1.0×10^{-3} mol L^{-1} (Dutton, 2007). Hence, it was decided that the CPC feed concentration should be maintained at 1.0 mM throughout this project. CPC was available as a powdered form inside the laboratory and hence, it must be dissolved in the distilled water. See Appendix for further details on how to make 1.0 mM CPC solution.

The CPC solution was then diluted to different concentrations ranging from 0 to 0.8 mM by adding distilled water. Only 3 mL of sample was made for each concentration as the UV-glass cuvette could only store this amount of sample. See Appendix for the different volumes of CPC and distilled water needed to vary the CPC concentration.

Mechanical pipettes were used to measure accurately the volume of these solutions. The CPC solution and distilled water were pipetted into a small vial for every concentration made. The vial was agitated using a rotamixer so that CPC molecules would distribute evenly in the diluted solution. The solution was transferred into a UV-glass cuvette. This cuvette was then placed inside the spectrophotometer to measure the absorbance.

Figure 18: UV-VIS Spectrophotometer. This device is used to measure the absorbance of samples collected from foam fractionation process. For example, in this case the absorbance is measured as -0.4757 ABS at 595nm wavelength.

According to the Beer Lambert Law, there is a linear relationship between the absorbance and concentration of an absorbing species at lower concentration of the species. Both CPC and water molecules are examples of absorbing species. CPC has a maximum absorbance peak at a wavelength of 259 nm. Water is also known to be absorbing some light at this wavelength.

It is necessary to measure the absorbance of distilled water first using the glass cuvette and then to zero the reading before recording the absorbance of different CPC concentrations. This would ensure the measurement of light absorbance from CPC molecules only. The CPC dilution method was repeated twice. There were 3 samples being measured in order to obtain the average absorbance for every CPC concentration. See Appendix for these average absorbance readings. The linear correlation between the average absorbance and CPC concentration was found and demonstrated in Figure 20. This correlation is useful in determining CPC concentration in the bottom and top reservoirs for batch and continuous foam fractionation studies.

4.3.2 Buffered Casein Solution Makeup and Calibration

It was initially suggested that a protein called bovine serum albumin (BSA) and CPC would make up the binary mixture of feed used in continuous stripping foam fractionation. However, it was found out that BSA has a well defined absorbance peak at wavelengths of 259 and 595 nm. This means that both BSA and CPC molecules would absorb light at 259 nm.

For this reason, BSA was replaced with another protein called Casein. Casein has a well defined peak at 595 nm and does not absorb light at 259 nm. See Appendix for further details on how to make buffered Casein solution. The buffered Casein solution was diluted to different concentrations forming standard samples of Casein. See Appendix on how to make these standard samples. The Coomassie Protein Assay Reagent was added to these standard samples. The Coomassie Reagent is a colorimetric method used for total protein quantification (Pierce, 2004). In order to measure the absorbance, a polystyrene cuvette was used as the Coomassie dye can permanently stain the glass cuvette. The correlation between the absorbance and Casein concentration was found and demonstrated in Figure 21.

4.3.3 Pump Calibration

A digital green-coloured peristaltic pump was used to pump the feed inside the large vessel continuously into the foam fractionation column. However, the digital reading displayed was in arbitrary units instead of $\mathrm{mL\,min^{-1}}$. It is important to convert the unit into $\mathrm{mL\,min^{-1}}$ as the feed flowrate was one of the operating conditions that need to be investigated in steady state without reflux experiments. See Appendix for further details on how to calibrate this pump.

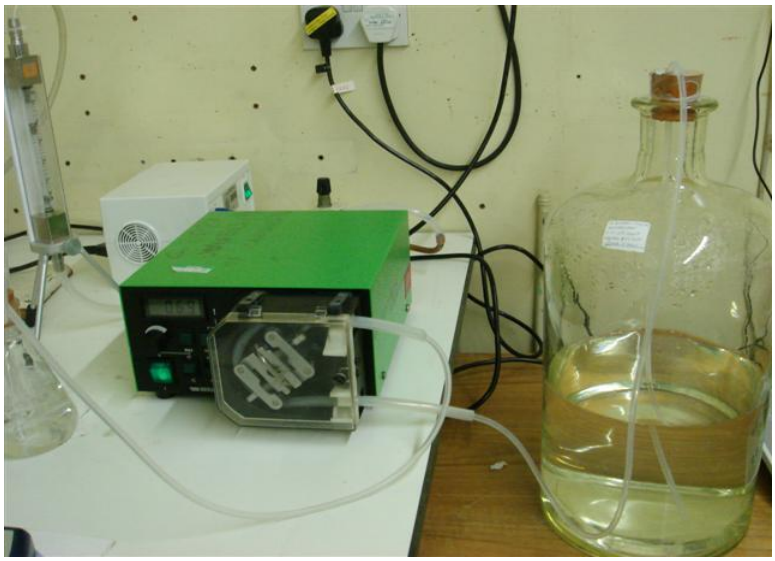

Figure 19: Pump was connected through a plastic tube to the large vessel and also the inverted J-shaped foam fractionation column

4.3.4 Batch foam fractionation

Before batch without reflux experiment began, the bottom reservoir was filled in with 800 mL of CPC solution while the top collector was totally emptied. The air pump and the motor operating the foam breaker were then switched on. The air flowrate was set as $1\,\mathrm{L\,min^{-1}}$. Using a pipette, 3 mL of samples were taken from the bottom and top reservoirs for every ten minutes. The changes in their absorbance and CPC concentration with time were measured.

Another three batch experiments were carried out but with total reflux this time. At the start of each experiment, the bottom and top reservoirs contained 800 mL and 150 mL of CPC solutions respectively. The air flowrate was again set as $1\,\mathrm{L\,min^{-1}}$. Each experiment lasted for about two hours with 3 mL of bottom and top product samples taken for every fifteen minutes. The bottom and top product reservoirs were supported on electronic mass balances which were connected to the PC data logger. The top reservoir was also connected to a peristaltic pump ran by a proportional derivative program in order to maintain 150 mL liquid level in the top collector.

4.3.5 Continuous foam fractionation

All of the continuous foam fractionation studies were conducted without total reflux. A feed pump was used for continuous movement of feed into the top of the column. CPC was present in the feed solution as a single surfactant. Its absorbance was measured before each experiment began to ensure that CPC initial concentration was $1.0\,\text{mM}$. Two operating conditions were varied; the feed flowrate and the air flowrate.

The air flowrate was maintained at $1\,\text{L min}^{-1}$ during the investigative studies of different feed flowrates. Three experimental runs were carried out at feed flowrates of 10.32, 23.11 and $46.21\,\text{mL min}^{-1}$. Each experimental run lasted for different times due to the limitation of the electronic mass balances. For example, the continuous experiment could only last for 80 minutes using the highest feed flowrate. The time taken was doubled to 160 minutes if the lowest flowrate was used. Samples were pipetted from the bottom and top reservoirs for every ten minutes. Each sample was diluted twice to give more accurate measurement of CPC absorbance and concentration using the spectrophotometer.

Another set of experiments were carried out using different air flowrates. The air flowrates used were 1, 2 and $4\,\text{L min}^{-1}$ (controlled by the air valve) while the feed flowrate was maintained constant at $10.32\,\text{mL min}^{-1}$. A lower feed flowrate was chosen so that the experiment could last longer and that the mass of overflow and top product collected were within the electronic mass balances limit. $1\,\text{mM}$ CPC feed solution was used at air flowrates of 1 and $4\,\text{L min}^{-1}$. The feed concentration was purposely lowered down to $0.1\ \text{mM}$ at an air flowrate of $2\ \text{L min}^{-1}$ to investigate its effect on the time taken to reach steady state concentration. Samples were pipetted from the bottom and top reservoirs for every ten minutes. These samples were diluted twice and the spectrophotometer was used to measure their absorbance.

Another continuous experiment was carried out using a mixture of CPC and protein Casein as the feed even though the Casein calibration curve could not be corrected due to time limitation. See Figure 21 for Casein calibration curve. The feed flowrate was set as $10.32\,\text{mL min}^{-1}$ while the air flowrate was maintained at $1\,\text{L min}^{-1}$. This experiment lasted for about 160 minutes with $3\,\text{mL}$ samples pipetted from bottom and top reservoirs for every 10 minutes. Every sample was dilute twice and their absorbance was measured using the spectrophotometer. This experiment was conducted in order to investigate the trend of CPC concentration in bottom and top reservoirs as time increased.

4.4 CPC Concentration, Enrichment and Recovery

The CPC concentration in the bottom and top reservoirs can be measured after knowing the linear relationship between its absorbance and concentration. However, this correlation was only valid if the sample's absorbance did not exceed the value of 2.0. In most experiments, the samples from both reservoirs need to be diluted so that their absorbance lied within the linear region of CPC calibration curve. See Figure 20 for this linear region.

The following equation was used to calculate the dilution factor of a sample.

$$\text{Dilution factor} = \frac{\text{Volume of CPC} + \text{Volume of distilled water}}{\text{Volume of CPC}} \text{---Equation 4}$$

A trial experiment for batch foam fractionation without reflux was carried out. The samples from bottom and top reservoirs were diluted by factors of 50, 100 and 200. It was found out that none of these factors were suitable as they caused very low absorbance of CPC (below 0.5 ABS). Using trial and error, it was discovered that the suitable dilution factors for samples from bottom and top reservoirs were $\times 2.5$ and $\times 10$ respectively.

The table below listed the volumes of CPC and distilled water used to make a 3 mL sample with different dilution factors.

Dilution Factor	Volume of CPC (mL)	Volume of distilled water (mL)
No dilution	3.00	0.00
× 2.50	1.20	1.80
× 10.00	0.30	2.70

Table 1: Dilution factors used for diluting samples collected from bottom and top reservoirs.

CPC enrichment ratio must be measured in order to know whether the foam fractionation process was efficient enough to increase CPC concentration in the top product compared to the bottom one. The enrichment ratio is defined as the ratio of CPC concentration in the top collector to its concentration in the bottom collector at the same sampling time. This ratio was calculated for every sampling time throughout an experiment. Samples were taken for every ten or fifteen minutes time interval.

$$\text{Enrichment ratio} = \frac{\text{CPC concentration in top product}}{\text{CPC concentration in bottom product}} \text{ --- Equation 5}$$

Meanwhile, the recovery of CPC is also an important indicator to know how much CPC in the feed stream was recovered in the top product stream. The mass flowrates of CPC in the bottom and top collectors must be measured from the data logger mass balances so that the recovery could be estimated for every sampling time. However, a high recovery of CPC does not necessarily mean that there is a high CPC enrichment taking place at the same time in the process.

In the equation below, values 1 and 2 indicate the feed and top product streams respectively.

$$\text{CPC recovery} = \frac{\dot{M}_2 c_2}{\dot{M}_1 c_1} \text{ --- Equation 6}$$

5 Experimental Results, Observations and Discussion

5.1 CPC Calibration

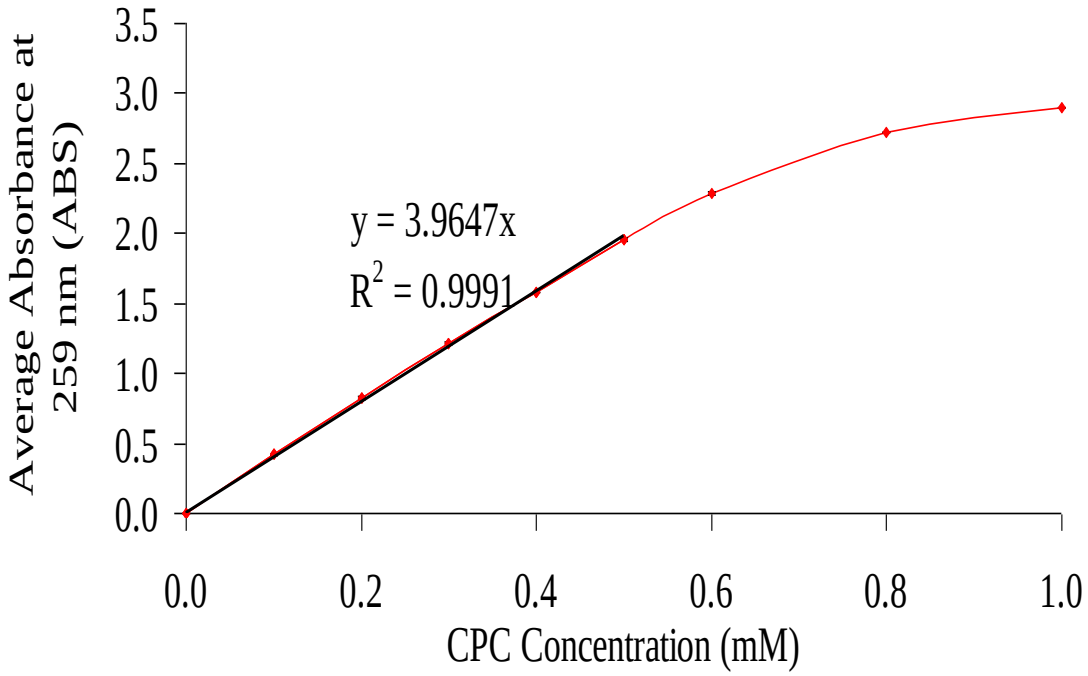

Figure 20: CPC Absorbance Curve at 259 nm. The linear region is represented by the black line while the non-linear region existed when CPC concentration was above 0.5 mM .

The results above showed that the absorbance became higher as the CPC concentration increased. The Beer-Lambert Law suggested a linear relationship between lower CPC concentration and also their absorbance (Wall, 2007). This is in line with the linear equation in Figure 20 which only holds when the CPC concentration lied between 0 and 0.5 mM with a maximum absorbance of 2.0.

The gradient of this linear equation was compared to those values obtained by Swain (2005) and Dutton (2007) which were 3.9793 and 4.2157 respectively. The gradient's value 3.9647 is closer to the one recorded by Swain (2005). The standard errors for all points in Figure 20 were also measured and they lied between the values of 0 and 0.02. This showed that there was a high level of competency in calibrating and determining the CPC absorbance curve at 259 nm wavelength.

5.2 Casein Calibration

Using the average absorbance measurements from the spectrophotometer, the standard calibration curve for buffered-Casein was plotted and shown below.

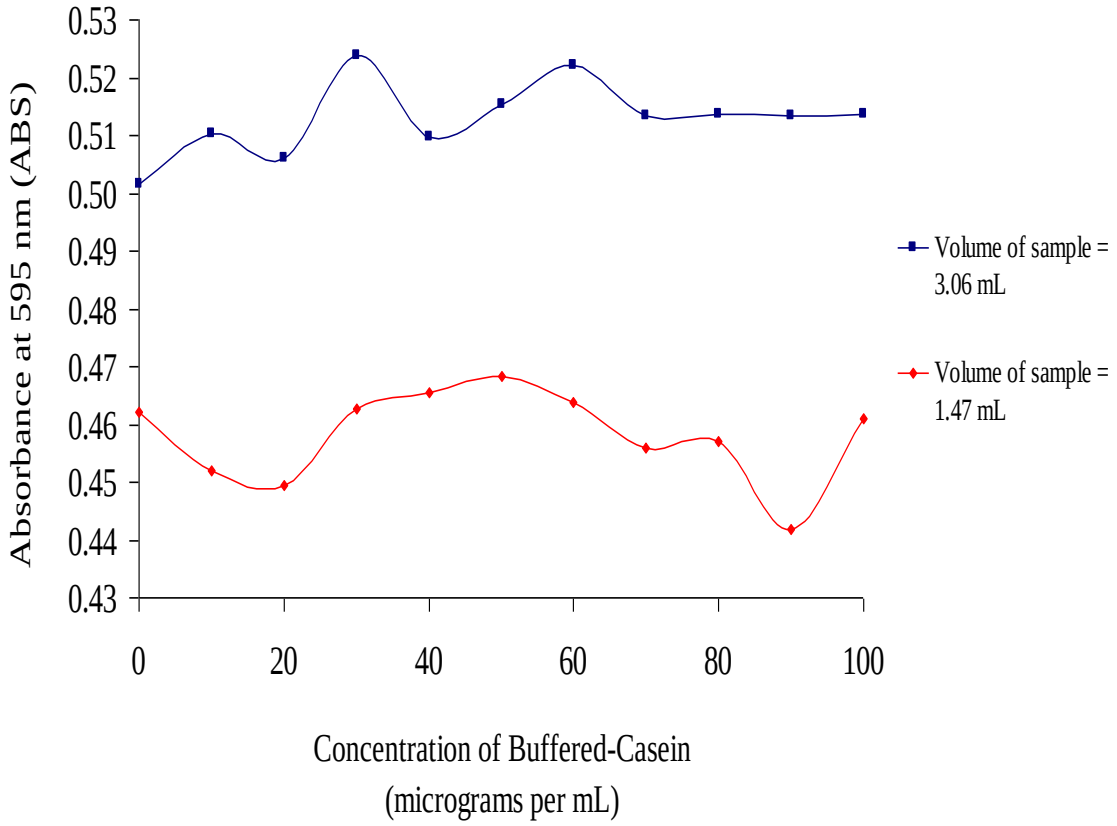

Figure 21: Absorbance curve for buffered-Casein solution using different sample volumes.

In Figure 21 there was an inconsistency in the absorbance readings causing both calibration curves to fluctuate as buffered-Casein concentration increased. No specific reports could be found on the Casein absorbance curve. However, the results above were compared to the standard calibration curve for BSA. The BSA calibration curve showed an increase in the absorbance as protein concentration increased causing the existence of both linear and non-linear regions similar to CPC calibration curve (Pierce, 2004).

A good explanation for the readings discrepancy is that the powdered Casein failed to dissolve properly in the solution despite being mixed for one day using the magnetic stirrer. It was observed that the Coomassie dye did not change its colour from brown to blue after being added to the protein samples and mixed for a few minutes. The brown Coomassie dye was supposed to turn blue to indicate the presence of Casein. It is likely that only a very small amount of Casein molecules was present in the pipetted samples.

Another possible reason for the inconsistencies is that the absorbance might be measured at an incorrect wavelength. The maximum absorption peak of a protein combined with the Coomasie dye should occur when the wavelength was between 465 and 595 nm (Pierce, 2004). The spectrophotometer might not have the maximum sensitivity and detection level for Casein at 595 nm. Due to time limitation, the Casein calibration curve was not corrected until it exhibits a similar pattern to BSA calibration curve.

5.3 Feed Pump Calibration

The calibration curve of the feeding pump was plotted using pump setting as the y-axis variable and water flowrate as the x-axis variable. The pump curve should be smooth and any obvious uneven points would indicate the pump was in need of an immediate maintenance. Pump cleaning, the replacement of diaphragm and seal are few examples of maintenance that can be done.

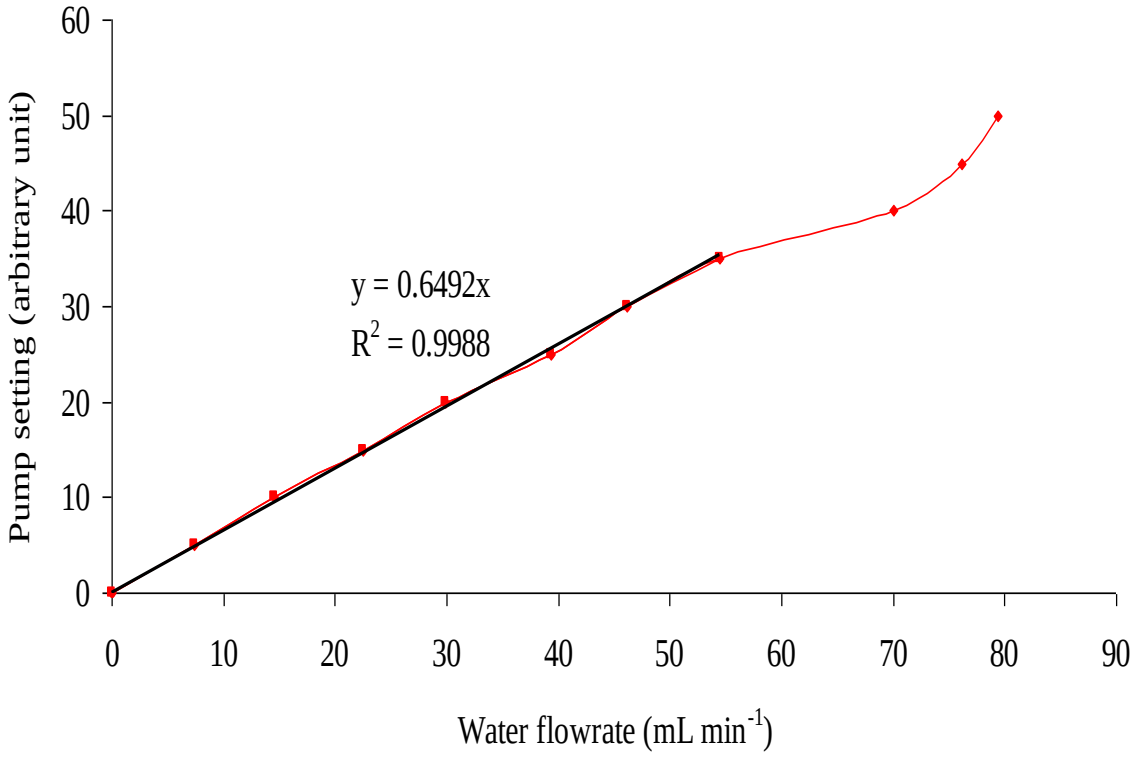

Figure 22: Calibration curve for the feed pump. The linear region is shown by the black line. The non-linear region is found above 35 arbitrary units.

The linear region of the curve was found when the pump setting lied between values of 0 and 35. This meant that there was a linear relationship between pump setting and water flowrate up to 35 arbitrary units. At higher arbitrary units, a non-linear correlation existed between pump setting and its output making it difficult to measure the water flowrate accurately.

In order to operate an experiment with higher feed flowrate, the pump setting should be adjusted to 40, 45 or 50 arbitrary units so that the feed flowrate was exactly known. However, the data logger failed to record the mass of reservoirs after one hour at high arbitrary units. Each of the electronic mass balances could only measure up to 7.0 kilograms. Therefore, for the continuous foam fractionation experiments the pump setting was set to 6.7, 15 and 30 arbitrary units which all lied within the linear region of the pump calibration curve.

5.4 Batch Foam Fractionation

The aim of performing batch foam fractionation was to develop confidence and most importantly the skills required to run a long experiment with minimal human error.

5.4.1 CPC Feed without Reflux

The first batch experiment took place for about an hour in the absence of total reflux. This experiment was meant to examine that the CPC concentration in the feed solution would decrease over time causing an increase in the top product concentration in the bottom reservoir.

The graphs below showed the variation of CPC concentration and enrichment over time.

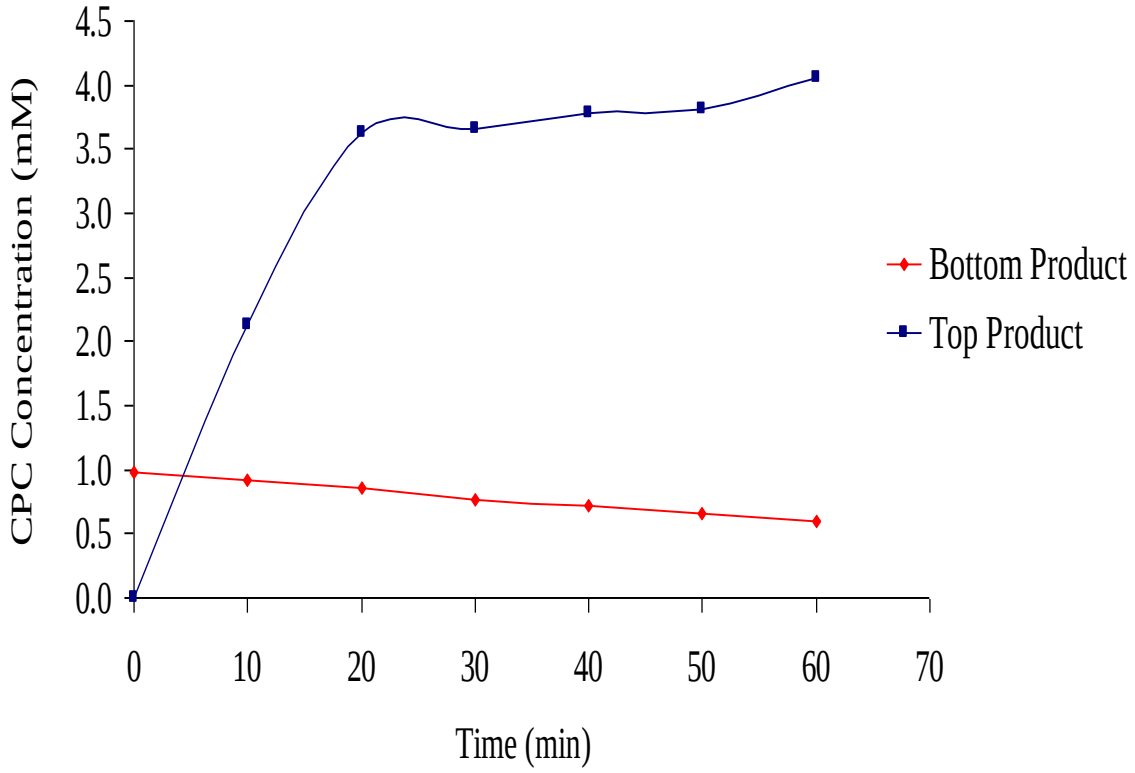

Figure 23: Changes in CPC concentration of the bottom and top products as time increased.

Figure 23 showed that after one hour the CPC concentration in the bottom product decreased from 1.00 to 0.60 mM while its concentration in the top product increased from 0 to 4.0 mM. These results were comparable with Darton et al. (2005) who investigated two-staged, batch foam fractionation column using CPC feed at the same air flowrate. Darton et al. (2005) mentioned that as time increased there was a linear decrease in CPC concentration at the bottom stage which represented the bottom reservoir and also an increase in CPC concentration at the top stage that is the top reservoir.

The introduction of air bubbles into the feed solution caused CPC molecules to be selectively adsorbed onto the bubbles surface. The bubbles rose up through the column together with adsorbed CPC molecules causing a decrease in their number of molecules in the feed solution. These bubbles were broken up using the foam breaker to release CPC enriched foamate inside the top reservoir.

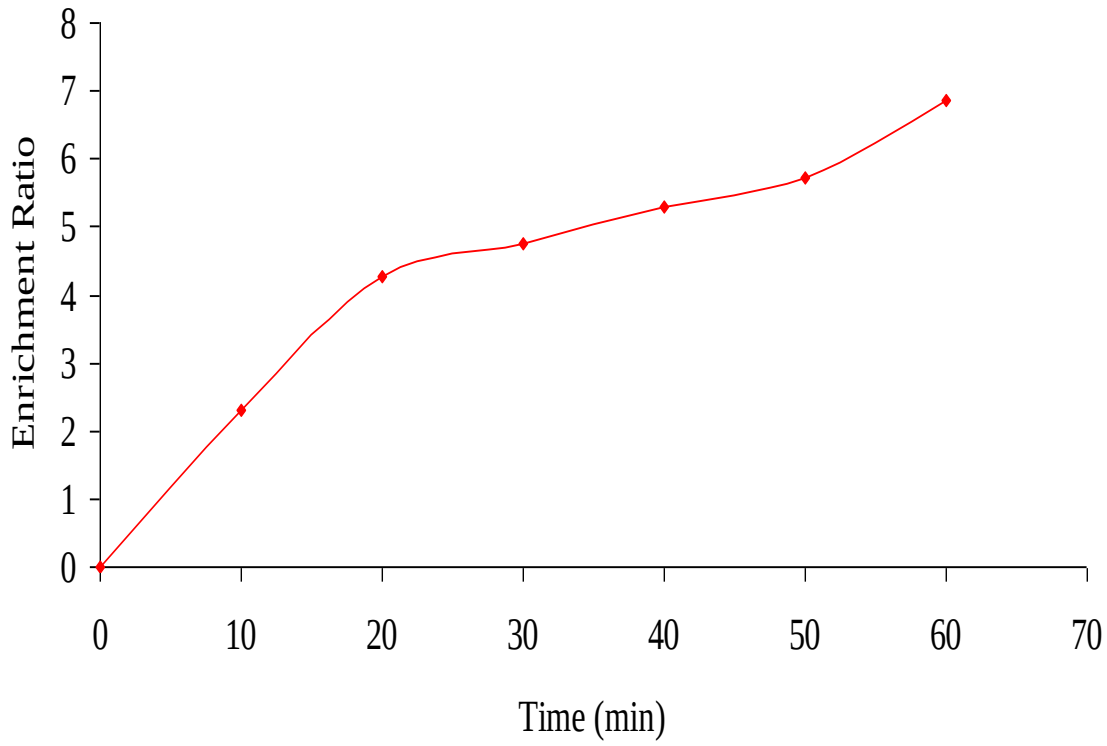

Figure 24: Changes in enrichment ratio for batch foam fractionation without reflux.

Figure 24 showed that the enrichment ratio increased from 0 to 7 after the first hour of the experiment. This result was comparable to Wall (2007) who had conducted continuous foam fractionation without reflux at the same gas flowrate $1\,\mathrm{L\,min^{-1}}$ with CPC as feed. Wall (2007) stated that the enrichment ratio increased from 0 to 8 after two hours.

In a batch operation the CPC molecules would decrease in the bottom reservoir and accumulated in the top reservoir over time. The enrichment may be assisted by internal reflux that occurred inside the foam fractionation column. This internal reflux can helped to cascade the number of adsorbed molecules on the bubbles surface. As a result, the difference between CPC concentration in the bottom and top reservoirs at the same sampling time became larger causing higher enrichment ratio.

From this experiment, it was being realised that the top collector should be initially filled in with $1.0\,\mathrm{mM}$ CPC solution. This is because the top product foamate took quite a long time to collapse and settle down as liquid causing difficulty in collecting sample in the beginning of the experiment.

5.4.2 CPC Feed with Total Reflux

Another three batch experiments with total reflux were carried out aiming to show that these experiments can be repeated with high confidence and the results produced did not vary that much. In addition, these experiments aimed to investigate the effect of total reflux on the CPC enrichment as time increased. Lemlich (1968) stated that the foam fractionation column is operating using the enriching mode if it involves in the use of reflux.

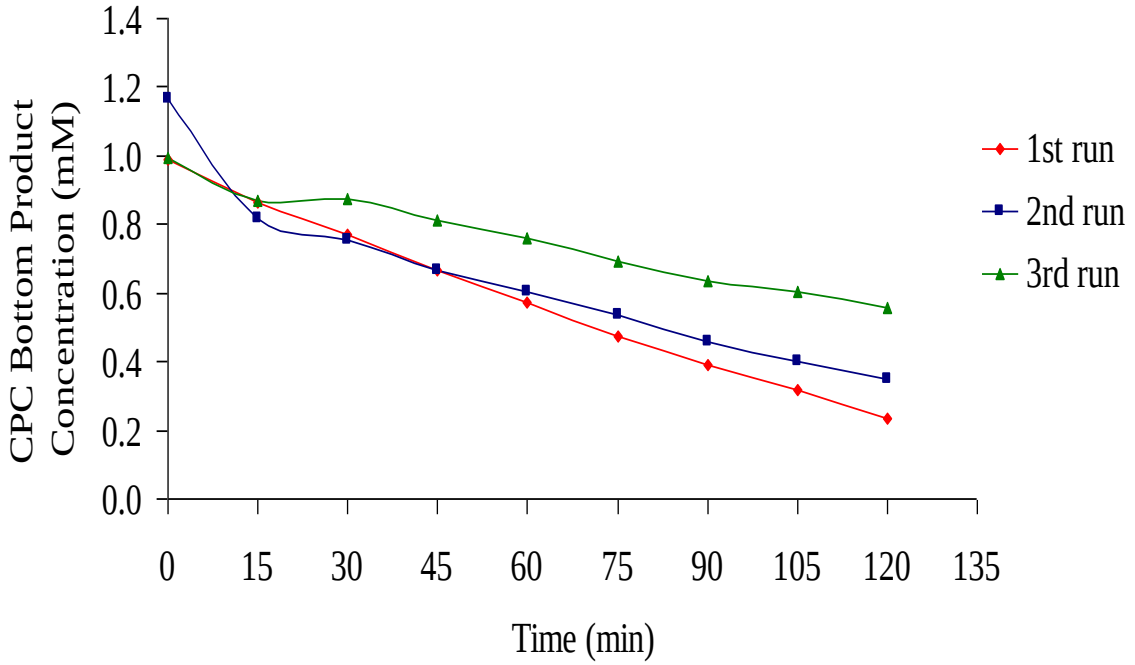

Figure 25: Changes in CPC concentration in the bottom collector for batch with external reflux.

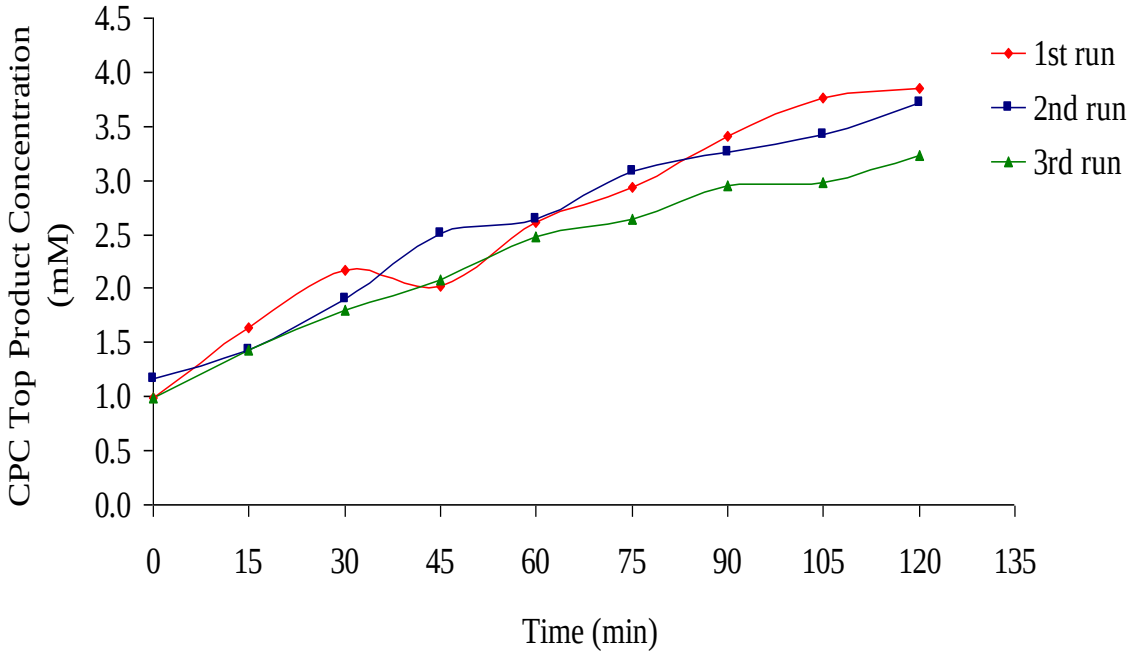

Figure 26: Changes in CPC concentration of the top product for batch with external reflux.

Figures 25 and 26 showed that there was a consistent trend in the results obtained. As time increased, the CPC concentration in the bottom feed decreased while its concentration in the collapsed foam increased. However, the final CPC concentration varied slightly with each other in the bottom and top reservoirs. This might be due to human error. One of the few identified sources of error included the initial CPC concentration for the second experiment which was around 1.20 mM .

The other reason was that the CPC molecules might not be evenly distributed in bottom and top reservoirs as stirring the solutions in these reservoirs was not an option. As a result, samples had to be taken from three same locations inside the reservoirs to reduce this possibility of uneven distribution. A large difference in the concentration gradient may affect the results of absorbance.

In addition, the errors might originate from the fluctuation of spectrophotometer's reading. The spectrophotometer often displayed an absorbance of -0.5863 ABS by excluding the absorbance of distilled water. There were several cases when the reading fluctuated greatly from -0.5863 ABS. These readings needed to be checked constantly and any deviations must be corrected immediately to increase the accuracy of absorbance measurements.

Extra care must also be taken when using the foam breaker as it was prone to be stuck onto the foam fractionation column. During the third experiment, the wirings connecting to the foam breaker's motor were accidentally being disconnected. This caused the top product to be less concentrated as foam was not being properly broken down for awhile.

Darton (2005) reported that the CPC concentration in the bottom and top products were decreasing and increasing linearly as the batch experiment progressed. However, the results in Figures 25 and 26 showed that there was at least one fluctuation point in the concentration curve. For example, the CPC concentration in the bottom product fluctuated at the fifteenth minute for second and third experiments. There were even greater inconsistencies for the points in Figure 26.

These results were compared with the continuous total reflux studies done by Martin et al. (2010) which showed similar fluctuating pattern at a gas flowrate of $1\,L\,min^{-1}$. However, their experiments were carried out using more concentrated CPC solution. One possible explanation for this pattern is that there was a time lag in the process control whose function is to detect an increase or decrease in the top product's volume and to restore it to 150 mL by controlling the reflux.

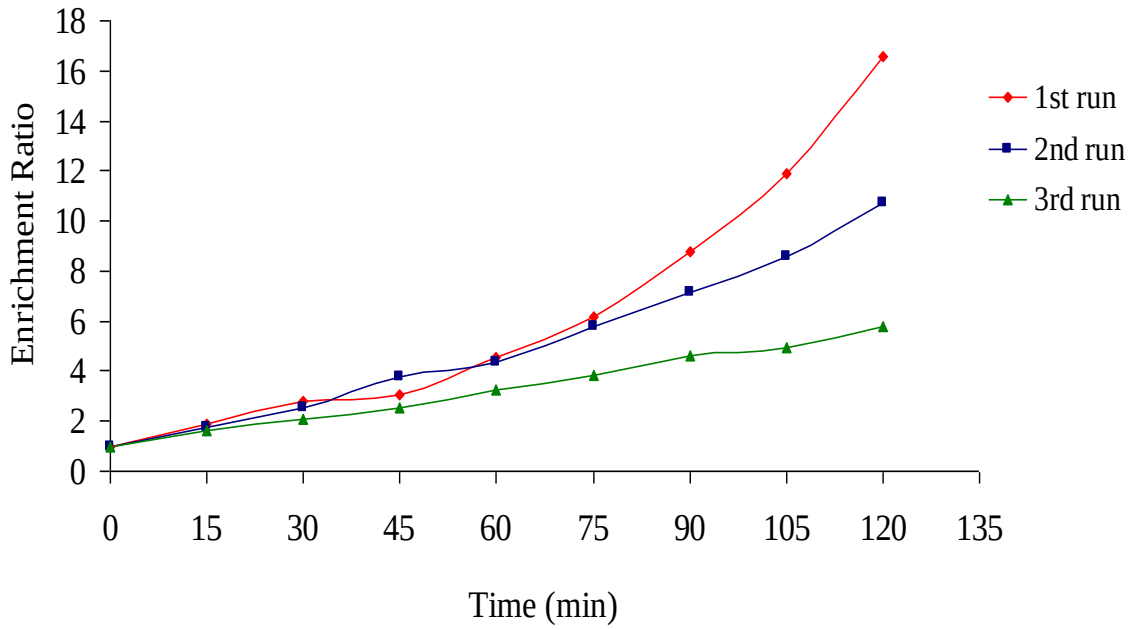

Figure 27: Changes in CPC enrichment ratio for batch foam fractionation with external reflux.

The CPC enrichment ratios for batch operations with and without reflux were also compared. It was found out that the enrichment ratio increased from 8.0 to 16.0 in the presence of reflux. Fanlo and Lemlich (1965) found out that there was a twofold improvement in protein concentration in their reflux batch operation. These results were in line with the theory that the returned portion of the top product contained higher CPC molecules which trickled down the column through the less enriched rising foam. Such countercurrent movement resulted in the mass transfer of CPC molecules from liquid into foam and thus, the CPC enrichment was multiplied yielding more concentrated top product compared to a no reflux operation.

5.5 Continuous Foam Fractionation

Continuous foam fractionation experiments were carried out using the stripping mode aiming to investigate the effects of air and feed flowrates on the enrichment and recovery of CPC as time increased.

5.5.1 CPC Feed with Varied Feed Flowrates

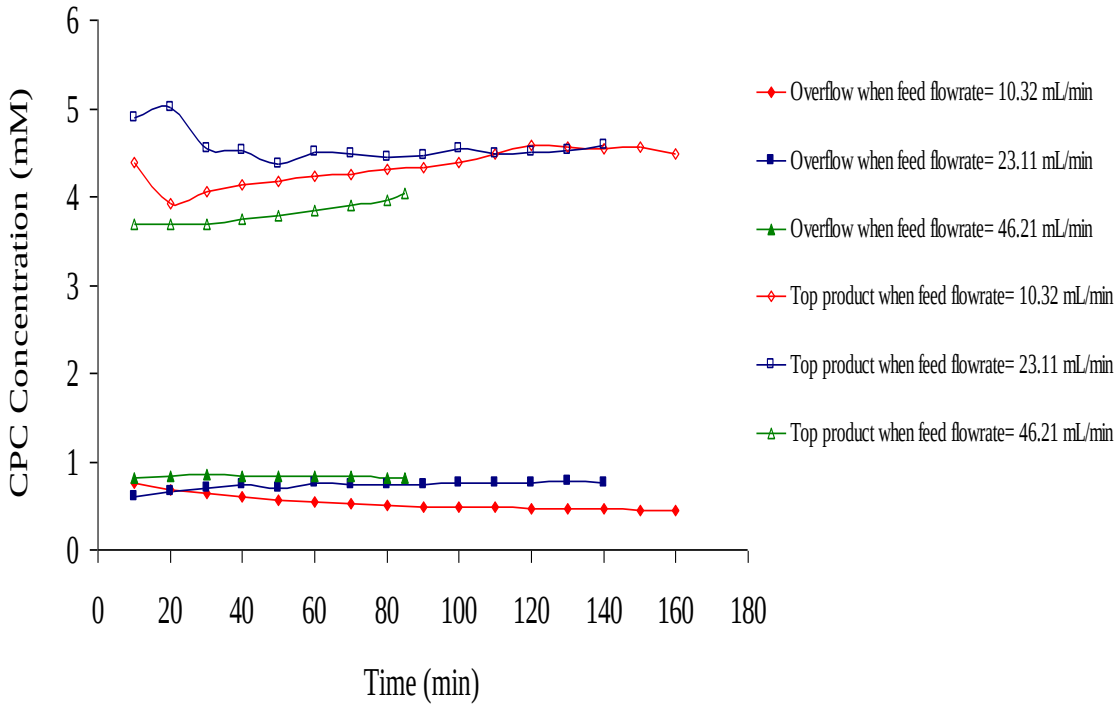

Figure 28: Changes in CPC concentration of overflow and top product when the feed flowrate is varied for a continuous stripping foam fractionation column.

For a continuous process, it was observed that the volume of liquid pool inside the column remained constant as some of the feed introduced into the column was withdrawn as the overflow from the column. Foaming began a few seconds after the bottom liquid pool was sparged with air.

Smaller bubbles started to coalesce at the bottom of the column and the bubble walls became thinner after some time. This may be due to the formation of plateau borders which should be initially filled in with interstitial liquid of bottom pool composition (Lemlich, 1968). It was observed that the bubbles grew larger as they reached the top of the column and more bubbles breakage took place.

The surviving bubbles at the top of the column may trap some of the downflowing liquid feed while the rest of the feed flowed into the plateau borders (Lemlich, 1968). This replaced the interstitial liquid of pool composition with interstitial liquid of feed composition improving the degree of stripping. The interstitial liquid of feed composition drained to the bottom of the column causing more CPC concentrated bottom pool composition (Lemlich, 1968).

The CPC concentration remained constant and reached its steady state value between the first and second hours for all experiments in Figure 28. It was expected that at the highest feed flowrate the least amount of CPC molecules were stripped from the down flowing liquid feed into the rising foam due to the lower residence time in the column. This meant that the rising foam contained lower number of adsorped CPC molecules at the gas-liquid interface and more CPC molecules reached the bottom pool through the plateau borders. Figure 28 showed that at the highest feed flowrate the top product collected was the least concentrated and the CPC overflow concentration was the highest.

However, the top product curve for the lowest feed flowrate should be found above all other top product curves in Figure 28. This did not happen as the foam breaker broke down during the first experimental run (at the lowest feed flowrate) and was replaced with another type of foam breaker. The more recent foam breaker was used in all continuous foam fractionation studies in order to maintain experimental consistencies. This foam breaker had not been used for a long time and hence, it might affect its efficiency in breaking down the top product foamate during the first use.

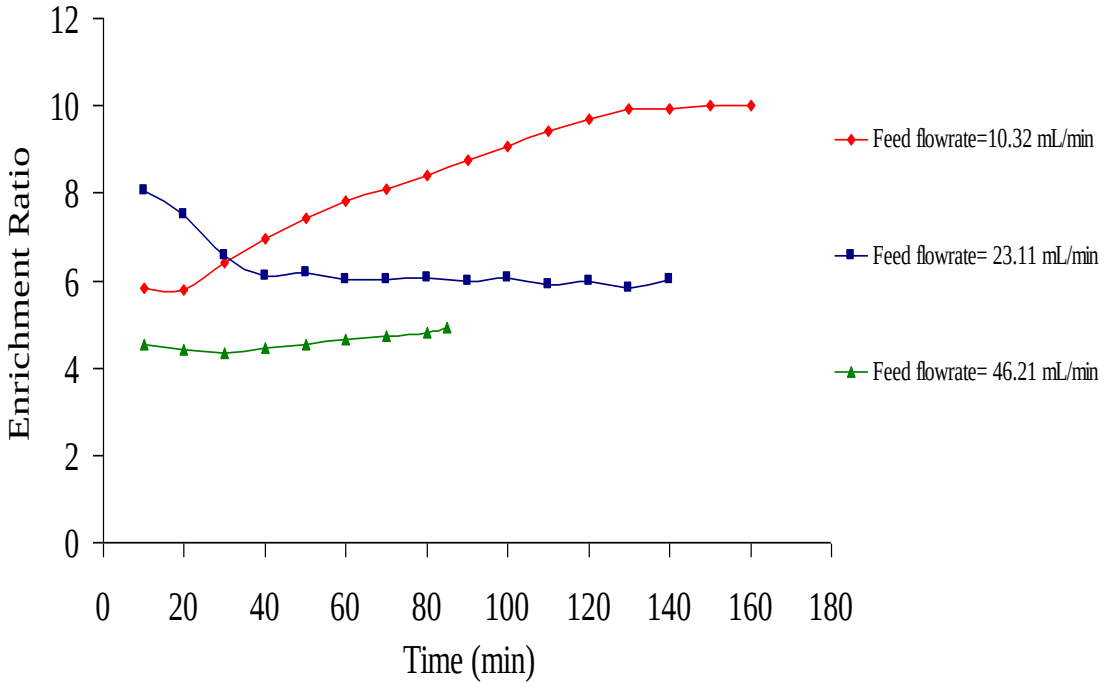

Figure 29: Enrichment ratio of CPC for different feed flowrates.

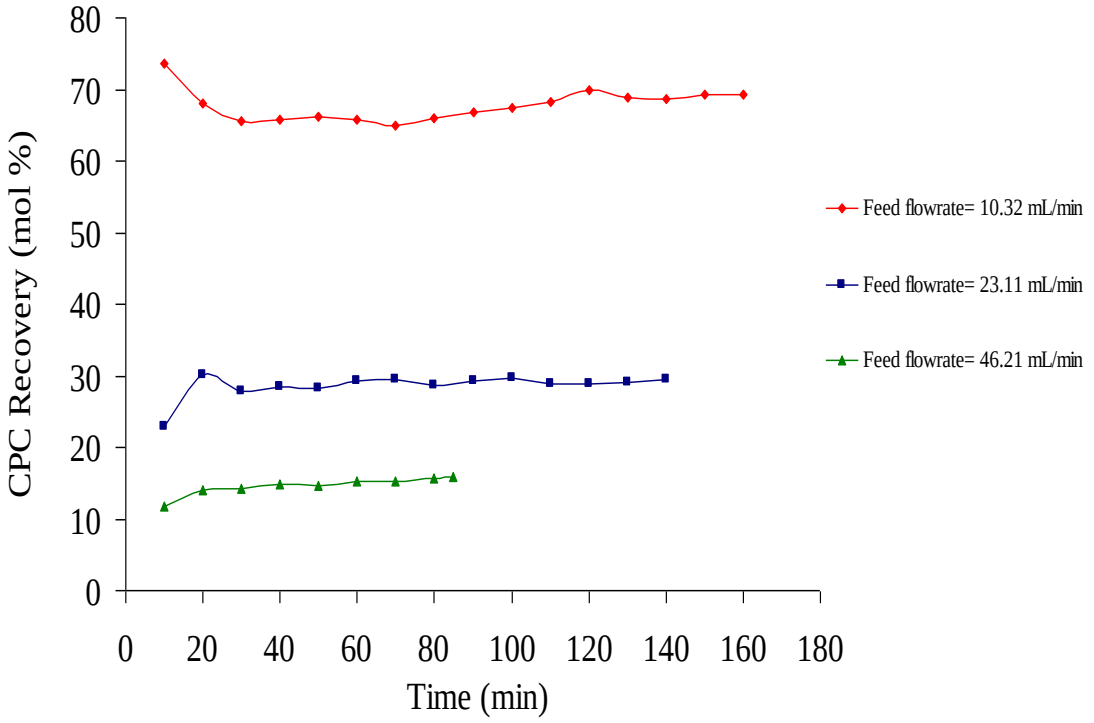

Figure 30: CPC recovery in the top product for different feed flowrates.

Figures 29 and 30 showed that as the feed flowrate was increased from 10.32 to 46.21 $mL\,min^{-1}$ the CPC enrichment ratio decreased from 10 to 4 while the molar percentage recovery of CPC also decreased from 70 to 10. The observed trends are in agreement with findings of Haryono (1994) and Brown *et al.* (1998).

Haryono (1994) found out that the BSA enrichment decreased as the feed flowrate was increased from 2.5 to $10.5\,mL\,min^{-1}$. Haryono (1994) also mentioned that the percentage recovery for BSA decreased at higher feed flowrates for continuous recovery of BSA. Brown *et al.* (1998) reported a decrease in the β-Casein enrichment ratio from 23.6 to 5.1 as the feed flowrate was increased from 2.5 to $10\,mL\,min^{-1}$. Meanwhile, the percentage of β-Casein recovery increased from 67.9 to 37.6 as the flowrate increased from 2.5 to 10 $mL\,min^{-1}$.

Uraizee and Narsimhan (1992) stated that the enrichment and recovery performance of a continuous foam fractionation column were affected by the hydrodynamics of bubbles coalescence in a foam bed. At lower feed flowrates, the CPC feed molecules travelled more slowly down the foam fractionation column through the rising foam and thus, allowing more time for the countercurrent mass transfer of CPC molecules to take place. As a result, the small bubbles interfaces were fully occupied with adsorped CPC molecules at the top of the column causing higher recoveries of CPC feed in the top reservoir.

London *et al.* (1954) mentioned that excess drainage of interstitial liquid in the foam fractionation column cause lower recoveries and purifications of surfactants. For these continuous studies, the excess drainage of liquid was likely to take place at higher feed flowrates that caused the lower CPC recoveries. This is also in line with the findings of Brown *et al.* (1998) who discovered that an increase in feed flowrate causes a significant change in the foam flowrate (volume of foam) due to larger liquid drainage rate resulting in lower β-Casein recovery.

5.5.2 CPC Feed with Varied Air Flowrates

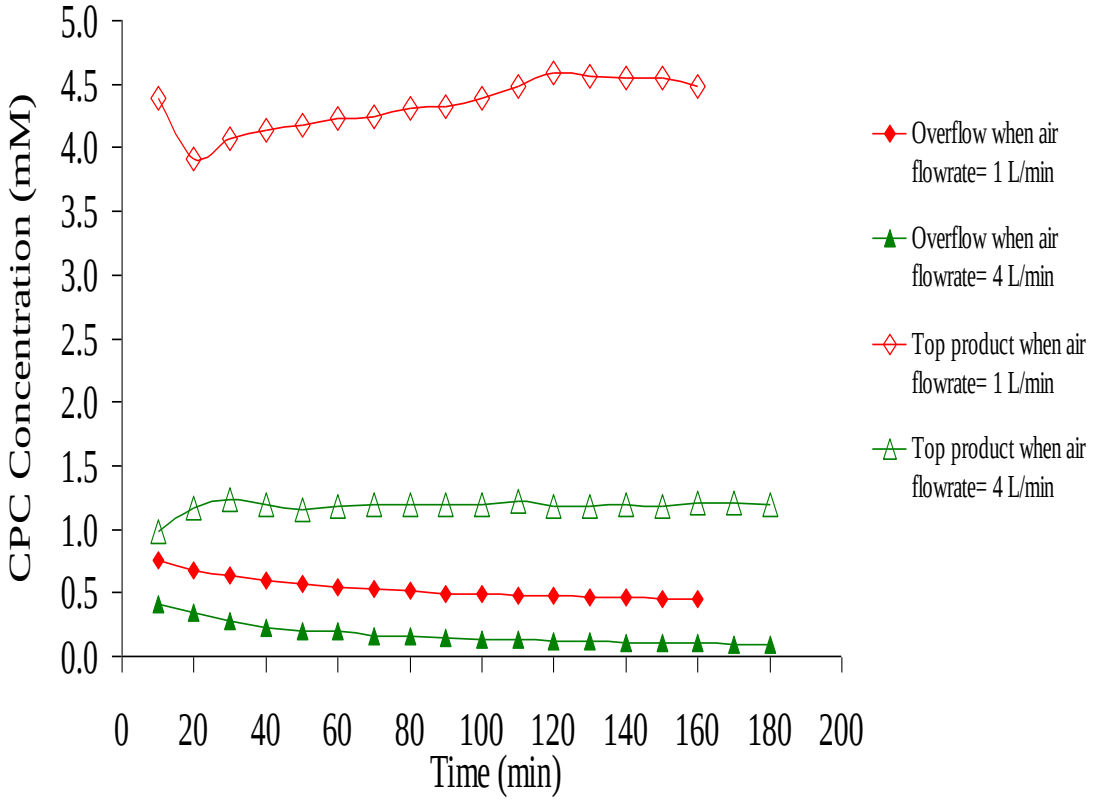

Figure 31: Changes in CPC concentration of overflow and top product for different air flowrates.

The results above showed that the top product concentration decreased from 4.5 to 1.2 mM while the overflow concentration decreased from 0.5 to 0.2 mM when the air flowrate was increased from 1 to 4 $L\,min^{-1}$. It was observed that a fourfold increase in the air flowrate caused a fourfold decrease in the concentration of the top product. Wall (2007) also investigated the variation of air flowrates in steady state without reflux experiments. However, his experiments were based on using more concentrated CPC feed solution. Wall (2007) showed that as the air flowrate was doubled to 2 $L\,min^{-1}$ the top product concentration decreased by a factor of 2 from 9.78 mM to 4.87 mM. There is a similarity in the pattern of the results obtained to Wall's (2007).

Figure 31 showed that as time increased the overflow concentration decreased from 1 to $0.5\,\text{mM}$ and from 1 to $0.2\,\text{mM}$ at air flowrates of 1and $4\,\text{L}\,\text{min}^{-1}$. The overflow originates from the CPC feed which has a concentration of $1\,\text{mM}$ at the start of the experiment. These results indicated that the degree of stripping was larger at higher air flowrates. The CPC concentration in bottom and top reservoirs remained constant after entering the second hour for different air flowrates.

It was also observed that at higher air flowrates more air bubbles were sparged through the column's bottom liquid pool at the same period of time. This caused higher rate of bubbles coalescence at the bottom of the column. The bubbles grew larger as they rose to the top of the foam fractionation column. An increase in the bubble size caused a decrease in the surface area to volume ratio. As a result, less CPC molecules were adsorbed onto the bubbles surface at higher air flowrates. These CPC molecules were released into the top reservoir causing less concentrated top product to be collected at higher air flowrates.

In Figure 32, the top product concentration decreased from 4.5 to $3.2\,\text{mM}$ when the air flowrate was increased from 1 to $2\,\text{L}\,\text{min}^{-1}$ despite using $0.1\,\text{mM}$ CPC feed solution. This supported the fact that the bubbles size increased at higher gas flowrates causing a reduction in the number of adsorbed surfactant molecules.

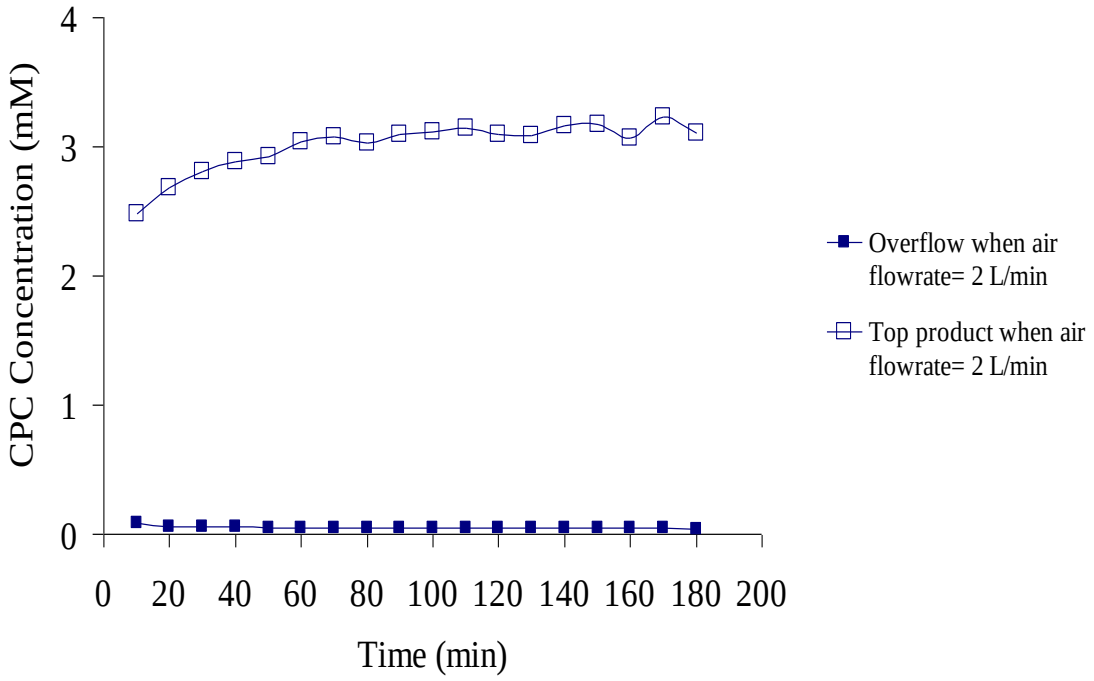

Figure 32: Changes in CPC concentration of overflow and top product at lower feed concentration.

At a lower CPC feed concentration (0.1 mM), the concentration of overflow and top product remained almost constant after the first hour. The gas flowrate used was $2\,\mathrm{L\,min^{-1}}$. This indicated that an experiment would reach its steady state condition faster if a lower feed concentration is used. In Figure 33 the CPC enrichment was comparably higher than other experiments for a lower inlet concentration. One possible explanation for this is that there was an increase in internal reflux inside the column. This may be due to more frequent rupture of the bubbles thin film caused by the Marangoni and Gibbs effects (Lemlich, 1968).

A limited supply of surfactants caused the bubbles surface to be incompletely covered by CPC molecules resulting in higher surface tension and the likelihood for film thinning and rupture (Lemlich, 1968). Such rupture released enriched CPC liquid down the column to be trapped by other rising foams found in the column. Therefore, at lower feed concentration the internal reflux cascaded the increase in surfactant concentration on the bubbles surface and hence, causing higher top product enrichment as time increased.

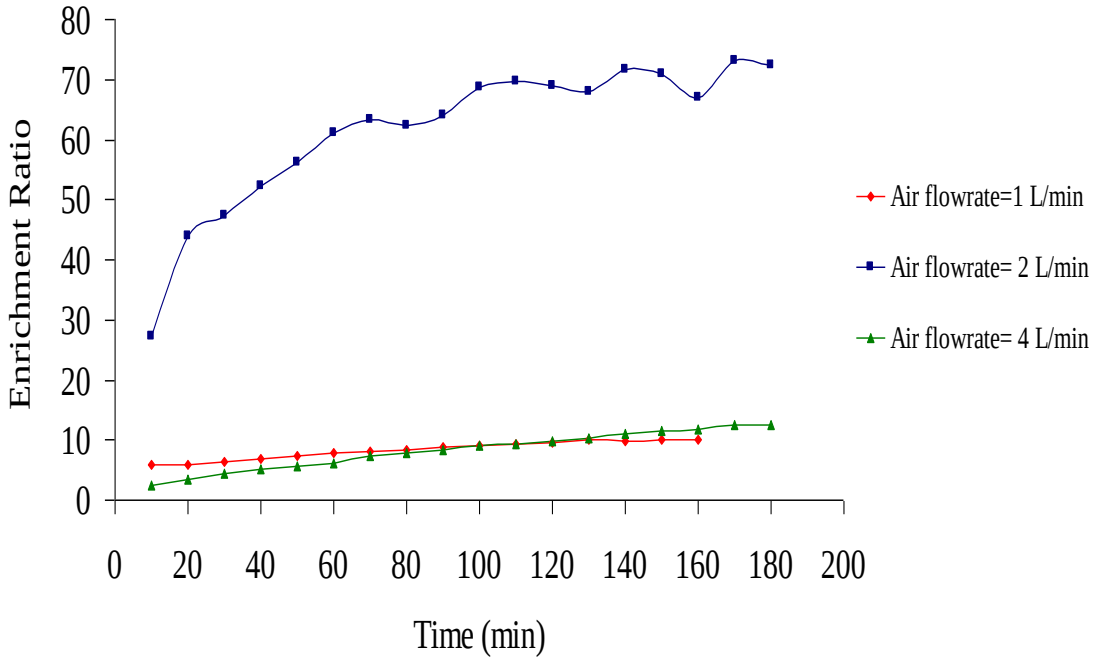

Figure 33: CPC enrichment ratio for different air flowrates.

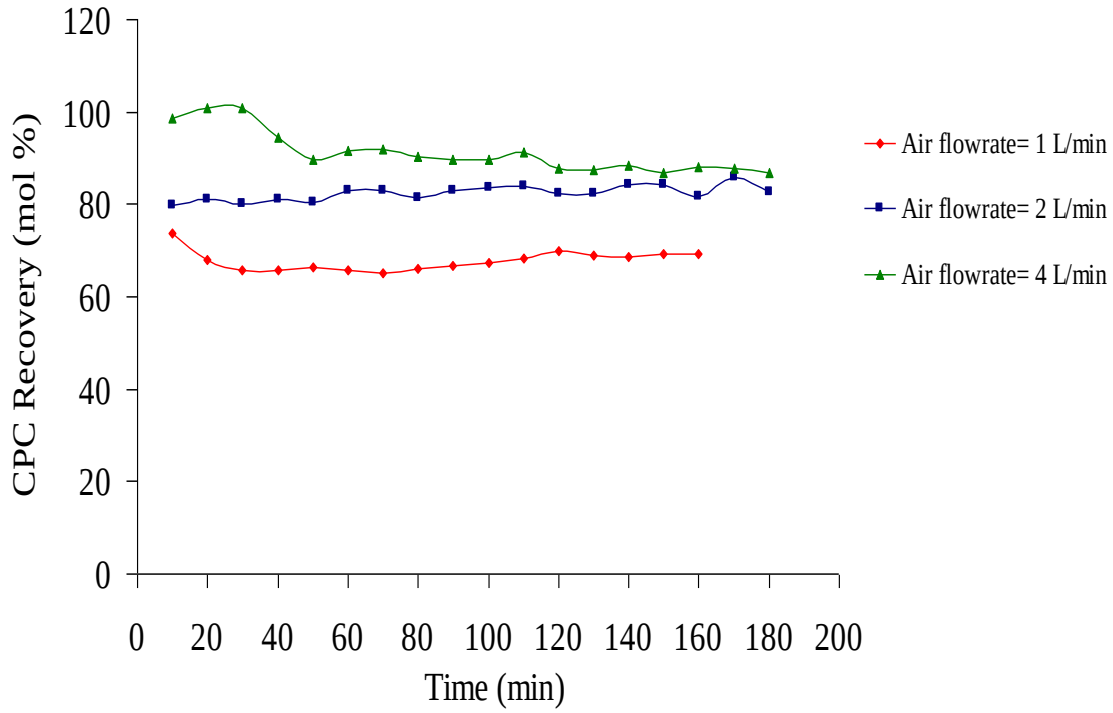

Figure 34: CPC recovery in the top product for different air flowrates.

Figures 33 and 34 showed that when the air flowrate was increased from 1 to 4 $L\,min^{-1}$ the molar percentage recovery of CPC also increased from 60 to 90. At a higher air flowrate, the CPC enrichment decreased at the beginning of the experiment but slowly increased exceeding the lower air flowrate enrichment. Uraizee and Narsimhan (1992) found out that the enrichment ratio decreased and recovery increased at a higher gas velocity. Uraizee and Narsimhan (1992) also stated that there is a stronger dependence of enrichment and recovery on coalescence especially for larger bubbles.

Brown *et al.* (1998) reported that when the air flowrate was increased from 60 to 100 $mL\,min^{-1}$ the β-Casein enrichment ratio decreased from 11.7 to 6.0 while its percentage recovery increased from 24.7 to 41.6. The observed trends in Figures 33 and 34 are in line with the previous work of Uraizee and Narsimhan (1992) and Brown *et al.* (1998).

Brown *et al.* (1998) also mentioned that when the air flowrate was increased the residence time of foam in the column was reduced limiting the internal reflux and liquid drainage. In addition, high levels of gas flowrate cause larger amount of liquid to be taken into the foam. These two factors can result in an increase in the volume of foam collected which leads to a reduction in protein concentration and hence, lower enrichment ratio.

Brown *et al.* (1998) also reported that at higher air flowrates the interfacial area available for protein adsorption and the amount of interstitial liquid taken into the foam increased causing a higher mass of protein entering the foam. The residence time of foam in the column also decreased limiting liquid drainage and coalescence. All of these factors help to increase the mass of protein entering the foam leading to higher protein recovery.

At a higher gas velocity, the bubble coalescence frequency increased causing the bubble to grow more rapidly. The number of bubbles per unit volume of the column decreased with the column height due to coalescence. It was also observed that the volume of bottom liquid pool inside the column decreased at higher air flowrates. This meant that the liquid drainage travelled down the column at a faster rate causing an increase in the level of bottom liquid pool first before it overflowed into the bottom reservoir.

5.5.3 Mixture of CPC and Buffered Casein As Feed

A steady state without reflux experiment was carried out for about 160 minutes using mixture of CPC and buffered Casein solutions as the feed. This experiment was meant to investigate the trend of CPC concentration in the bottom and top reservoirs as time increased. Only the CPC concentration was analysed since the calibration curve for Casein failed to be corrected on time. The feed flowrate was set as 10.32 mL min^{-1} while the air flowrate was maintained at 1 L min^{-1}.

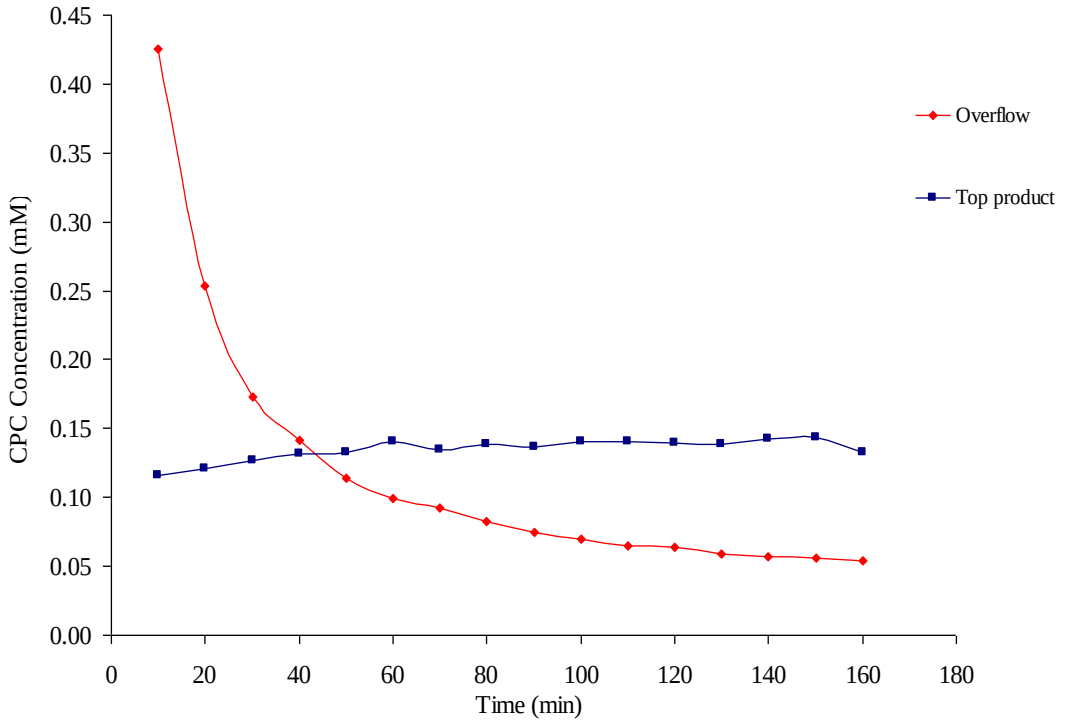

Figure 35: Changes in CPC concentration of overflow and top product using a feed mixture containing 1 mM CPC and 0.1 gL^{-1} buffered Casein solutions.

In Figure 35, the CPC concentration in the overflow decreased from 0.43 to 0.05 mM while its concentration in the top product increased from 0.11 to 0.13 mM between the 10^{th} and 160^{th} minute. These results were compared with the continuous foam fractionation studies in Figure 28. For the same period of time, the CPC overflow concentration was reduced from 1 to 0.5 mM while the CPC concentration in the top product increased from 4 to 4.5 mM at a feed flowrate of 10.32 mL min^{-1}.

The top product in Figure 35 took a shorter time to reach its steady state condition in comparison to the overflow. The CPC concentration in the overflow has a decreasing pattern even towards the end of the experiment. These unexpected results may be due to the fact that powdered Casein failed to dissolve properly inside the feed mixture solution. The powdered Casein might hinder the movement of CPC molecules in the feed solution. Hence, a smaller number of CPC molecules were pumped into the foam fractionation column and adsorbed onto the bubbles surface. It is predicted that the Casein concentration in the bottom and top reservoirs would decrease and increase respectively over time if they are able to dissolve in the solution.

Time is the only major limitation in this research project. It took at least six hours to set up an experiment, run it properly and obtained good, reliable results. The sample dilution and CPC concentration measurement were very tedious processes. At least 100 clean vials must be prepared and properly labeled before each experiment began. These vials were used to store the samples collected from bottom and top reservoirs and also the diluted samples.

Other examples of experimental limitations include the electronic mass balances and also the foam breaker used. The mass balances could only weigh up to 7.0 kilograms. There were many times when the experiment needed to be stopped and started all over again as the mass balance reached its maximum limit even before entering the first experimental hour. Different foam breakers were used for the batch and continuous foam fractionation studies. The foam breakers were found to be very delicate and susceptible to breaking down or even getting stuck onto the column in the middle of running an experiment.

6 Further Work

The continuous foam fractionation studies can be extended by using a mixture of CPC and protein as the feed for different air and feed flowrates. Their effects on the enrichment and recovery can be compared to those continuous studies conducted using CPC feed only.

If time permits, it is recommended to repeat the casein calibration curve again. However, the protein casein must be fully dissolved in the acetate buffer solution before the calibration can be done. Another option is to use a different powdered protein that is easily dissolved in distilled water rather than casein for the continuous experiments. The alternative protein should not interfere with the CPC absorbance at the 259 nm wavelength.

The bulk concentration of CPC is another example of experimental operating parameter that can be varied. Only 1 mM CPC feed solution was used throughout this research project as it represents the critical micelle concentration of CPC. It is interesting to observe the effects of using more concentrated CPC solution on the enrichment and recovery of CPC.

Other types of experiments that can be possibly done included continuous foam fractionation with total reflux. As a starting point, the air and feed flowrates can be varied under the total reflux condition. These studies may be useful for the bio-processing industries as they are still finding ways to operate foaming at a larger scale but with cheaper cost. In addition, the surface tension of starting solutions and foamate can also be measured using the pendant drop imaging method.

7 Conclusion

As a conclusion, the results from the foam fractionation studies showed that the column should be operated with reflux at a low feed flowrate and a high gas velocity. This will help to maximise the top product enrichment and recovery and also to ensure more consistent product quality due to the steady state concentration.

The CPC enrichment ratio increased from 8 to 16 in the presence of reflux for a batch foam fractionation process. Most of the continuous foam fractionation experiments attained their steady state conditions after the first experimental hours. The overflow and top product are able to reach their steady state concentrations at a faster rate by using a lower feed concentration.

The performance of a continuous foam fractionation column can be measured by the enrichment and recovery parameters which depend on the bubbles coalescence. When the feed flowrate was increased from 10.32 to 46.21 $mL\ min^{-1}$ the CPC enrichment and recovery decreased from 10 to 4 and 70 mol% to 10 mol% respectively. This shows that the highest enrichment and recovery of CPC could be obtained at an air flowrate of 10.32 $mL\ min^{-1}$. These results are in line with the work of Haryono (1994) and Brown *et al.* (1998) who had obtained the same trends.

At a higher gas velocity, the molar percentage recovery of CPC increased while the CPC enrichment ratio also slowly increased over time. For example, the CPC recovery increased from 60 mol% to 90 mol% when the air flowrate was increased from 1 to 4 $L\ min^{-1}$. The enrichment ratio is the same at the 100^{th} minute for air flowrates of 1 and 4 $L\ min^{-1}$. By the end of the experiment, the enrichment ratio was slightly higher for the larger air flowrate which may be due to an increase in internal reflux. The result for CPC recovery agrees with previous findings of Brown *et al.* (1998) and Uraizee and Narsimhan (1992).

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9 Appendix

CPC Solution Makeup and Calibration

The molecular formula of CPC is $C_{21}H_{38}ClN.H_2O$ and its relative molecular mass is 358.01 grams per mole. The following procedures were used in making 1.0 mM CPC solution.

(a) Measure the mass of powdered CPC needed to form 1.0 mM CPC solution. In this case, 0.35801 g of CPC needs to be dissolved in 1 L of distilled water.

$$\text{concentration} = \frac{\text{no. of moles}}{\text{volume}} \quad \text{--- Equation 7}$$

$$\text{no. of moles} = \text{concentration} \times \text{volume}$$

$$\text{no. of moles} = 1\times10^{-3}\,\frac{\text{M}}{\text{L}} \times 1\text{L}$$

$$\text{no. of moles} = 1\times10^{-3}\text{M}$$

$$\text{no. of moles} = \frac{\text{mass}}{\text{relative molecular mass}} \quad \text{--- Equation 8}$$

$$\text{mass} = \text{no. of moles} \times \text{relative molecular mass}$$

$$\text{mass} = 1\times10^{-3}\text{M} \times 358.01\frac{\text{g}}{\text{M}}$$

$$\text{mass} = 0.35801\text{g}$$

(b) Switch on a mass balance and place an empty plastic petri dish on top of it.

(c) Zero the scale reading and slowly add powdered CPC into the dish using a spatula until the mass balance displayed a reading of 0.3580 g. The mass balance used could only record readings up to 4 decimal places.

(d) Place the powdered CPC into a small beaker containing distilled water and slowly stirred the mixture using a glass stirrer avoiding any liquid spillage.

(e) Transfer the beaker solution into a 1 L volumetric flask.

(f) Top up the volumetric flask with distilled water until it was reaching the meniscus level of the flask.

(g) Seal the open end of the flask using a rubber stopper and shake the flask for a few minutes allowing CPC to distribute evenly inside it.

The following table shows the volumes of 1.0 mM CPC solution and also distilled water used to vary the CPC concentration.

CPC Concentration (mM)	Volume of CPC (mL)	Volume of distilled water (mL)
0.00	0.00	3.00
0.10	0.30	2.70
0.20	0.60	2.40
0.30	0.90	2.10
0.40	1.20	1.80
0.50	1.50	1.50
0.60	1.80	1.20
0.80	2.40	0.60
1.00	3.00	0.00

Table 2: Volumes of CPC and distilled water used to dilute 1.0 mM CPC solution.

The table below shows the absorbance data for different CPC concentrations.

CPC Concentration (mM)	ABSORBANCE (ABS)			
	RUN 1	RUN 2	RUN 3	AVERAGE
0.00	0.0000	0.0000	0.0000	0.0000
0.10	0.4220	0.4264	0.4109	0.4198
0.20	0.8055	0.8392	0.8243	0.8230
0.30	1.1906	1.2057	1.2306	1.2090
0.40	1.5811	1.5863	1.5811	1.5828
0.50	1.9686	1.9319	1.9686	1.9564
0.60	2.2960	2.2697	2.2960	2.2872
0.80	2.7219	2.7219	2.7219	2.7219
1.00	2.8981	2.8981	2.8981	2.8981

Table 3: Absorbance for different concentration of CPC solutions at a wavelength of 259 nm

Buffered Casein Solution Makeup and Calibration

The following steps were taken to make buffered Casein solution. The buffer solution was made up of a weak acetic acid and a salt called sodium acetate trihydrate.

(a) Calculate the mass of sodium acetate trihydrate and also the volume of acetic acid needed to produce 1 M buffer solution. It was found out that 37.14 mL of acetic acid and 47.72 g of sodium acetate trihydrate were needed to make the buffer solution.

(b) Measure the pH of the 1 M buffer solution using the pH metre. Ensure that the pH metre was calibrated before it was used.

(c) Pipette 1 mL of the 1 M buffer solution into a 1 L volumetric flask. Add distilled water into the flask to make up a solution of one litre. By doing this, the concentration of the original buffer solution was diluted to 1 mM. It was easier to dilute the 1 M buffer solution rather than making 1 mM buffer solution each time the protein solution was made.

(d) Measure the pH of the 1 mM buffer solution.

(e) Add acetic acid or alkaline sodium hydroxide to adjust the pH of the buffer to around pH 6. It was reported that casein tends to dissolve at acidity values above the isoelectric point.

(f) Add 0.1 g of powdered casein directly into the volumetric flask that contained 1 mM buffer solution.

(g) Again measure the pH of the buffered casein solution using the pH metre.

(h) If casein refused to dissolve in the buffer solution, use a rotamixer to mix the solution for several hours. The rotamixer could be switched on and left overnight in the laboratory if the protein persisted not to dissolve.

There were two types of polystyrene cuvettes available; one that could store a 1.5 mL sample and the other could store a 4 mL sample. The tables below showed the different volumes of buffered casein and distilled water used to make

Dilution factor	Concentration of buffered casein(µg/mL)	Volume of buffered casein (µL)	Volume of distilled water (µL)
0.00	0	0	30
0.10	10	3	27
0.20	20	6	24
0.30	30	9	21
0.40	40	12	18
0.50	50	15	15
0.60	60	18	12
0.70	70	21	9
0.80	80	24	6
0.90	90	27	3
1.00	100	30	0

Table 4: Volumes of buffered casein and distilled water used to prepare the smaller volume of casein standard samples. 1.47 mL of Coomassie dye was added to each of the protein samples above producing a final 1.48 mL sample.

Dilution factor	Concentration of buffered casein(µg/mL)	Volume of buffered casein (µL)	Volume of distilled water (µL)
0.00	0	0	60
0.10	10	6	54
0.20	20	12	48
0.30	30	18	42
0.40	40	24	36
0.50	50	30	30
0.60	60	36	24
0.70	70	42	18
0.80	80	48	12
0.90	90	54	6
1.00	100	60	0

Table 5: Volumes of buffered casein and distilled water used to prepare the larger volume of casein standard samples. 3 mL of Coomassie dye was added to each of the protein samples above producing a final 3.06 mL sample.

The standard procedures for mixing the buffered-casein with the Coomassie Reagent were explained below.

(a) Pipette the required volumes of buffered casein and distilled water into a 5 mL vial.

(b) Add an appropriate volume of the Coomassie Reagent into the bottle using a pipette.

(c) Incubate the samples for ten minutes at room temperature in order to produce consistent results.

(d) Set the wavelength of the spectrophotometer to 595 nm. Place a polystyrene cuvette filled with distilled water only inside the spectrophotometer. Zero the instrument's reading in order to eliminate the water absorbance at 595 nm.

(e) Measure the absorbance of all protein samples mixed with the Coomassie reagent.

(f) Repeat all of the steps above one more time to get the average absorbance of samples at different concentrations of buffered-casein.

(g) Repeat steps (a) until (f) by using different volumes of buffered casein, distilled water and the reagent.

Pump Calibration

The following steps were adapted to calibrate the feed pump.

(a) Isolate the pump from other laboratory apparatus in the steady state system.

(b) Zero the reading of bottom and top mass balances which should be connected to the data logger.

(c) Place two conical flasks on the mass balances whereby one of the flasks must be totally emptied while the other was almost filled with water.

(d) Decide on the type of plastic tubing to be used throughout the steady state experiments. Place the middle section of the single tube in the pump. Ensure that one end of the tube was inserted into the water-filled flask while the other end was inserted into the emptied flask.

(e) Turn on the pump. Set the pump output by adjusting the black manual knob which controlled the displayed reading values ranging from 0 to 54.6.

(f) The pump was set for every 5 arbitrary unit intervals. Make sure that the data logger was recording the time and mass of the flasks once the pump output was being set.

(g) For every pump setting, allow at least 5 minutes for water to be transferred from the filled flask to the empty one.

The flowrate of water was measured for each pump setting using the results recorded from the data logger. The pump calibration data was shown below.

Pump setting (Arbitrary units)	Pump output
	Water flowrate (mL /min)
0	0.00
5	7.34
10	14.54
15	22.46
20	29.88
25	39.34
30	46.16
35	54.42
40	69.99
45	76.14
50	79.38

Table 6: Water flowrate obtained for different pump settings.