The Effect of Fermentation Broth Components and Added Compounds on Mass Transfer During Liquid-Liquid Extraction

By

Mark Robert Pursell

A Thesis Submitted for the Degree of Doctor of Philosophy of the University of London and The Diploma of Imperial College.

Department of Chemical Engineering and Chemical Technology,
Imperial College of Science, Technology and Medicine,
University of London.

October 2001

Abstract

The recovery and purification of biologically produced compounds is often complicated by the complex and undefined nature of fermentation media. Liquid-liquid extraction is a common method of choice, however, extractor performance can be severely reduced by the presence of surface-active compounds that arise from the fermentation of micro-organisms. This work examined the effect that compounds present in fermentation media, and those produced by micro-organisms, or those added during fermentation had on mass transfer. Two modes of extraction were investigated; physical extraction based on physiochemical partitioning, and reactive extraction using an ion exchange reagent in the solvent.

The antibiotic chloramphenicol, which is produced extracellularly by *Streptomyces venezuelae*, was used as the model solute in the study of physical extraction. The media components used to produce chloramphenicol were found not to affect extraction rates, however, soluble and non-soluble compounds produced during the fermentation did reduce extraction rates by up to 70%. Micro-organisms are capable of producing many large surface-active molecules such as proteins and lipids, and the presence of these in a pure form was found to reduce mass transfer rates to a similar extent to the fermentation broth. The addition of anti-foam was found to have both positive and negative effects, however, the addition of ionic surfactants, which are used in novel extraction processes, caused interfacial turbulence and increased extraction rates by up to 174%.

The amino acid phenylalanine, which is produced extracellularly by *Corynebacterium glutamicum*, was used as the model solute in the study of reactive extraction, where the liquid ion exchange reagent was Aliquat 336. Aliquat 336, which extracts anionic molecules, is non-specific and co-extraction of other anions present in the fermentation medium will occur. A model was developed to predict the level of co-extraction, and excellent agreement with experimental data was found. During mass transfer the flux of reactants and products are related. This relationship indicates that during mass transfer the conditions at the interface are equal to the final equilibrium conditions for the extraction system, thus the interfacial concentration can be determined. Mass transfer was modelled, and for extraction from fermentation broth the film mass transfer coefficient was reduced by 75%.

Acknowledgements

I would like to begin by thanking my supervisors, Dr Alcina M. Mendes-Tatsis and Dr. David C. Stuckey, for their support and guidance throughout this project, especially during the later stages when it seemed that there was no end was in sight.

This project was financially supported by a studentship from EPSRC, to whom I am grateful for allowing me this rewarding opportunity.

To my colleagues in the BSEA group I wish all the best for the future. Special thanks goes to Lucy Freese, Darrell Patterson, Ste Lamb, Steve Doig, Justin Scarpello, Georg Stenig, Fiona Oloo, Darren Palmer, Dinesh Nair and Daniel Kune for all the chats, coffees and beers that made the time fly by!

Thanks to my fellow stewards, staff and management of the union bar, and especially my team-mates in ICUnited, for providing many hours of distraction which helped me to maintain some form of sanity after endless hours of laboratory work.

Last, but not least, I wish to thank my mum and dad, for their unwavering love and support in what ever I do.

Contents

Title 1

Abstract 2

Acknowledgements 3

Contents 4

List of Figures 10

List of Tables 20

Nomenclature 21

Chapter1: Introduction

1.1 Introduction 23

1.2 Biological Production of Compounds 24

1.3 Recovery and Purification of Fermentation Products 25

1.4 Liquid-Liquid Extraction 26

1.5 Aim of the Project 28

1.6 Overview of Thesis 28

Chapter 2: Literature Review

2.1 Introduction 31

2.2 Surface-Active Compounds 31

2.2.1 Surfactants 32

2.2.2 Biosurfactants 34

2.3 Mass Transfer in Liquid-Liquid Systems 39

2.3.1 Effect of Surfactants 39
2.3.2 Effect of Biosurfactants 41
2.4 Mass Transfer in the Lewis Cell 42
2.4.1 Mechanism of Interphase Mass Transfer 43
2.4.2 Modelling Mass Transfer 44
2.4.3 Reactive Extraction 47
2.4.3.1 Characteristics of Reactive Extraction 48
2.4.3.2 Modelling Mass Transfer 52
2.5 Project Objectives 55

Chapter 3: Interfacial Processes

3.1 Introduction 58
3.2 Description of the Interface 58
3.3 Mass Transfer Across the Interface 60
3.3.1 Introduction 60
3.3.2 Mass Transfer by Diffusion 61
3.3.2.1 Diffusion Coefficients 62
3.3.2.2 Mass Transfer Coefficients 63
3.3.3 Solutions of Fick's Law 64
3.3.3.1 Transfer Across a Thin Film 64
3.3.3.2 Transfer Into a Semi-Infinite Medium 66
3.3.4 Interphase Mass Transfer 68
3.3.4.1 Two Film Theory 68
3.3.4.2 Other Theories of Mass Transfer 70
3.4 Adsorption at the Interface 71
3.4.1 Introduction 71
3.4.2 Adsorption to Interfaces 71
3.4.3 Behaviour of Surfactants in Solution 73
3.4.4 Dynamic Adsorption 74
3.5 Interfacial Convection 75
3.5.1 Introduction 75

3.5.2 Marangoni Phenomena 76
3.5.3 Gravitational Convection 79

Chapter 4: Liquid-Liquid Extraction

4.1 Introduction 80
4.2 Solute Partitioning 81
4.3 Enhancing Partitioning 83
4.3.1 Solute Dissociation 83
4.3.2 Ion Pair Extraction 84
4.4 Solvent Selection 85

Chapter 5: Selection of Experimental Liquid-Liquid Systems

5.1 Introduction 88
5.2 Experimental Extraction Methods 88
5.3 System Selection 90
5.3.1 Criteria for Solute Selection 91
5.3.2 Physical Extraction 92
5.3.3 Reactive Extraction 93
5.4 Physical Extraction System 94
5.5 Reactive Extraction System 95
5.6 Fermentation of Solutes 98
5.6.1 Chloramphenicol Production 99
5.6.2 Phenylalanine Production 100
5.7 Overview of Experimental Study 101

Chapter 6: Experimental Methods

6.1 Introduction 103
6.2 Extraction Equilibrium 103
6.3 The Lewis Cell 104
6.4 The Schlieren Optical System 105
6.4.1 Schlieren Mass Transfer Cell 107
6.4.2 Combined Optical Set Up 109
6.5 Analytical Methods 111
6.5.1 Physical Extraction System 111
6.5.2 Reactive Extraction System 112
6.5.2.1 Phenylalanine 112
6.5.2.2 Chloride 112
6.5.2.3 Other Anions 113
6.5.2.4 pH 114
6.5.3 Other Techniques 114
6.5.3.1 Biomass Concentration 114
6.5.3.2 Surface and Interfacial Tension 115
6.5.3.3 Total Carbon Content 115
6.5.3.4 Ultrafiltration 115
6.5.3.5 UV absorbence Spectrum 115
6.5.3.6 Viscosity 116

Chapter 7: Physical Extraction: Results and Discussion

7.1 Introduction 118
7.2 Equilibrium Extraction 119
7.2.1 The Effect of pH on the Partition Coefficient 119
7.2.2 The Effect of Concentration on the Partition Coefficient 120
7.2.3 The Effect of Added Compounds on Extraction Equilibrium 122
7.3 Analysis of Dynamic Mass Transfer Using Two-Film Theory 122
7.4 Mass Transfer Control in the Dynamic System 123

7.5 The Effect of Concentration Driving Force on Dynamic Mass Transfer 126
7.6 The Effect of Media Components on Solute Extraction 127
7.7 The Effect of Fermentation Broth on Solute Extraction 128
7.7.1 Filtered Fermentation Broth 129
7.7.2 Weight Fractionated Fermentation Broth 130
7.7.3 Biomass 135
7.8 The Effect of Biosurfactants on Solute Extraction 138
7.9 The Effect of Antifoam Agents on Solute Extraction 143
7.10 The Effect of Surfactants on Solute Extraction 147
7.10.1 SDS 147
7.10.2 DTAB 151
7.10.3 Surfactant and Biomass 154
7.10.4 Surfactant and Protein 159
7.11 Summary of Results and Discussion 160
7.11.1 Effects of Fermentation Broth Components On Mass Transfer 160
7.11.2 Effect of Biosurfactants on Mass Transfer 161
7.11.3 Effect of Surfactants on Mass Transfer 162

Chapter 8: Reactive Extraction: Results and Discussion

8.1 Introduction 165
8.2 Extraction Equilibrium 165
8.2.1 Co-Extraction 166
8.2.2 The Equilibrium Constant 167
8.3 Modelling Extraction Equilibrium 170
8.4 The Effect of System Conditions on Extraction Equilibrium 174
8.4.1 The Effect of Feed Composition on Solute Extraction 174
8.4.2 The Effect of the Value of the Equilibrium Constant 178
8.4.3 The Effect of a Second Competing Anion 179
8.4.4 Extraction of Multi-Valent Anions 182
8.4.5 The Effect of Phase Volume Ratio on Extraction Equilibrium 182
8.5 Co-Extraction During Mass Transfer 183

8.6 Mass Transfer Modelling 186
8.6.1 Interfacial Flux Balance 187
8.6.2 Mass Transfer Mechanism 188
8.6.3 Determining Film Mass Transfer Coefficients 190
8.7 The Effect of Co-Extraction on Mass Transfer 192
8.8 Extraction from Fermentation Broth 195
8.8.1 The Effect of Filtered Fermentation Broth 196
8.8.2 The Effect of Biomass 198
8.9 The Effect of Surfactants on Extraction 199
8.10 Summary of Results and Discussion 201
8.10.1 Equilibrium Extraction 201
8.10.2 Mechanism of Mass Transfer 202
8.10.3 Effects of Added Compounds 203

Chapter 10: Conclusions and Recommendations for Future Work

9.1 Introduction 206
9.2 Conclusions 207
9.2.1 Physical Extraction 207
9.2.2 Reactive Extraction 208
9.3 Recommendations for Future Work 209
9.3.1 Broth Characterisation 209
9.3.2 Fermentation Conditions 210
9.3.3 Characterisation of the Interface 210
9.3.4 The Effect of Dynamic Adsorption on Mass Transfer 211
9.3.5 Reactive Extraction from Fermentation Broth 211

References 213

Appendix A Concentration Analysis Using UV-Visible Absorbance Measurements. 224

List of Figures

Chapter 2

Figure 2-1	Examples of the structure of the four different classes of surfactants, a) anionic, b) cationic, c) zwitterionic and d) non-ionic.	33
Figure 2-2	The molecular structures of biologically produced surfactants, a) surfactin, b) phosphatidylcholine, where R_1 and R_2 are hydrocarbon chains.	36
Figure 2-3	The concentration profile for solute transfer from the bulk (b) aqueous (aq) across the interface (i) to the bulk solvent (sol) phase; based on the two-film model of the physical extraction process.	44
Figure 2-4	Concentration profiles close to the interface (i) for all the species involved in the reactive extraction of phenylalanine (Phe) with Aliquat 336 (QCl). Where solute transfer will occur from the bulk (b) aqueous (aq) across the interface to the bulk solvent (sol) phase; based on the two-film model of the reactive extraction process. Also shown is the profile for hydroxide (OH) which may be co-extracted.	53

Chapter 3

Figure 3-1	Schematic showing how a physical property (e.g. concentration) would vary across the liquid-liquid interfacial region, depending on the existence of either an interface (solid line) or an interphase (dotted line).	59
Figure 3-2	The concentration profile across a thin stagnant film between an interface and the bulk of the phase.	65
Figure 3-3	Effect of increasing surfactant concentration on interfacial/surface tension (CMC = critical micelle concentration).	73
Figure 3-4	The effect of the interfacial tension gradient on flow at the interface for a) a single element reaching the interface and b) sustained interfacial convection.	77

Chapter 5

Figure 5-1	Schematic of the interfacial ion exchange reaction occurring during reactive extraction; where X^- is the dissociated solute, Q^+ the ion exchange reagent, and Cl^- the counter ion of the ion exchange reagent.	89
Figure 5-2	The chemical structure of chloramphenicol.	95
Figure 5-3	The chemical structure of (a) phenylalanine, and (b) Aliquat 336.	95

Chapter 6

Figure 6-1	Schematic diagram of the Lewis cell.	105
Figure 6-2	Schematic of the Schlieren optical system.	106
Figure 6-3	Two images of the drop interface during mass transfer, taken using the Schlieren optical system, (a) without the presence of interfacial turbulence and (b) with interfacial turbulence present.	106
Figure 6-4	Exploded view of the Schlieren cell.	108
Figure 6-5	Schematic of the combined optical set up (Agble, 1998).	109

Chapter 7

Figure 7-1	The effect of aqueous phase pH on the partition coefficient of chloramphenicol between de-ionised water and 1-octanol. Initial chloramphenicol concentration of 250μm, temperature = 25°C, and PVR = 1.	120
Figure 7-2	Equilibrium extraction isotherm for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 0 to 250μm, pH = 5.5, temperature = 25°C, and PVR = 1.	121
Figure 7-3	Plots of the concentration function used to determine overall mass transfer coefficients, for different solvent side stirrer speeds (N_{Sol}): 160 rpm (●), 220 rpm (◆) and 280 rpm (▲), for extraction of chloramphenicol from de-ionised water to 1-octanol. Initial chloramphenicol concentration of 250μm, N_{Aq} = 90 rpm, temperature = 25°C, and PVR = 1.	123

Figure 7-4 The effect of phase hydrodynamics on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. For organic phase variation, ●, N_{Sol} = 160 to 280 rpm while N_{Aq} = 90 rpm. For aqueous phase variation, ◆, N_{Aq} = 30 to 120 rpm while N_{Sol} = 250 rpm. Initial chloramphenicol concentration of 0 to 250μm, pH = 5.5, temperature = 25°C, and PVR = 1. 125

Figure 7-5 The effect of the initial concentration of chloramphenicol on the overall mass transfer coefficient for extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 0 to 1000μm, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 126

Figure 7-6 The effect of media components on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 128

Figure 7-7 The effect of different grades of fermentation broth on the overall mass transfer coefficient for chloramphenicol extraction into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 129

Figure 7-8 The effect of diluting the filtered fermentation broth on the solution surface tension. Initial chloramphenicol concentration of 250μm, pH = 4.7-5.5, temperature = 25°C. 131

Figure 7-9 The effect of diluting the filtered fermentation broth on the overall mass transfer coefficient for chloramphenicol extraction by 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 4.7-5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR=1. 131

Figure 7-10 The effect of molecular weight fractions on the solution surface tension. Initial chloramphenicol concentration of 250μm, pH = 5.5, temperature = 25°C. 133

Figure 7-11 The effect of molecular weight fractions on the overall mass transfer coefficient for chloramphenicol extraction into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR=1. 133

Figure 7-12 The distribution of carbon across the molecular weight fractions. 134

Figure 7-13 The effect of biomass concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR=1. 136

Figure 7-14 The effect of biomass concentration on the surface tension of chloramphenicol solutions. Initial chloramphenicol concentration of 250μm, pH = 5.5, temperature = 25°C. 136

Figure 7-15 The effect of adding BSA during extraction of chloramphenicol from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR=1. 139

Figure 7-16 The effect of cytochrome c concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 140

Figure 7-17 The effect of β – casein concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 140

Figure 7-18 The effect of BSA concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 141

Figure 7-19 The effect of phospholipid concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 141

Figure 7-20 The effect of biosurfactant concentration on the interfacial tension between PBS and 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, temperature = 25°C, and a PVR = 1. Biosurfactants used were; cytochrome c (■), β-casein (◆), and BSA (●). 142

Figure 7-21 The effect of added PPG 400 concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 144

Figure 7-22 The effect of added PPG 2000 concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 144

Figure 7-23 The effect of added SDS concentration on the surface tension of chloramphenicol solutions. Initial chloramphenicol concentration of 250μm, pH = 7, temperature = 25°C. 148

Figure 7-24 The effect of added SDS concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 148

Figure 7-25 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water into 1-octanol, at (a) 0 seconds, (b) 30 seconds, (c) 60 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C. 150

Figure 7-26 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with 0.1 g/l SDS added, into 1-octanol, at (a) 0 seconds, (b) 10 seconds, (c) 30 seconds, and (d) 40 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C. 150

Figure 7-27 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with 5 g/l SDS added, into 1-octanol, at (a) 0 seconds, (b) 5 seconds, (c) 20 seconds, and (d) 30 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C. 150

Figure 7-28 The effect of added DTAB concentration on the surface tension of chloramphenicol solutions. Initial chloramphenicol concentration of 250μm, pH = 7, temperature = 25°C. 152

Figure 7-29 The effect of added DTAB concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 152

Figure 7-30 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with 0.5 g/l DTAB added, into 1-octanol, at (a) 0 seconds, (b) 10 seconds, (c) 30 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C. 153

Figure 7-31 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with 5 g/l DTAB added. into 1-octanol, at (a) 5 seconds, (b) 10 seconds, (c) 30 seconds, and (d) 60 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C. 153

Figure 7-32 The effect of added SDS concentration on the surface tension of biomass solutions. Initial chloramphenicol concentration of 250μm, a biomass concentration of 0.14 g/l, pH = 5.5, temperature = 25°C. 155

Figure 7-33 The effect of added SDS concentration on the overall mass transfer coefficient for chloramphenicol extraction from a biomass solution into 1-octanol. Initial chloramphenicol concentration of 250μm, a biomass concentration of 0.14 g/l, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR=1. 155

Figure 7-34 The effect of added DTAB concentration on the surface tension of biomass solutions. Initial chloramphenicol concentration of 250μm, a biomass concentration of 0.14 g/l, pH = 5.5, temperature = 25°C. 157

Figure 7-35 The effect of added DTAB concentration on the overall mass transfer coefficient for chloramphenicol extraction from a biomass solution into 1-octanol. Initial chloramphenicol concentration of 250μm, a biomass concentration of 0.14 g/l, pH = 5.5, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR=1. 157

Figure 7-36 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with biomass added, into 1-octanol, at (a) 0 seconds, (b) 30 seconds, (c) 60 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, a biomass concentration of 0.14 g/l, pH = 5.5, temperature = 23°C. 158

Figure 7-37 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with biomass and SDS added, into 1-octanol, at (a) 5 seconds, (b) 10 seconds, (c) 30 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, a biomass concentration of 0.14 g/l, and a SDS concentration of 5 g/l, pH = 5.5, temperature = 23°C. 158

Figure 7-38 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with biomass and DTAB added, into 1-octanol, at (a) 0 seconds, (b) 30 seconds, (c) 60 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, a biomass concentration of 0.14 g/l, and a DTAB concentration of 5 g/l, pH = 5.5, temperature = 23°C. 158

Figure 7-39 The effect of added SDS concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS with BSA, into 1-octanol. Initial chloramphenicol concentration of 250μm, a BSA concentration of 0.01 g/l, pH = 7, N_{Aq} = 90 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 159

Chapter 8

Figure 8-1 Variation in the equilibrium concentrations of the reaction products (Aliquat-phenylalanine, •, and chloride, ♦) from the phenylalanine extraction reaction where the initial phenylalanine concentration was varied from 10mM to 50mM. Initially, 100 mM sodium hydroxide was added to the aqueous phase, and 200 mM Aliquat 336 added to the solvent phase, temperature = 25°C, PVR = 1. 167

Figure 8-2 Plot to determine the equilibrium constant for the phenylalanine extraction reaction, K^*_{Phe}. Initially; 10-50 mM phenylalanine and 50-100mM sodium hydroxide added to the aqueous phase, 200mM Aliquat 336 added to the solvent phase, temperature = 25°C, and PVR = 1. 168

Figure 8-3 Plot to determine the equilibrium constant for the hydroxide extraction reaction, K^*_{OH}. Initially; 10-50 mM phenylalanine and 50-100mM sodium hydroxide added to the aqueous phase, 200mM Aliquat 336 added to the solvent phase, temperature = 25°C, and PVR = 1. 169

Figure 8-4 Comparison of experimental results to model predictions for the extent of reaction; E_{Phe}, phenylalanine extraction reaction, o, and, E_{OH}, hydroxide extraction reaction, •. Initial phenylalanine anion concentration, 10-50mM, Initial hydroxide anion concentration, 10-60mM, initial Aliquat 336 concentration = 200mM, temperature = 25°C, and PVR = 1. 173

Figure 8-5 The change in E_{Phe}, the extent of the phenylalanine extraction reaction, with increasing initial phenylalanine anion concentration for initial hydroxide concentrations of 30mM (• and —), 60mM (♦ and —), and 90mM (■ and ---). Initial Aliquat 336 concentration = 200mM, and PVR = 1. Markers denote experimental data and the lines are the model predictions. 175

Figure 8-6 The change in E_{OH}, the extent of the hydroxide extraction reaction, with increasing initial hydroxide anion concentration for initial phenylalanine concentration of 10mM (• and —), 30mM (♦ and —), and 50mM (■ and ---). Initial Aliquat 336 concentration = 200mM, temperature = 25°C, and PVR = 1. Markers denote experimental data and the lines are the model predictions. 176

Figure 8-7 The effect of the Aliquat 336 concentration on the extent of the extraction, •, and the co-extraction, ■, reactions. Plotted on the second y-axis is the phenylalanine fraction of all extracted species, ♦. Initially, 30mM phenylalanine and 30mM hydroxide anions in the aqueous phase, and a PVR of 1. Results were based on the model only. 177

Figure 8-8 The effect of the equilibrium constant for both extraction and co-extraction reactions on the extraction yield of the solute. Initially, 30mM phenylalanine and 30mM hydroxide anions in the aqueous phase, 200mM Aliquat 336 in the solvent phase, and a PVR of 1. Results were based on the model only. 179

Figure 8-9 Plot to determine the equilibrium constant for the bromide extraction reaction, K^*_{Br}. Initially; 35mM phenylalanine, 25mM hydroxide anions and 10-70mM bromide anions added to the aqueous phase, 200mM Aliquat 336 added to the solvent phase, temperature = 25°C, and PVR = 1. 181

Figure 8-10 The effect of varying the initial bromide concentration from 10 to 70mM, on the extent of the phenylalanine extraction reaction (■, 1st y-axis) and the value of the co-extraction constant (•, 2nd y-axis). Initially: 35mM phenylalanine and 25mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase,, temperature = 25°C, and PVR = 1. 181

Figure 8-11 The effect of the phase volume ratio on the determination of the equilibrium constant for the phenylalanine reaction. Initially: 10-50mM phenylalanine and 10-50mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, temperature = 25°C. 183

Figure 8-12 Plot of the aqueous phase concentration of chloride against the moles of phenylalanine extracted. Initial conditions: 20mM phenylalanine and 40mM hydroxide anions in the aqueous phase and 200mM Aliquat 336 in the solvent phase, N_{AQ} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 184

Figure 8-13 The effect of the initial hydroxide anion concentration on the value of the co-extraction constant determined from equilibrium experiments, •, Lewis cell experiments, ♦, and the extraction equilibrium model, —. Initial conditions were 35mM phenylalanine and 10 to 50mM hydroxide anions in the aqueous phase, and 200mM Aliquat 336 in the solvent phase, N_{AQ} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1. 186

Figure 8-14 Example of plots used to determine the film mass transfer coefficient for phenylalanine (•) and chloride (♦). Initial phenylalanine concentration of 50mM, hydroxide anion concentration of 50mM, Aliquat 336 concentration of 200mM, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1. 192

Figure 8-15 The effect of initial bromide concentration (10mM (♦), 35mM (■) and 70mM (•)), on the extent of the phenylalanine extraction reaction (hollow markers) and combined extents of all extraction reactions (filled markers). Initially: 35mM phenylalanine and 25mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1. 193

Figure 8-16 The effect of initial bromide concentration on the film mass transfer coefficient (•, 1st y-axis) and the co-extraction constant (♦, 2nd y-axis). Initially: 35mM phenylalanine, 25mM hydroxide anions and 10-70mM bromide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1. 194

Figure 8-17 The effect of dilutions of filtered fermentation broth on the film mass transfer coefficient for phenylalanine extraction (♦) and the extent of all extraction reactions (•). Initially: 35mM phenylalanine and 25mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1. 197

Figure 8-18 The effect of biomass concentration on the film mass transfer coefficient for phenylalanine extraction. Initially: 35mM phenylalanine and 25mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1. 198

Figure 8-19 The effect of the addition of Softanol 30 to the solvent phase on the film mass transfer coefficient (•, 1st y-axis) and the co-extraction constant (♦, 2nd y-axis). Initially: 35mM phenylalanine, 25mM hydroxide anions and in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1. 199

Figure 8-20 The effect of the addition of Softanol 30 to the solvent phase on the film mass transfer coefficient (•, 1st y-axis) and the co-extraction constant (♦, 2nd y-axis). Initially: 35mM phenylalanine, 25mM hydroxide anions and in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1. 200

Appendix A

Figure A-1 UV-visible absorbance spectrum of the aqueous and solvent phases arising from a chloramphenicol equilibrium extraction experiment. Initially the aqueous phase was distilled water, pH 5.5, with a chloramphenicol concentration of 250µM, the solvent phase was 1-octanol, temperature = 25°C, and PVR = 1. De-ionised water and 1-octanol were used as the reference solutions for the aqueous and solvent phase scans, respectively. Cuvette path length = 10mm. 221

Figure A-2 UV-visible absorbance spectrum of the aqueous and solvent phases arising from a chloramphenicol equilibrium extraction experiment from filtered fermentation broth into 1-octanol. The initial aqueous phase was filtered fermentation broth with a chloramphenicol concentration of 250µm, pH 7, temperature = 25°C, and a PVR = 1. De-ionised water and 1-octanol were used as the reference solutions for the aqueous and solvent phase scans, respectively. Cuvette path length = 10mm. 221

Figure A-3 Calibration plots obtained for chloramphenicol concentration-absorbance measurements: (a) aqueous phase measurements (o) for initial chloramphenicol concentrations of 0-250µM in distilled water, and (b) solvent phase measurements (•) after extraction with 1-octanol, PVR = 1. De-ionised water and 1-octanol were used as the reference solutions for the aqueous and solvent phase measurements, respectively. Cuvette path length = 10mm. 222

Figure A-4 Calibration plot obtained from the measurement of absorbance at a wavelength of 257nm for phenylalanine concentrations of 0-50mM, with a sodium hydroxide concentration of 100mM. Reference phase was de-ionised water and the cuvette had a path length of 5mm. 224

Figure A-5 Calibration plot obtained from the measurement of absorbance at a wavelength of 460nm for chloride concentrations of 0-50mM, with a phenylalanine concentration of 50mM, a sodium hydroxide concentration of 100mM, a sample dilution of 10 ($^{v}/_{v}$). Reference solution was a blank assay solution, and the cuvette had a path length of 10mm. Other experimental details are given in Section 6.5.2.2. 225

List of Tables

Chapter 5

Table 5-1	Composition of the media used for small-scale (≈ 100mls) fermentation for chloramphenicol production.	99
Table 5-2	Composition of the media used for small-scale (≈ 100mls) fermentation for phenylalanine production.	100
Table 5-3	Composition of the media used for large-scale (≈ 20 litre) fermentation for phenylalanine production.	100

Chapter 6

Table 6-1	Details of the components used in the combined Mach – Zehnder interferometer / Schlieren optical method set-up. Component index numbers relate to those designated in Figure 6-5.	110
Table 6-2	Nominal molecular weight cut-off of membranes used for ultrafiltration of fermentation broth.	116

Nomenclature

Symbol	Definition	Units
A	Interfacial area (of Lewis cell)	cm^2
C	Concentration	mM
Cl^-	Chloride anion	—
D	Diffusion Coefficient	cm^2 / s
E	Extent of reaction at equilibrium	mM
J	Molar Flux	mM / cm^2 s
k	Film Mass Transfer Coefficient	cm / s
K	Overall Mass Transfer Coefficient	cm / s
K^*	Equilibrium Reaction Constant	—
K_P	Equilibrium Partition Coefficient	—
M_{Wt}	Molecular Weight	g / mol
N	Lewis cell stirrer speed	rpm
OH^-	Hydroxide anion	—
Phe^-	Phenylalanine anion	—
PVR	Phase Volume Ratio	—
Q^+	Aliquat 336 cation	—
QCl	Aliquat 336-chloride ion pair	—
QOH	Aliquat 336-hydroxide ion pair	—
QPhe	Aliquat 336-phenylalanine ion pair	—
R	Resistance to Mass Transfer	s/cm
R	Universal Gas Constant	kJ / kmol K
T	Temperature	°C or K
t	Time	Seconds
V	Volume	cm^3

Greek Symbols

α	Co-extraction Constant	—
δ	Solubility Parameter	$cal^{1/2}/cm^{3/2}$
δ	Interfacial Film Thickness	nm
γ	Interfacial Tension	mN/m
μ	Chemical Potential	—
μ	Viscosity	kg / m s
ν	Kinematic Viscosity	cStokes
ρ	Density	g / l
τ	Integration Constant in Equation 3-26	—
Γ	Surface Excess Concentration	mol / m^2

Subscripts

0	Initial Condition
a	Phase a
Aq	Aqueous Phase
b	Phase b
e	Equilibrium Condition
i	Component i
i	Interface
j	Component j
R	Ratio of two equilibrium constants
Sol	Solvent Phase

Chapter 1

Introduction

1.1	Introduction	23
1.2	Biological Production of Compounds	24
1.3	Recovery and Purification of Fermentation Products	25
1.4	Liquid-Liquid Extraction	26
1.5	Aim of the Project	28
1.6	Overview of Thesis	28

1.1 Introduction

In this Chapter a brief summary of the production and processing of compounds obtained via biochemical pathways is presented. This forms the background for the experimental study that is presented later in this thesis. In Section 1.2 an overview of the production of compounds using biological cells is given, and particular emphasis is placed on the recovery and purification of these compounds. The recovery and purification of compounds obtained from cells is one of the greatest challenges facing the biotechnological industries, as factors associated with this will have a key influence on the processes viability. In Section 1.3 a brief review of the methods available to recover and purify compounds is presented. This thesis will specifically examine the recovery of compounds by liquid-liquid extraction, and in Section 1.4 the problems associated with the use of liquid-liquid extraction in bioprocessing are discussed. Some of the areas highlighted in Section 1.4 will be addressed in this investigation, and in Section 1.5 the aims of this study are defined. In Section 1.6 a summary of the contents of each Chapter is given.

1.2 Biological Production of Compounds

The science of biotechnology encompasses the production of a wide variety of compounds through the careful cultivation of micro-organisms, animal cells, and plant cells. In the broadest sense this technology has been used to the benefit of humans for thousands of years through the production of bread, beer, cheese and yoghurt. However, a conventional description of bioprocessing would refer mainly to the production of chemically defined compounds. In this sense biotechnology and bioprocessing has only come into being in the last 100 years, and particularly since the First World War where acetone was produced by fermentation for the munitions industry.

As a by-product of their growth and reproduction cells produce a variety of compounds which may be useful to modern society such as antibiotics, amino acids, enzymes and vitamins. By cultivating micro-organisms in a closed environment they can be manipulated to maximise the production of the desired compound. Then after periods ranging from hours to months the culture medium is harvested and the desired product is recovered.

Many of the compounds that are produced biochemically can also be synthesised using chemical pathways. However, the biochemical process is often more economical due to the ambient processing conditions, thus avoiding the use of extremes of temperature and pressure that can be hazardous and costly. Furthermore, a single bioreaction step may replace numerous intermediary reaction stages required to obtain the same compound via a chemical pathway.

The end product of a bioprocess must meet varying and understandably strict demands of regulatory bodies before it can be sold in the marketplace. Recent advances in genetic engineering, and hybridoma technology have made the production of novel proteins economically viable, however, the nature of cells used in these production methods has lead to increased purity requirements. A pure pharmaceutical product should satisfy the demands of no immunogenic substances, no unwanted biological

activity, no microbiological contamination, and has no enzymatic activity present that is harmful to the product.

One can imagine that in the coming years the number of compounds devised or discovered to enhance the quality of life will increase dramatically. This will require that complimentary technologies of separation and purification keep pace with the new challenges presented by increasingly complex molecules so that the benefits of these novel compounds can be realised by all societies.

1.3 Recovery and Purification of Fermentation Products

In biotechnological applications the main cost of the total process lies in the down stream processing stages. The recovery of some bioproducts offer challenges not encountered in traditional chemical process. Particular care must be taken in the recovery of compounds with three-dimensional protein structures; loss of conformation during processing often gives rise to a loss of biological activity and specificity. Subsequently, the high cost of separation and purification prevents some biological processes from becoming viable, cost effective, and successful (Sadana, 1996).

Despite the diversity of products available through biotechnology the general process outline for post-fermentation operations are essentially similar, and downstream operations can be summarised into four key categories (Belter *et al.*, 1996),

- ◊ ***Removal of Insolubles.*** Filtration and centrifugation are the principal unit operations used in this segment.
- ◊ ***Isolation of Product.*** These steps, which are relatively non-specific, remove materials of widely divergent properties compared to the desired product. Appreciable concentration and product quality increases usually occur. Adsorption and liquid-liquid extraction are typical operations.
- ◊ ***Purification.*** These processing techniques are highly selective for the product and remove impurities of similar chemical functionality and physical properties. Chromatography, electrophoresis and precipitation are good examples

◊ ***Polishing.*** The end use of the product dictates the final sequence utilised: crystallisation is often key, and most products must also be dried.

An underlying feature common to nearly all bioprocesses is the low concentration of product in the harvested culture fluid. Typically the concentration will be in the range of 1-2 g/l for proteins and above 10 g/l for some antibiotics, with the major components of the medium being the biomass (cells) and water, other components present will include unused media components and other metabolites. As the downstream process proceeds through the above categories the concentration and purity of the product will increase until the process requirements are met.

The second category in the list above, isolation of the product, is the area that is the focus of this study, and in particular the use of liquid-liquid extraction. A liquid-liquid extraction process is used to isolate the product into a second liquid phase; through this technique it should be possible to increase the compound's concentration and remove a significant quantity of other compounds. The next Section will examine the use of liquid-liquid extraction for the recovery of compounds from biological solutions.

1.4 Liquid-Liquid Extraction

Liquid-liquid extraction has been used widely in the chemical and hydrometallurgical industries for a considerable length of time; therefore, it is perceived to be a mature technology (Baird, 1991). However, the liquid-liquid extraction applications are considerably more developed than the underlying design data, and some of the fundamental mechanisms involved are still poorly understood (Stuckey, 1996). A number of areas where problems arise in solvent recovery of biologically derived products have been identified by Liddel (1994).

◊ The complexity of fermentation broth makes characterisation difficult to achieve, furthermore, the presence of solids is a feature not generally encountered in conventional extraction.

- ◊ The presence of surfactants are known to influence the mass transfer performance of liquid extraction processes, and biological systems are known to contain a large number of interfacially active materials, both soluble and insoluble.
- ◊ The surface-active material will also influence the rate of separation and the extent of phase recovery.

Many components of fermentation broth are known to be surface active and the above list highlights the effect that they can have on the overall liquid-liquid extraction process. Surfactants have long been known to decrease mass transfer rates (Lewis, 1954), and their effects are ascribed to mechanisms such as;

- ◊ Increasing film rigidity.
- ◊ Reducing levels of interfacial turbulence.
- ◊ Blockage of the interface.
- ◊ Altering interfacial tension, preventing/damping interfacial convection.
- ◊ Altering the equilibrium partition of the solute.

However, surface active agents have also been associated with initiating or enhancing interfacial convection which can increase mass transfer rates (Agble and Mendes-Tatsis, 2000). Surface active compounds may also cause emulsion formation leading to problems during phase separation of the broth and the organic solvent phase (Liddel, 1994). Effective separation of the phases is required to prevent contamination of the aqueous and solvent streams, maximise yields and minimise solvent losses.

The traditional scheme of liquid-liquid extraction involves the use of non-polar solvents, which will have a low miscibility with the aqueous phase and thus minimise solvent losses. The use of non-polar solvents precludes the use of liquid-liquid extraction for the recovery of polar solutes, such as amino and carboxylic acids, which will not significantly partition into them (Stuckey, 1996). However, this problem may be overcome through reactive extraction where the solute reacts with a long hydrocarbon chain to make it a less polar compound that is more soluble in the solvent phase.

In this Section the problems and challenges associated with the liquid-liquid extraction of compounds from biological solution have been highlighted. However, despite the numerous problems it is still a viable separation technique and forms an integral part of many bioprocesses. The problems presented here should been seen as challenges to be overcome that will allow the technique to be understood in more depth and developed for use in novel situations.

1.5 Aims of the Project

A number of areas were highlighted in the previous Section where there is a lack of fundamental understanding of the liquid-liquid extraction process. In particular the role of surface-active compounds in mass transfer is an area that requires further characterisation.

Hence, the overall aim of this study was to investigate and quantify the role of surface active fermentation broth constituents on interphase mass transfer and solute partitioning during both whole and filtered broth extraction, and also to investigate the effect of these compounds on a reactive extraction mechanism.

1.6 Overview of Thesis

The background information for the thesis has been divided into three Chapters. In Chapter 2 a review of literature relating to the effects that chemical and microbial surface active agents can have on mass transfer is presented, also included in that Chapter is a review of mass transfer in the Lewis cell. In Chapter 3 the theories behind interphase mass transfer and the adsorption of surface-active compounds to fluid interfaces is presented, also the phenomenon of interfacial turbulence is examined. Chapter 4 looks at the chemical basis for liquid-liquid extraction and explores methods that can be used to enhance extraction.

The remainder of the thesis details the experimental investigation conducted. To meet the aims of the project the experimental study was conducted using two different liquid-liquid extraction systems, these two systems are detailed in Chapter 5. In Chapter 6 the

experimental apparatus is described together with details of analytical techniques that were used. In Chapter 7 experimental results are presented for the investigation of fermentation broth components on mass transfer and partitioning using a conventional liquid-liquid extraction process. In Chapter 8 experimental results are presented for the investigation of the effect of fermentation broth constituents on the reaction mechanism of a reactive liquid-liquid extraction process. Finally, in Chapter 9 the main conclusions of this work are presented, and some recommendations for future work are given.

Chapter 2

Literature Review

2.1	Introduction	31
2.2	Surface-Active Compounds	31
	2.2.1 Surfactants	32
	2.2.2 Biosurfactants	34
2.3	Mass Transfer in Liquid-Liquid Systems	39
	2.3.1 Effect of Surfactants	39
	2.3.2 Effect of Biosurfactants	41
2.4	Mass Transfer in the Lewis Cell	42
	2.4.1 Mechanism of Interphase Mass Transfer	43
	2.4.2 Modelling Mass Transfer	44
	2.4.3 Reactive Extraction	47
	2.4.3.1 Characteristics of Reactive Extraction	48
	2.4.3.2 Modelling Mass Transfer	52
2.5	Project Objectives	55

2.1 Introduction

In Chapter 1 an overview was given of the recovery and purification of compounds produced during fermentation using liquid-liquid extraction. A number of areas were identified that require clarification so that liquid-liquid extraction processes can be developed and designed properly. One of these areas, which was the main focus of this study, was the influence of broth components on solute partitioning and mass transfer. It is anticipated that surface active broth components will have an important effect on solute extraction, and therefore, a review of the occurrence and effect of surface active compounds on liquid-liquid extraction is presented in this Chapter. In Section 2.2 the nature of the surface activity is examined, and in particular the appearance of these compounds in biological systems is reviewed. Section 2.3 details previous studies where surfactants and biosurfactants were found to affect liquid-liquid extraction. During this study a two-phase contactor called a Lewis cell was used to investigate mass transfer; the mechanism of mass transfer occurring in the Lewis cell, and the modelling procedure used to determine mass transfer coefficients are presented in Section 2.4. A reactive extraction mechanism was also investigated in this study; during reactive extraction, co-extraction of other compounds can occur and reports of this effect and models developed for mass transfer in a reactive system are also reviewed in Section 2.4. Finally in Section 2.5 the specific scientific objectives of this project are presented.

2.2 Surface Active Compounds

A surface active compound is a substance that, when present in low concentrations in a system, has the property of adsorbing onto the surfaces or interfaces of the system and of altering to a marked degree the amount of work required to expand these surfaces/interfaces (Rosen, 1978). In Section 2.2.1 the cause of surface activity within a molecule is discussed. In Section 2.2.2 a review of surface-active compounds present in biological systems is presented. It is common place to shorten the term "surface active compound" to surfactant, and for biologically produced surface active compounds the term biosurfactant is used.

2.2.1 Surfactants

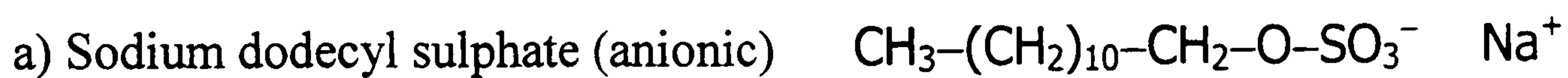
When an interface is expanded the minimum work required to create the additional amount of area is the product of interfacial tension and the increase in area. Surfactants usually decrease the interfacial tension, and thus reduce the energy required to expand the interface; as a result of this property surfactants are often used in processes where emulsification is desirable, such as in the manufacture of foods and cosmetics (Rosen, 1978).

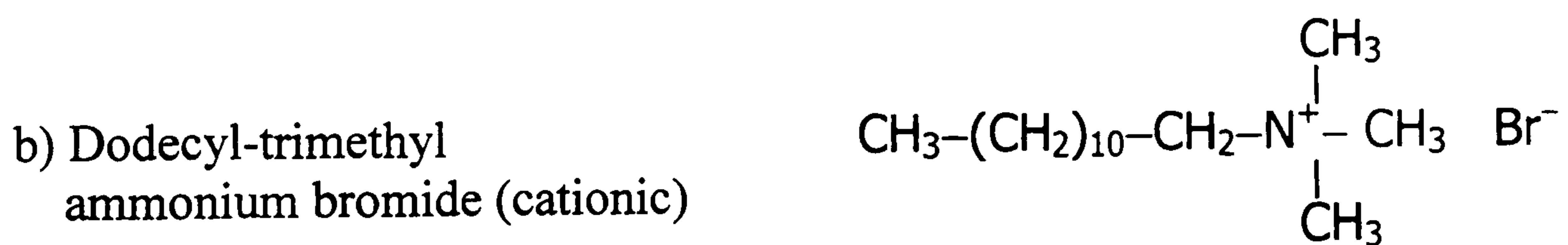
Surfactants have a characteristic structure consisting of a structural group that has very little attraction for the solvent, known as the lyophobic group, together with a group that has a strong attraction for the solvent, the lyophilic group. Collectively this is known as an amphipathic structure (Rosen, 1978). When a surfactant is dissolved in a solvent the lyophobic group causes a distortion of the liquid structure causing an increase in the free energy of the system. This means that less energy is required to bring a surfactant molecule to the surface than a solvent molecule. Thus the surfactant concentration at the interface increases and the amount of work needed to create a unit area of surface decreases, i.e. the interfacial tension decreases. The presence of the lyophilic group prevents the surfactant from being completely expelled, as that would require the desolvation of the lyophilic group. The molecule is orientated at the surface with the lyophilic group in the solvent and the lyophobic group orientated away from it. Further details of surfactant adsorption at fluid interfaces is given in Section 3.4

The structures of groupings suitable as the lyophilic/lyophobic portions of the molecule vary with the nature of the solvent and the conditions of use. In a highly polar solvent such as water the lyophobic part may be a hydrocarbon, fluorocarbon or siloxane. The lyophilic part may be an ionic or highly polar group in water, whereas in non-polar solvents such as heptane they may act as the lyophobic group. Surfactants are usually classified into four groups depending on the nature of the lyophilic group in water (normally called the hydrophilic group to indicate the interaction with water),

- ◊ Anionic - the surface-active portion of the molecule bears a negative charge,
- ◊ Cationic - the surface active portion of the molecule bears a positive charge.
- ◊ Zwitterionic - both positive and negative charges may be present in the surface active portion,
- ◊ Non-ionic - the surface-active portion bears no apparent charge.

Examples of the structures of each of these groups are given in Figure 2-1 (Rosen, 1978):





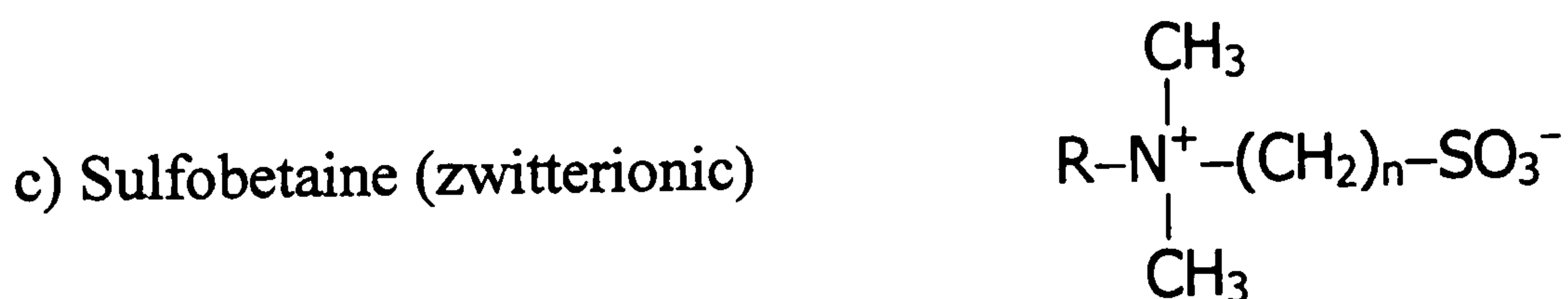


Figure 2-1 Examples of the structure of the four different classes of surfactants, a) anionic, b) cationic, c) zwitterionic and d) non-ionic.

Differences in the nature of hydrophobic groups are usually less pronounced, generally they are long chain hydrocarbon residues, however they include different structures such as (Rosen 1978),

- ◊ Straight chain long alkyl groups (C_8 - C_{20})
- ◊ Branched chain long alkyl groups (C_8 - C_{20})
- ◊ Long chain alkyl benzene residues (C_8 - C_{15})
- ◊ Alkylnapthalene residues (C_3 and greater alkyl groups)
- ◊ Rosin derivatives
- ◊ High molecular weight propylene oxide polymers
- ◊ Long chain perfluoroalkyl groups
- ◊ Polysiloxane groups

2.2.2 Biosurfactants

The term biosurfactant is used to refer to any compound that is synthesised by microorganisms and has some influence on interfaces (Desai *et al.*, 1994). A lot of interest surrounds biosurfactants as they have many advantages over their chemically synthesised counterparts in that they are highly specific, biodegradable and less toxic. Recently, a number of reviews have been published concerning biosurfactant production and their potential industrial applications (Georgiou *et al.*, 1992; Desai *et al.*, 1994; Lin, 1996; Desai and Banat, 1997; Rosenberg and Ron, 1999; Bognolo, 1999).

Amphipathic molecules, such as polysaccharides and proteins, are omnipresent in biological systems, with a relatively large proportion of the molecules present located at the interfaces between different bulk phases. These molecules are involved in the formation of complex organelles and membrane structures and the stabilisation of globular proteins that are essential in the evolution and maintenance of life processes (Jones and Chapman, 1995). Biosurfactants may be synthesised by bacteria, yeasts, and fungi during growth on various carbon sources, particularly during growth on hydrophobic substrates such as hydrocarbons. When production of the biosurfactant is extracellular they may cause emulsification of the hydrocarbon to aid uptake, when they

are cell wall associated they facilitate the penetration of the hydrocarbon into the periplasmic cavity (Desai and Banat, 1997).

Biosurfactants are commonly differentiated on the basis of their biochemical nature and the producing organism. In general biosurfactants are microbial metabolites with a typical amphiphilic structure and are either non-ionic or anionic (Bognolo, 1999). The hydrophobic moiety consists of a hydrocarbon chain of a fatty acid, while the hydrophilic moiety may be (i) an ester or alcohol function of a neutral lipid, (ii) the carboxyl group of a fatty acid or amino acids, (iii) phosphate containing part of a phospholipid, (iv) carbohydrate part of glycolipids.

There are six major classes of biosurfactants all of which show, under different systems and conditions, the ability to lower the surface or interfacial tension (Bognolo, 1999).

◊ Fatty acids are surfactants of moderate potency, as found in soaps as their sodium/potassium salts. They are normally studied as an intermediate in the synthesis of more complex molecules such as lipids, although they do sometimes appear free in biological media and exert their surface-active properties. An examples of surface active a fatty acids is corynomycolic acid.

◊ Glycolipids are the most commonly characterised biosurfactants, with the best known classes being rhamnolipids, trehalolipids and sophorolipids. They are carbohydrates in combination with long chain aliphatic acids or hydroxyaliphatic acids.

◊ Polysaccharides, which are large molecules containing many saccharide groups and the associated hydrocarbon chains, may complex with protein and lipid molecules to become very effective biosurfactants.

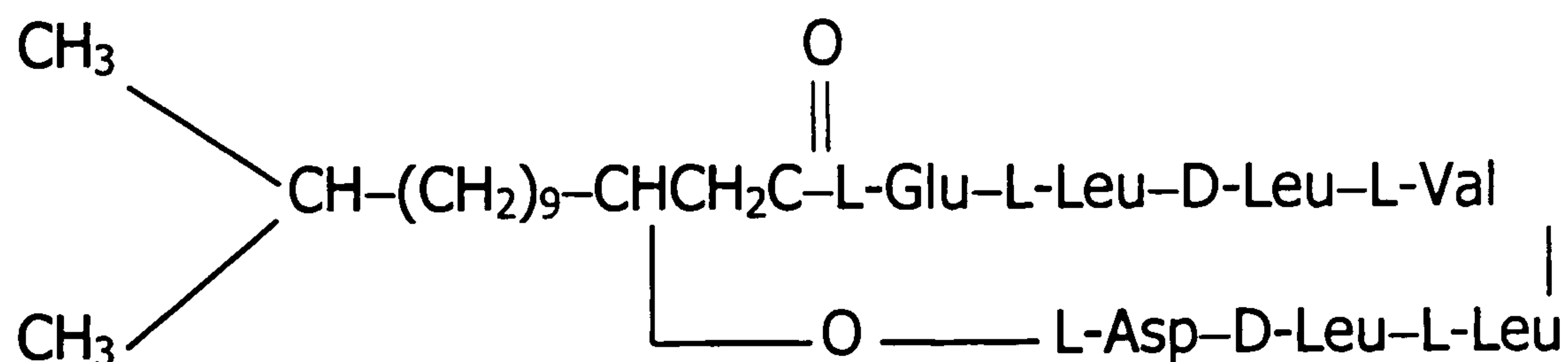
◊ Lipoproteins consisting of chains or rings of peptide groups combined with long chain hydrocarbons. The peptide groups themselves may have polar or non-polar side chains, such as glutamic acid which will have a positive charge at physiological pH.

◊ Phosphoglycerides (which are more commonly called phospholipids) are the result of esterification of one of the hydroxyl groups of glycerol to create a phosphatidic backbone. Two fatty acids chains are usually attached to the glycerol by esterifying the two remaining hydroxyl sites. One of the two hydroxyl groups on the phosphatidic acid back bone can be replaced by esterification of an alcohol to phosphoric acid.

◊ Whole cells and cell debris are formed from surface-active compounds such as phospholipids and proteins; therefore they may inherently have surface-active properties.

In Figure 2-2 the structural configuration of some biosurfactants are presented; a common feature of biosurfactants are their large size and complex structure, and in the case of proteins their ability to alter their surface-active properties through refolding.

a) Surfactin



b) Phosphatidylcholine

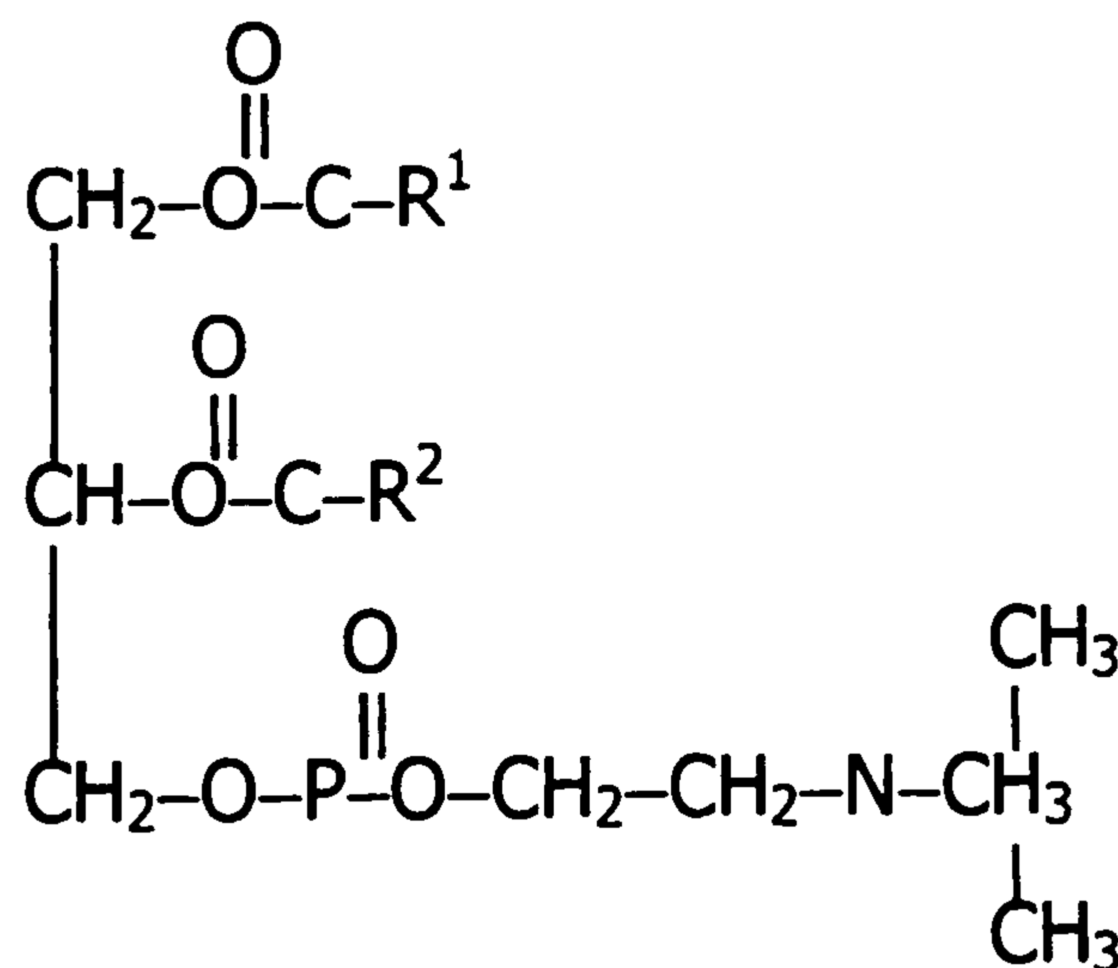


Figure 2-2 The molecular structures of biologically produced surfactants, a) surfactin, b) phosphatidylcholine, where R_1 and R_2 are hydrocarbon chains.

The complex structure of biosurfactants, and their large size compared to many chemical surfactants gives biosurfactants unique interfacial properties. The critical micelle concentration of biosurfactants can be much lower than conventional surfactants, with concentrations as low as 0.02 mg/l being reported (Georgiou *et al.*, 1992). Adsorption of biosurfactants to the interface is slow and will be controlled by diffusion (Fainerman *et al.*, 1998). At the interface proteins are known to undergo conformational changes which can further reduce the interfacial tension of the system, and denature the protein (Graham and Phillips, 1979). In combination with other surfactants, competitive adsorption can occur whereby the adsorption of one compound is favoured. This process does not necessarily favour the most surface-active molecule, and other conditions such as temperature, pH, viscosity, density and ionic strength can be important (Millar *et al.*, 2000).

When a protein adsorbs to an interface its conformation may change dramatically. The second phase (gas or oil, and type of oil) will influence the change in structure, and for a protein these changes can be seen as a form of interfacial denaturation. Proteins may displace one another at the interface, and there effectiveness at doing so is based on their ability to change conformation upon adsorption (Bos and van Vliet, 2001). Flexible (soft) proteins change conformation easily and will displace globular (hard) proteins. However, adsorbed monolayers of hard proteins have a high surface shear, which implies strong lateral interactions between molecules and is reflected in the increase in surface shear with time, a process which is also called ageing.

Proteins form more stable emulsions than low molecular weight surfactants, that have higher adsorption energies per m^2 than proteins, however proteins can adsorb at the interface with several segments and through conformational changes more segments may adsorb. The displacement of proteins by surfactants from solid and liquid surfaces can be described by two extreme mechanisms (Bos and van Vliet, 2001).

◊ *Solubilisation*: Water soluble surface active compounds binds to the protein to form a soluble complex. The surface active compound does not necessarily need to adsorb to the interface but must interact strongly with the protein.

◊ *Replacement*: Surface active compound adsorbs to the interface and displaces the protein because the Gibbs interfacial energy is lower (interfacial tension) than that of the protein. The surfactant need not interact with the protein but must adsorb to the interface.

In practice the situation will be a combination between these two extremes. Ionic surfactants will mainly interact with the protein, whereas non-ionic surfactants will mainly displace proteins from the interface.

For many situations the production of biosurfactants during fermentation is desirable and a great deal of work has been done on optimising yields. Much of this work is directed towards the production of biosurfactants for use in enhanced oil recovery and environmental clean up operations in situations such as oil spills and contaminated land. A number of recent reviews (Cooper and Zajic, 1980; Desai and Banat, 1997) detail compounds which have been discovered and characterised; these reviews demonstrate the wealth and variety of compounds that may be produced during fermentation, and a number of notable discoveries include:

◊ Surfactin, a cyclic polypeptide produced by *Bacillus subtilis*, is one of the most effective biosurfactants discovered so far. It is superior to commonly used surfactants such as sodium dodecyl sulphate (SDS), and can reduce the surface tension of distilled water to 27.9 mN/m at a concentration as low as 50 mg/l (Cooper and Zajic, 1980).

◊ Emulsan, a high molecular weight compound composed of fatty acids linked to a heteropolysaccharide backbone produced by *Acinetobactor calcoaceticus*. Emulsan is a very effective emulsifier of hydrocarbons in water even at a concentration as low as 10mg/l (Desai and Banat, 1997).

The production of biosurfactants depends primarily on the micro-organism, although it has been shown that factors like substrate source, temperature, pH and aeration will affect the yield of biosurfactant (Desai, 1987). Water soluble carbon sources such as glycerol and glucose are inferior to immiscible substrates like n-alkanes and olive oil for

producing biosurfactants, and nitrogen limitations can give rise to over production of biosurfactants (Desai, 1987).

2.3 Mass Transfer in Liquid-Liquid Systems

2.3.1 The Effect of Surfactants

In liquid-liquid extraction systems the effects of surfactants on mass transfer have been studied on many occasions. Surfactants may affect the solute transfer both positively and negatively due to a number of changes that they may bring about in the system. Unfortunately, there is no predictive method to determine the effect that a particular surfactant may have on a particular extraction system. The nature of effects at low concentrations may be interfacial rigidification, blocking of the available surface area, damping surface movement, physico-chemical interactions between the solute and the surfactant, and the induction of Marangoni instabilities (Agble, 1998). At higher surfactant concentrations micelle formation and the aggregation of surfactant molecules can have a varying effect on the transport process. In addition, at all concentrations the equilibrium of extraction may be altered leading to a change in the concentration driving force.

Surfactants can alter the rate of solute transfer by altering the mass transfer driving force if the equilibrium distribution of the two solutes is affected. Garner and Hale (1953) observed that increasing amounts of Teepol first decreased the equilibrium partitioning of diethylamine extraction from toluene, and then increased it at higher surfactant concentrations.

Rigidification, or immobilisation, of the interface as a result of a surfactant monolayer can reduce solute mass transfer. Lewis (1954) observed a transfer reduction in the presence of a rigid protein layer, and this was attributed to the damping of turbulent eddies close to the interface. Davis and Mayers (1961) suggested the retardation of surface renewal by a film of plasma albumin as the cause of solute transfer reduction.

Physico-chemical interactions can change the ability of a solute to cross the interface through the creation of an energy barrier. Also referred to as barrier effects or interfacial resistance, physico-chemical interactions can be differentiated from the previous effect that changed the hydrodynamic nature of the interface. Hutchinson (1948) concluded that a form of solute-surfactant interaction caused the solute to adsorb to the interfacial surfactant film creating a change in the concentration driving force. In the study it was noted that the transfer of n-butyl alcohol and n-butyl amine was retarded more than the transfer of ether and methyl ethyl ketone in the presence of sodium cetyl sulphate. This was attributed to differing molecular configurations with the presence of hydrophilic groups increasing the retarding effect. Garner and Hale (1953) reached a similar conclusion by showing that neither hydrodynamics or a blocking effect could be responsible for the reduction of transfer of diethylamine in the presence of Teepol.

Lindland and Terjesen (1956) studied the transfer of iodine to solvent drops in the presence of sodium oleyl-p-anisidine sulphonate. It was found that the mass transfer rate decreased rapidly with increasing surfactant concentration up to the critical micelle concentration (CMC (Section 3.4.3)), beyond which no further change occurred. They proposed a solute-surfactant interaction governed by the equilibrium between the solute and interfacial surfactant concentration; furthermore, a relationship was formulated to relate the interfacial resistance to interfacial surfactant concentration.

Mudge and Heideger (1970) studied the effect of SDS and sodium tetradecyl sulphate on the transfer of n-butanol and iso-butanol from water to carbon tetrachloride. A resistance was found in all systems, with it being significantly pronounced for the transfer of n-butanol; this was attributed to a barrier effect and possibly a solute-surfactant interaction. Using interferometry measurements of the bulk concentration they tried to extrapolate back to the interfacial concentration to show that there was a departure from equilibrium and thus a barrier effect.

Marangoni instabilities occur when localised interfacial tension gradients are created at the interface. The system will then respond by expanding the interface that will cause the fluid layers adjacent to the interface to be dragged with the interface. Depending on the physical properties of the system the fluid motion may then escalate into sustained

interfacial convection. The Marangoni phenomenon can give rise to mass transfer rates greater than those predicted by general mass transfer theories. Sherwood and Wei (1957) studied ternary liquid-liquid systems. Localised stirring, rippling and twitching of the interface was observed along with measured mass transfer rates which were greater than theory predicted. Since then a number of studies (e.g. Blokker 1957; Bakker *et al.* 1966, 1967) have found similar results in selected ternary systems. Further information on Marangoni phenomena is given later in Section 3.5 where the cause and mechanism is detailed.

2.3.2 The Effect of Biosurfactants

Considering the extensive use of liquid-liquid extraction in processes such as penicillin recovery there is very little literature concerning the effects of broth constituents on the extraction process. Crabbe *et al.* (1986) investigated the effect of yeast cells on transfer rates of ethanol from water into n-decanol. An 86% reduction in transfer rates was observed at a concentration of 0.1g/l of yeast cells. This reduction was attributed to physical blocking of the liquid-liquid interface by adsorption of cells. Furthermore, in relation to the study of Lewis (1954) on protein films it was postulated that the presence of the adsorbed cells might act to prevent turbulence transfer across the interface.

Weatherley *et al.* (1993) compared ethanol extraction from yeast and mycelial culture fermentation broth as well as examining the effect of varying biomass concentration on extraction rates. At zero biomass concentration the extraction rate was lower from mycelial culture broth, this was attributed to the effect of soluble surface-active compounds on rheology and damping of interfacial turbulence. With increasing biomass concentration the transfer rate was seen to decrease for both yeast and mycelial culture broth, with the mycelial culture extraction rates always lower than those for yeast broth. The effect of biomass concentration on equilibrium partitioning was also examined, and over the concentration range considered no variation was observed although it was suggested that the concentrations examined were too low to be thermodynamically significant.

Weatherley *et al.* (1994) investigated the effect of *Aueobasidium pullans* broth, which contains both unicellular yeast and filamentous cells, on partitioning of iso-propanol into n-decanol. Interestingly, the presence of biomass increased the partition coefficient with the highest value found for the presence of yeast cells only. Furthermore, when the yeast cells where re-suspended in buffer a higher partition was observed compared to broth with yeast cells leading to the conclusion that the yeast cells increased partitioning, although it was suggested that higher concentrations should be examined before any definitive conclusions are drawn.

Hamilton and Weatherley (1995) investigated the effect of biomass on interfacial tension. Increasing biomass concentration was seen to reduce the interfacial tension to a minimum at around 2 g/l. With no cells present the broth/oil interfacial tension was lower than the water/oil interfacial tension indicating the presence of soluble surface-active compounds in the broth.

On a larger scale, Anderson and Lau (1955) compared the performance of a Podbielniak centrifugal contactor on extraction of clarified and whole broth. Extraction efficiency was reduced by 10-15% for whole broth extraction, although in comparison to a non-biochemical extraction both clarified and whole broth extraction performance was much lower.

2.4 Mass Transfer in the Lewis Cell

In this study the effect of broth components on mass transfer was investigated using a fixed area contactor. This type of contactor, based on the original design of Lewis (1954), has been used extensively over the past five decades to study liquid-liquid mass transfer e.g. Davis and Mayers, (1961); Haensal et al (1986); Scarpello and Stuckey (2000). Details of the design and operation of the Lewis cell used in this study are given in Section 6.3. In Section 2.4.1 the accepted mechanism of mass transfer in the Lewis cell is described. Based on this mechanism mass transfer can be modelled to allow mass transfer coefficients to be determined, an overview of this modelling procedure is given in Section 2.4.2. In this study a reactive extraction mechanism has also been investigated to recovery a polar solute using an ion exchange reagent, in

Section 2.4.3 reports of co-extraction by the ion exchanger are presented and the most recent model of the reactive mass transfer process is reviewed.

2.4.1 Mechanism of Interphase Mass Transfer

The Lewis cell was originally used by Lewis (1954) to investigate the effect of insoluble protein films on mass transfer. Lewis proposed that transfer to the interface occurred entirely by eddy diffusion, with no contribution from molecular diffusion. However, Gordon and Sherwood (1954), Mayers (1961) and McManamey (1961) disagreed proposing that close to the interface molecular diffusion controlled mass transport. Since then it has been generally accepted that transport in a Lewis cell can be described using the two-film theory of Whitman (1927) where transport by molecular diffusion is assumed. An outline of this theory is given below, and further details are given in Section 3.3.4.1.

In the liquid-liquid extraction process, the solute will transfer from the feed phase to the receiving phase, and the final extent of extraction will be governed by the equilibrium conditions of the system. The controlling resistance in the transfer process will be diffusion of the solute across stagnant liquid films either side of the interface. Outside of the stagnant films diffusional mass transfer will be aided by bulk convection, which is achieved by independently stirring each phase. This results in a homogenous concentration in the bulk of a phase, and the only concentration gradients that exist are located in a thin film close to the two-phase interface. Steady state transfer will occur in each film, therefore, the solute flux on either side of the interface will be equal, and there will be no accumulation of mass at the interface. Figure 2-3 shows the concentration profile across the interface based on two-film theory, where the only change in concentration occurs in stagnant films either side of the interface (King, 1980; Cussler, 1997).

The configuration of the Lewis cell will result in physical conditions similar to that encountered in conventional separation processes, such as mixer settlers, where mixing causes homogenous bulk phases and interfacial films with transient properties (Cussler, 1997). Experimentally the Lewis cell will allow the solute concentration to be determined from samples taken from each phase at known times after initial contact.

The concentration-time data obtained from experiments will be analysed using models based on the proposed transport mechanism to yield values for mass transfer coefficients.

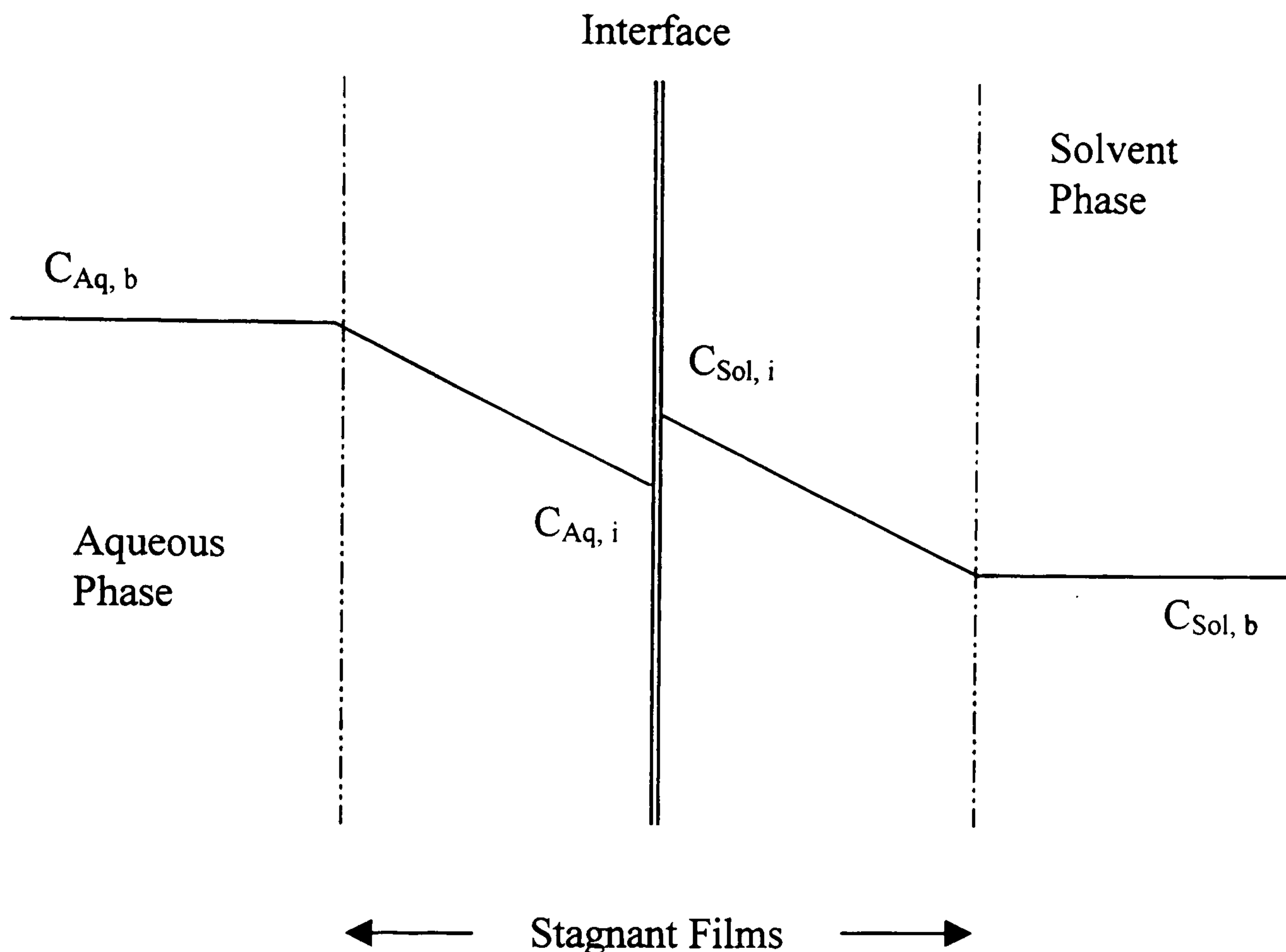


Figure 2-3 The concentration profile for solute transfer from the bulk (b) aqueous (aq) across the interface (i) to the bulk solvent (sol) phase; based on the two-film model of the physical extraction process.

2.4.2 Modelling Mass Transfer

Mass transfer theory for transport across a film gives a simple description of the solute flux, and is described in Section 3.3.3.1. According to this theory the solute flux is linearly related to the concentration driving force, with the film mass transfer coefficient being the constant of proportionality, (Equation 2-1; King, 1980). This expression for the solute flux can then be used to describe the transfer of a solute in a differential mass balance on one of phases. The film model of mass transfer has been successfully employed in previous investigations, allowing the modelling, and analysis of experimental data obtained from stirred cells of similar design to the cell used in this

investigation (Davis and Mayers, 1961; Haensel *et al.*, 1986; Scarpello and Stuckey 2000).

$$J = k\,(C_b - C_i) \qquad (2\text{-}1)$$

To allow evaluation of the mass transfer coefficient the interfacial solute concentration must be determined; however, it is usually not possible to do this. The problem can be circumvented by assuming that the solute concentrations either side of the interface are in equilibrium, and then an expression for the equilibrium condition is used to describe the interfacial concentration in terms of measurable bulk phase concentrations (King, 1980).

For the case of physical extraction the interfacial concentration can be described by the equilibrium partition coefficient (King, 1980). The interfacial concentration on one side of the interface is assumed to be in equilibrium with the bulk concentration in the other phase. Using this method to describe the interfacial concentration gives rise to an overall mass transfer coefficient, which is a combination of the film mass transfer coefficients for the two phases. For the case of reactive extraction the equilibrium constant for the ion exchange reaction may be used to describe the interfacial concentrations, this is discussed separately in Section 2.4.3

From the mechanism described in Section 2.4.1 the solute flux across each film will be equal, and can be described by a film model,

$$J = k_{Aq}\,(C_{Aq,b} - C_{Aq,\,i}) = k_{Sol}\,(C_{Sol,\,i} - C_{Sol,\,b}) \qquad (2\text{-}2)$$

As mentioned the interfacial concentration of the solute is usually not known, and the flux equation (Equation 2-2) is redefined for a concentration change between the bulk phase and a concentration that is in equilibrium with the other bulk phase. The constant of proportionality between the flux and the concentration difference is now called the overall mass transfer coefficient, and is a function of the film mass transfer coefficients on both sides of the interface,

$$J = K_{Aq}\,(C_{Aq,b} - C_{Aq,\,e\text{-}Sol}) = K_{Sol}\,(C_{Sol,\,i} - C_{Sol,\,e\text{-}Aq}) \qquad (2\text{-}3)$$

Equation 2-3 may be substituted into the differential mass balance, but to allow the expression to be evaluated, $C_{i,\,e\text{-}j}$, the concentration in phase i that is in equilibrium with the phase j, should be expressed as a measurable bulk phase concentration. This can be done using the equilibrium partition coefficient, where,

$$K_P = \frac{C_{Sol}}{C_{Aq}} = \frac{C_{Sol,\,e\text{-}Aq}}{C_{Aq,\,b}} = \frac{C_{Sol,\,b}}{C_{Aq,\,e\text{-}Sol}} \qquad (2\text{-}4)$$

Using these definitions of the equilibrium partition coefficient (Equation 2-4), the solute flux through the interface can be written in terms of bulk phase concentrations (as no other concentrations remain the subscript b is omitted),

$$J = K_{Aq}\left(C_{Aq} - \frac{C_{Sol}}{K_P}\right) = K_{Sol}\left(K_P\, C_{Aq} - C_{Sol}\right) \qquad (2\text{-}5)$$

In this form Equation 2-5 can be used to model the solute flux across the interface, and in the absence of concentrations other than those for bulk phases will allow the model to be evaluated using experimental concentration measurements.

In the experimental work conducted during the present study the solute concentration was generally measured in the solvent phase, therefore the model will be developed for that situation. A differential mass balance on the solvent phase would equate the accumulation of the solute with time to the flux of solute crossing the interface by mass transfer, and is written as,

$$V\frac{dC_{Sol}}{dt} = JA = A\,K_{Aq}\left(C_{Aq} - \frac{C_{Sol}}{K_P}\right) \qquad (2\text{-}6)$$

To allow this expression to be integrated, the aqueous phase concentration should be expressed in terms of the solvent phase concentration, which can be achieved by considering an overall mass balance for the two phases.

$$C_{Aq} = C_{Aq,0} - C_{Sol} \tag{2-7}$$

This expression is then substituted in Equation 2-6, which upon rearranging may be integrated between the limits, $C_{Sol} = 0$ at $t = 0$, and $C_{Sol} = C_{Sol}$ at $t = t$, to yield,

$$K_{Aq}\, t = \frac{V\, K_P}{A\,(K_P + 1)} \ln\left(C_{Aq,0} - C_{Sol} - \frac{C_{Sol}}{K_P}\right) \tag{2-8}$$

Equation 2-8 is in the form of an equation of a straight line such that,

$$f(C_{Sol}) = K_{Aq}\, t \tag{2-9}$$

and,

$$f(C_{Sol}) = \frac{V\, K_P}{A\,(K_P + 1)} \ln\left(C_{Aq,0} - C_{Sol} - \frac{C_{Sol}}{K_P}\right) \tag{2-10}$$

By plotting $f(C_{Sol})$ vs. t using experimental concentration-time data a straight line should be obtained whose gradient is the overall mass transfer coefficient, K_{aq}. By conducting mass transfer experiments under different conditions, the K_{aq} values determined can be compared to assess whether the imposed condition had an effect on mass transfer.

2.4.3 Reactive Extraction

Extraction of acids from dilute aqueous solutions is a challenging task because of hydrophilic nature of the compounds; the partition coefficient of the acid with many organic solvents is often less than unity. However, the addition of organophosphates or aliphatic amines to the solvent phase can greatly increase acid extraction, where aliphatic amines are slightly more effective and less expensive than organophosphates (Yang *et al.*, 1991). Primary amines have a high solubility in aqueous solutions and give rise to considerable extractant losses (or recovery costs), and secondary amines are subject to amide formation during regeneration. Therefore, most investigations have

focused on long-chain tertiary and quaternary amine compounds. King and co-workers (Ricker *et al.*, 1979 and 1980; Tamada *et al.*, 1990; Dai and King, 1996) have examined the extraction of various carboxylic acids by a range of tertiary amines. Extraction of an acid molecule by a tertiary amine occurs through the formation of a solvent soluble complex between the undissociated acid and the amine compound (Keertes and King, 1986). Tong *et al.* (1998) compared the extraction efficiencies of a number of tertiary amines and a quaternary amine, Aliquat 336, in an extractive fermentation system for lactic acid production. The quaternary amine was found to be superior to the tertiary amines at extractions conducted at the pH of the fermentation process. Although Aliquat 336 was found to be more toxic to the micro-organism a cleaning step was successfully employed to remove residual Aliquat 336 from the fermentation medium before it was returned to the fermenter.

In this study a reactive extraction mechanism was used to facilitate the recovery of a polar solute into a non-polar solvent phase. The model system used was the extraction of the amino acid phenylalanine using the trioctylmethylammonium chloride, a quaternary ammonium ion exchange reagent that is commonly called Aliquat 336; further details of the extraction mechanism are given in Chapter 5. In this Section a review of the findings from previous extraction studies where Aliquat 336 has been used to recover bioproducts is given, where particular emphasis is given to the effect of others species present in the extraction medium on solute extraction.

2.4.3.1 Characteristics of Reactive Extraction

Quaternary amines, and in particular Aliquat 336, have been shown to be effective extractants for a number of biotechnological separations including carboxylic acids (Coelhoso *et al.*, 1997), amino acids (Scarpello and Stuckey, 2000), antibiotics (Bora *et al.*, 1997) and vitamins (Calvarin *et al.*, 1992). The extraction mechanism of the quaternary amine Aliquat 336 differs from that found for tertiary amine compounds, in this case the acid is extracted in a dissociated anion state through an ion exchange reaction. Due to the insolubility of the solute and the extractant in the others phase the reaction will occur at the two-phase interface, and is consider instantaneous in comparison to the mass transfer kinetics found in liquid-liquid extraction systems (Haensel *et al.*, 1986). In the reaction the phenylalanine anion is exchanged for the

chloride ion associated with Aliquat 336, forming a phenylalanine-Aliquat 336 solvent soluble compound, and releasing chloride anions into the aqueous phase. Phenylalanine is a zwitterionic compound, therefore, to allow extraction by Aliquat 336 it must exist in an anionic form (Thien *et al.*, 1987). This can be achieved by adding a buffer to raise the solution pH above its pKa of 9.27, and at a pH of 11.3 greater than 99% of the acid will be dissociated into the anionic form.

The extraction performance of Aliquat 336 has been utilised in a number of liquid-liquid extraction techniques including emulsion liquid membranes (Thien *et al.*, 1987) and supported liquid membranes (Molinari *et al.*, 1992). Although these techniques differ through the composition of the solvent phase they can provide important information on the overall behaviour of Aliquat 336 as an extractant. The nature of the specificity of the ion exchanger for a solute was demonstrated by Thien *et al.* (1987) using an emulsion liquid membrane system where the initial flux of a range of amino acids was found to be a linear function of the solute's hydrophobicity. A similar result was also reported by Hano *et al.* (1991), who found that the equilibrium constant for the extraction reaction was a linear function of the solute's hydrophobicity for a similar range of amino acids.

Hano *et al.* (1991) also investigated whether the solvent used to dilute Aliquat 336 had an effect on the extraction equilibrium. This is an aspect that is known to affect extraction using tertiary amines where the polarity of the solvent can influence the size of solvent phase aggregates (Keertes and King, 1986). No effect was reported by Hano *et al.* (1991), and similarly by Molinari *et al.* (1992). However, the solvent did, as would be expected, alter the solute flux as the transport properties of the phase were altered.

Behr and Lehn (1973) showed that a liquid membrane system using Aliquat 336 would extract a range of amino acids and di-peptides. It was also reported that the transport of hydroxide, added to buffer the system, would compete with the solute for the extraction through the membrane. Hano *et al.* (1991) showed that Aliquat 336 was capable of extracting inorganic anions of ionic valencies –1 (OH^-), –2 (SO_4^{2-} and CO_3^{2-}) and –3 (PO_4^{3-}), and the stoichiometry of the extraction reaction for each anion correspond to the ionic valance of the anion. Thien *et al.* (1987) examined a similar system to Behr and

UCL LONDON UNIV.

Lehn (1973) and showed that the addition of SO_4^{2-} anions to the feed phase (to simulate ions present in a fermentation medium) reduced the total extraction flux of phenylalanine by 40%. Molinari *et al.* (1992) found similar results for a supported liquid membrane system, an NO_3^- concentration of 10mM reduced the flux of phenylalanine to 15% of the original value and similar concentrations of SO_4^{2-} and PO_4^{3-} reduced the flux to 35%.

The performance of a liquid-liquid extraction operation may be characterised by the equilibrium distribution coefficient, which describes the ratio of solute in the solvent phase to that in the aqueous phase at equilibrium. The solute distribution coefficient for phenylalanine extraction using Aliquat 336 has been reported to vary with the initial concentration of species involved in the reaction (Haensal *et al.*, 1986; Molinari, *et al.*, 1992; Coelhoso *et al.*, 1996 and 1997; Escalante *et al.*, 1998). Haensal *et al.* (1986) attributed this variation to the co-extraction of buffer ions, however, they did not consider the effect to be important and neglected co-extraction in species mass balances. An alternative method of characterising the extraction system is through the Law of Mass Action. It describes the equilibrium balance of species concentration involved in the extraction reaction, and defines the equilibrium constant for the reaction as,

$$K^*_{Phe} = \frac{C_{QPhe,i}\, C_{Cl,i}}{C_{Phe,i}\, C_{QCl,i}} \qquad (2\text{-}11)$$

The thermodynamic basis of this equilibrium constant predicts that it should remain constant for a given temperature, irrespective of the initial concentration of reaction species (Smith and Van Ness, 1987). Therefore, as it remains constant with concentration it can be used to describe the equilibrium distribution of species at the interface for mass transfer modelling (Haensal *et al.*, 1986; Yang *et al.*, 1991; Chan and Wang, 1993; Coelhoso *et al.*, 1996 and 1997). An important consideration when determining the equilibrium reaction constant is the interaction of other reactions that may involve the ion exchange reagent. Initially suggested by Behr and Lehn (1973), the idea of competitive extraction has not been fully appreciated until the recent study of Scarpello and Stuckey (2000) where the co-extraction of buffer anions was considered. As indicated by the reports detailed above, where Aliquat 336 has been used to extract many different compounds (carboxylic acids, amino acids, peptides, antibiotics and

inorganic salts), Aliquat 336 is not specific to phenylalanine and may extract a range of compounds. Therefore, it is logical to assume that during phenylalanine extraction co-extraction of other compounds will occur, especially when extraction from a complex medium like fermentation broth is required. In relation to the determination of the equilibrium constant any co-extraction reaction will contribute to the mass balances used to determined unmeasured concentrations, usually Aliquat 336 and chloride. Failure to take into account these contributions will lead to the incorrect determination of the equilibrium constant, which in turn will have implications on results obtained from mass transfer models where the equilibrium constant was used to express interfacial conditions (Scarpello and Stuckey, 2000).

Scarpello and Stuckey (2000) investigated the effect of buffer (hydroxide) anion co-extraction on equilibrium extraction and mass transfer of phenylalanine extraction using Aliquat 336. During mass transfer experiments they found that the extraction of phenylalanine was linearly related to the production of chloride, but not equal as would be the case if no co-extraction occurred, therefore, co-extraction of the buffer was occurring. The constant of proportionality between these two concentration changes was termed the co-extraction constant; when there is initially no chloride present in the aqueous phase the relationship was written as,

$$C_{Cl} = \alpha\,(C_{Phe} - C_{Phe,0}) \qquad (2\text{-}12)$$

With decreasing initial phenylalanine concentration Scarpello and Stuckey (2000) found the level of co-extraction increased recording an α value of –4.9 for an initial phenylalanine concentration of 4mM. This indicates that there was extensive co-extraction, with nearly four times as much hydroxide being extracted over phenylalanine. In previous studies of this extraction system co-extraction has been assumed to be negligible, where the change in moles of Aliquat 336 and chloride has been taken as equal to the change in moles of the aqueous phase solute (Haensal *et al.* (1986); Uddin *et al.* (1990 and 1992); Chan and Wang (1993); Coelhoso *et al.* (1996 and 1997).

The work of Scarpello and Stuckey (2000) focused on buffer co-extraction, however, realistically the extraction medium may contain a number of anionic fermentation media

compounds of appreciable quantity which will all compete for extraction. Furthermore, the studies of Behr and Lehn (1973), Hano *et al.* (1991) and Thien *et al.* (1987) have demonstrated the potential for co-extraction of compounds commonly found in fermentation media. Co-extraction will effect the extraction efficiency, mass transfer performance and design of the separation process, and therefore, characterisation of its effect on solute extraction is required

2.4.3.2 Modelling Mass Transfer

Many of the studies reported in the previous section have also modelled the mass transfer of phenylalanine extraction by Aliquat 336 (Haensal *et al.*, 1986; Uddin *et al.*, 1990 and 1992; Chan and Wang, 1993; Coelhoso *et al.*, 1997; Scarpello and Stuckey, 2000). The objective of these modelling processes has been to determine a mass transfer coefficient from experimental measurements, which has then been used to assess the effect of process conditions and added compounds on the extraction of phenylalanine. This section details the methods and assumptions previously used to model the reactive extraction of phenylalanine by the ion exchange reagent Aliquat 336. The previous models were developed for a contactor of similar design to the Lewis cell used in this present study where a two-film theory model of interphase mass transfer was assumed.

In addition to the assumptions previously stated in Section 2.4.1 for two-film theory, it is assumed that for reactive extraction using Aliquat 336, the extraction reaction rate is not mass transfer limiting (Haensal *et al.*, 1986; Uddin *et al.*, 1990; Coelhoso *et al.*, 1997; Scarpello and Stuckey, 2000). Figure 2-4 shows a schematic of the concentration profiles either side of the interface according to the two-film theory for the case under study. The flux of phenylalanine to the interface is described using a film model, Section 3.3.3.1, where the flux is proportional to the concentration driving force across the film, and the proportionality constant is the film mass transfer coefficient,

$$J_{Phe} = k_{Phe} \left(C_{Phe,\,b} - C_{Phe,\,i} \right) \qquad (2\text{-}13)$$

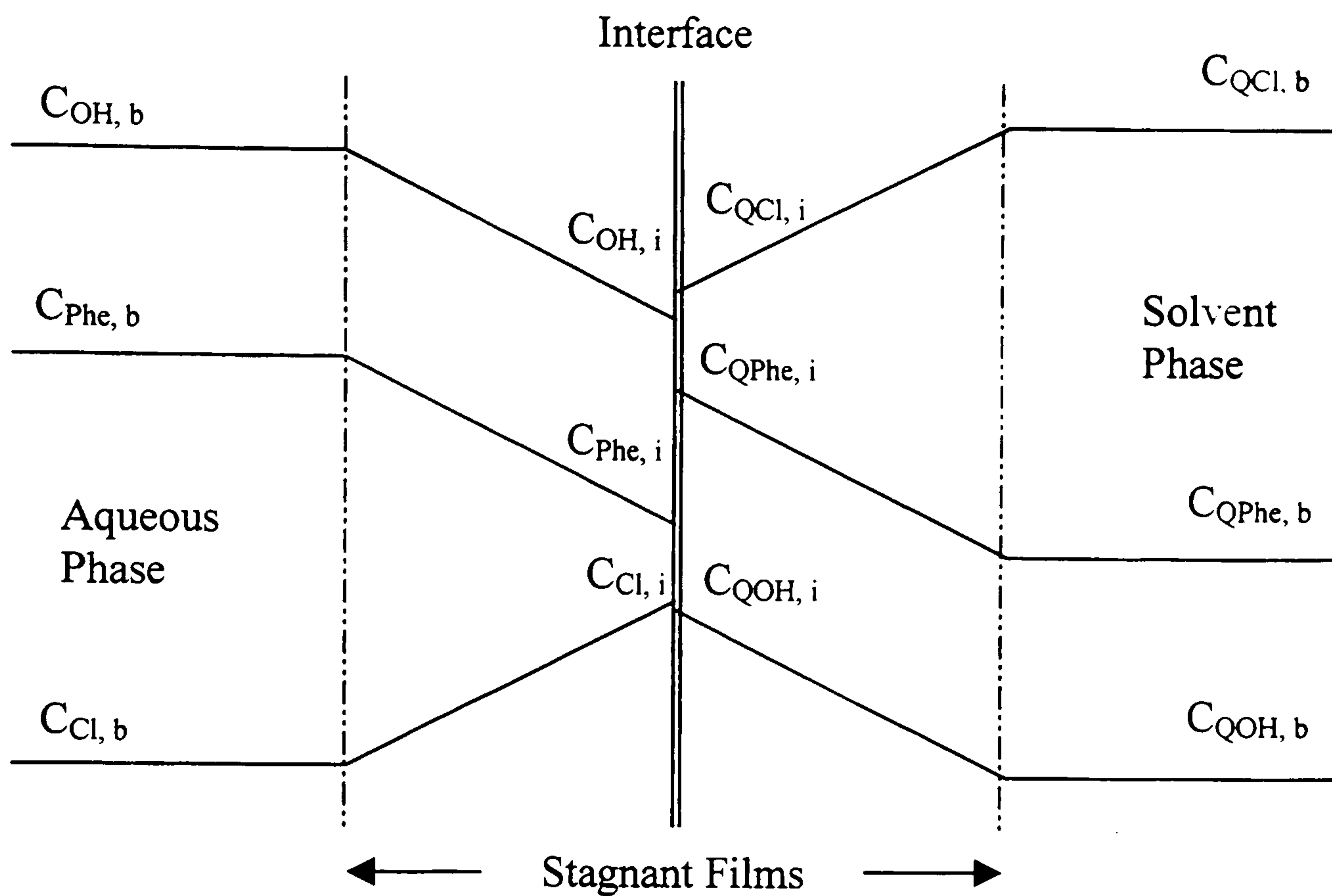


Figure 2-4 Concentration profiles close to the interface (i) for all the species involved in the reactive extraction of phenylalanine (Phe) with Aliquat 336 (QCl). Where solute transfer will occur from the bulk (b) aqueous (aq) across the interface to the bulk solvent (sol) phase; based on the two-film model of the reactive extraction process. Also shown is the profile for hydroxide (OH) which may be co-extracted.

The fluxes of the other species involved in the extraction reaction (chloride, Aliquat-phenylalanine, Aliquat-chloride) are also described in a similar manner,

$$J_{Cl} = k_{Cl} (C_{Cl, i} - C_{Cl, b}) \quad (2\text{-}14)$$

$$J_{QPhe} = k_{QPhe} (C_{QPhe, i} - C_{QPhe, b}) \quad (2\text{-}15)$$

$$J_{QCl} = k_{QCl} (C_{QCl, b} - C_{QCl, i}) \quad (2\text{-}16)$$

According to the two-film theory, steady state mass transfer occurs, and therefore there will be no accumulation of transferring species at the interface. As a result of this the fluxes of the species involved in the extraction reaction will be equal (Haensal *et al.*,

1986; Uddin *et al.*, 1990; Chan and Wang, 1993; Coelhoso *et al.*, 1997; Scarpello and Stuckey, 2000),

$$J = k_{Phe}\ (C_{Phe,b} - C_{Phe,i}) = k_{QPhe}\ (C_{QPhe,i} - C_{QPhe,b}) = k_{QCl}\ (C_{QCl,b} - C_{QCl,i}) \quad (2\text{-}17)$$

The two-film theory also assumes that the interfacial concentrations are in equilibrium, so a suitable expression describing equilibrium was used to eliminate the interfacial concentration from Equation 2-17. Haensal *et al.* (1986), Uddin *et al.* (1990 and 1992), Chan and Wang (1993), and Scarpello and Stuckey (2000) used the equilibrium constant for the extraction reaction, K^*_{Phe}, (Equation 2-11) for this purpose, however, assumptions about the aqueous phase species had to be made.

Haensal *et al.* (1986), Uddin *et al.* (1990 and 1992), and Scarpello and Stuckey (2000) assumed that the rate of the chloride ion transfer was not mass transfer limiting due its small size compared with the other species; Scarpello and Stuckey (2000) extended this assumption further to include phenylalanine. These assumptions imply that the main resistance to mass transfer lies on the solvent side, i.e. k_{Phe}, k_{Cl} >> k_{QP}, k_{QCl}, which was shown experimentally by Scarpello and Stuckey (2000) to be true. Equation 2-11 could then be written in terms of the interfacial concentration of the Aliquat 336,

$$C_{QCl,i} = \frac{C_{QPhe,i}\ C_{Cl,b}}{C_{Phe,b}\ K^*_{Phe}} \quad (2\text{-}18)$$

By equating the flux of Aliquat-phenylalanine to Aliquat-chloride (Equation 2-17) and substituting Equation 2-18, a definition for the interfacial concentration of Aliquat-phenylalanine was obtained. This was then used to define the flux of phenylalanine, and construct a differential mass balance for the aqueous phase, in terms of bulk concentrations, equilibrium constants and mass transfer coefficients (Scarpello and Stuckey, 2000),

$$J_{Phe} = -\frac{V}{A}\frac{dC_{Phe}}{dt} = k_{QPhe}\left(\frac{K^*_{Phe}\ C_{QCl}\ C_{Phe} - C_{QPhe}\ C_{Cl}}{K^*_{Phe}\ \dfrac{k_{QPhe}}{k_{QCl}}\ C_{Phe} + C_{Cl}}\right) \quad (2\text{-}19)$$

Scarpello and Stuckey (2000) showed that the contribution of co-extraction to species mass balances should not be ignored when determining the equilibrium reaction constant. This observation was also applied to their mass transfer analysis, Equation 2-19, where the co-extraction constant (Equation 2-12) was used to relate all other species concentrations to the aqueous phase phenylalanine concentration. Using these expressions Equation 2-19 was directly integrated to yield an expression for the film mass transfer coefficient for Aliquat-phenylalanine.

Although Scarpello and Stuckey (2000) took into account co-extraction when defining species mass balances, they did not notice that the flux of co-extracted material across the interface should also be considered when equating fluxes (Equation 2-17). The concentration profile shown in Figure 2-4 also shows a concentration profile for the co-extracting hydroxide anions. It can be seen that if co-extraction occurs then its flux across the interface should also be considered in the flux balance, and therefore the implications of the co-extraction flux on solute mass transfer should be considered further.

2.5 Project objectives

During fermentation surface-active compounds (biosurfactants) can be produced, and it has been reported that some of these compounds can have a strong influence on the interface and interfacial processes. Furthermore, surface-active compounds have been identified as causing both reductions and increases in mass transfer during liquid-liquid extraction. Therefore, specific objectives of this study were to,

◊ **Investigate the effect of soluble and non-soluble components of fermentation broth on mass transfer and equilibrium conditions during liquid-liquid extraction.**

◊ **Investigate the effect of surfactant and biosurfactant compounds on mass transfer and equilibrium conditions during liquid-liquid extraction.**

It is possible to extract polar solutes into non-polar solvents by using an ion exchange reagent. The ion exchange reagent can be non-specific, and other components of the medium may be co-extracted, therefore, specific objectives of this study were to,

◊ **Study and characterise the role of co-extraction during reactive liquid-liquid extraction.**

◊ **Investigate the effect of soluble and insoluble broth components and surfactant compounds on reactive liquid-liquid extraction.**

Chapter 3

Interfacial Processes

3.1	Introduction		58
3.2	Description of the Interface		58
3.3	Mass Transfer Across the Interface		60
	3.3.1	Introduction	60
	3.3.2	Mass Transfer by Diffusion	61
		3.3.2.1 Diffusion Coefficients	62
		3.3.2.2 Mass Transfer Coefficients	63
	3.3.3	Solutions of Fick's Law	64
		3.3.3.1 Transfer Across a Thin Film	64
		3.3.3.2 Transfer Into a Semi-Infinite Medium	66
	3.3.4	Interphase Mass Transfer	68
		3.3.4.1 Two Film Theory	68
		3.3.4.2 Other Theories of Mass Transfer	70
3.4	Adsorption at the Interface		71
	3.4.1	Introduction	71
	3.4.2	Adsorption to Interfaces	71
	3.4.3	Behaviour of Surfactants in Solution	73
	3.4.4	Dynamic Adsorption	74
3.5	Interfacial Convection		75
	3.5.1	Introduction	75
	3.5.2	Marangoni Phenomena	76
	3.5.3	Gravitational Convection	79

3.1 Introduction

This project was concerned with investigating the effect of surface-active compounds on solute recovery using liquid-liquid extraction, where the solute was present in the aqueous phase at a low concentration. The aim of the separation process will be to isolate the solute of interest from impurities present in the fermentation medium, and also to increase the solute concentration. The selectivity of the solvent for the solute over other compounds and the distribution of the solute between the two liquid phases will affect the separation process. However, for downstream operation of a continuous extraction process, solute mass transfer rates will dictate the contact time, liquid flow rates, and ultimately the size of process plant equipment. Therefore, the effect of surface active compounds on dynamic mass transfer needs to be investigated.

This chapter presents a summary of the current understanding of interfacial processes that may occur during liquid-liquid extraction. In Section 3.2 a description of the two-phase interface that separates the feed and receiving phase is presented. In Section 3.3 mass transfer of the solute is examined where transport is considered for diffusional and convective mass transfer. One of the objectives of this study was to examine the effect that the adsorption of compounds to the interface has on extraction, in Section 3.4 the process of adsorption to interfaces is examined. In some instances spontaneous interfacial convection may occur and this will affect the mass transfer process, the cause and effect of this phenomena is discussed in Section 3.5.

3.2 Description of the Interface

The boundary region between the two adjacent phases is known as the interface. In a liquid-liquid extraction system the interface arises due to the immiscibility of the two phases, with molecules at a liquid interface experiencing an attraction towards their bulk liquids and also across the interface between the two phases. Matter at an interphase will usually have different physical properties from that in the bulk. In the interfacial region it is envisaged that the physical properties of the interface change from that of one phase to that of the other phase, with the boundaries of the interface having properties equal to the bulk of the adjacent phase. This change may be considered to be

gradual or abrupt; an abrupt change would indicate a flat two-dimensional interface, whereas a gradual change would be consistent with a three-dimensional interfacial region, or commonly called an interphase, see Figure 3-1.

It is convenient to regard the interface between two phases as a mathematical plane, such as in Figure 3-1. This approach is unrealistic especially if an adsorbed film is present. Not only will such a film have a certain thickness, but also its presence may influence nearby structures (e.g. by dipole-dipole orientation) and result in an interphase of varying composition with an appreciable thickness in terms of molecular dimensions.

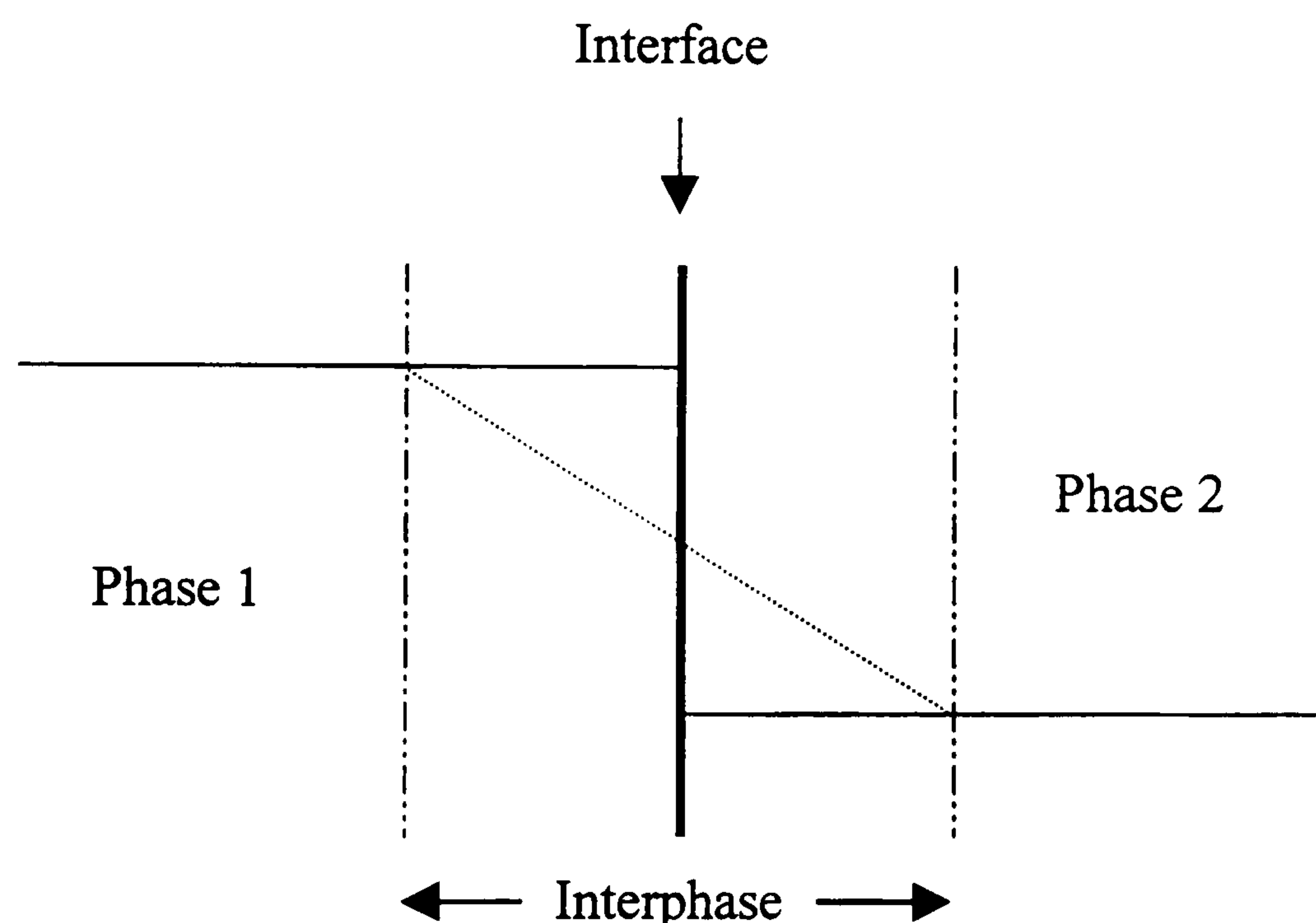


Figure 3-1 Schematic showing how a physical property (e.g. concentration) would vary across the liquid-liquid interfacial region, depending on the existence of either an interface (solid line) or an interphase (dotted line).

Each molecule at the interface possesses an excess free energy due to the different surrounding forces compared to similar molecules in the bulk of the solution. The interfacial molecules will experience attractive and repulsive forces from molecules on both sides of the interface. The energy required to bring a molecule to the interface, and expand the interface, is equal to the excess free energy of the surface. The balance of these forces at the interface is expressed as the interfacial tension, and is the work

required to increase the interfacial area between the two phases. When the interfacial tension of the system is lowered to zero the two phases will become miscible and the interface will disappear. Surface or interfacial tension is commonly used to characterise the interaction and effect of molecules adsorbing at the interface.

3.3 Mass Transfer Across the Interface

3.3.1 Introduction

In liquid-liquid extraction mass will transfer through the bulk of the feed solution until it reaches the two-phase interface. The mass will cross the interface and then move away into the bulk of the receiving phase. The basic process underlying this movement of mass is diffusion, which will be described in Section 3.3.2. In Section 3.3.3 the fundamental equations for diffusion are solved for situations relevant to experimental apparatus used in this study. In most instances, mass transfer by diffusion is slow and often the rate limiting process, therefore it is normal to aid the diffusional process with convection. In Section 3.3.4 theories that examine the role of convection on interphase mass transfer are examined.

The discussion below is developed based on diffusion of ideal gases where the driving force is the molar concentration gradient. In liquid systems the total molar concentration may vary considerably as the individual component concentrations change; however, in liquid systems the mass concentration (density) will vary less and can be assumed to be constant. Thus, for the liquid phase, diffusion will give rise to equal and opposite mass fluxes, rather than molar fluxes as is the case for gas phase transfer. Due to the effect of concentration on liquid phase diffusion coefficients, liquid phase transfer is theoretically considered for dilute solutions only. For dilute solutions the total molar concentrations may be assumed to be constant and thus molar units can be used for liquid phase calculations with confidence.

3.3.2 Mass Transfer by Diffusion

Mass transfer by diffusion is due to random molecular motion, with each molecule having an equal chance of moving in any direction. In areas of high solute concentration there will be more solute molecules and hence more molecules that are likely to move into the area of lower concentration than will move in the opposite direction. Thus, there will be a net movement of the solute towards the area of lower concentration.

The driving force for mass transfer by diffusion will be the concentration gradient. In 1855 Fick proposed that the steady state mass transfer rate is a linear function of the concentration driving force. When equimolecular counter diffusion occurs, or the concentration is sufficiently dilute, then the steady state molar solute flux due to diffusion is given by Fick's 1st Law of Mass Transfer,

$$J_A = -D\frac{\partial C_A}{\partial y} \qquad (3\text{-}1)$$

Where the molar solute flux, J_A, is proportional to the concentration gradient, which increases in the positive y direction. The constant of proportionality, D, is the diffusion coefficient for the solute in the solvent. The negative sign in Equation 3-1 takes account of the fact that mass transfer occurs in the opposite direction to the concentration gradient, therefore the direction of mass transfer will be in the negative y direction.

In practical mass transfer processes the solute concentration and thus the solute concentration gradient changes with time and position. If we consider a mass balance on a fluid element of unit surface area, and of thickness δy, then the accumulation of the solute within the element equals the difference between the solute flux entering and leaving the element. Using Equation 3-1 it may be shown that the change in the molar flux is,

$$\left(\frac{\partial J_A}{\partial y}\right)\delta y = D\left(\frac{\partial^2 C_A}{\partial y^2}\right)\delta y \qquad (3\text{-}2)$$

The accumulation of mass within the element equals the change in solute concentration with time multiplied by the volume of the element (equal to 1 x δy); equating this with the right hand side of Equation 3-2 gives rise to the following expression,

$$\frac{\partial C_A}{\partial t} = D\frac{\partial^2 C_A}{\partial y^2} \qquad (3\text{-}3)$$

Equation 3-3 is commonly referred to as Fick's 2nd Law of mass transfer that describes unsteady state mass transfer. In Section 3.3.3 this equation will be solved for two cases relevant to the experimental apparatus used in this study.

3.3.2.1 Diffusion Coefficients

The diffusion coefficient, D, is the proportionality factor in Fick's laws of mass transfer (Equations 3-1 to 3-3), and relates solute flux to the concentration driving force. The magnitude of the diffusion coefficient is dependent on the molecular size of the solute and the physical properties of the medium through which diffusion occurs. In the liquid phase diffusion coefficients are approximately four to five orders of magnitude lower than in the gas phase, this is due to the smaller intermolecular distances involved in liquids. The diffusion of solutes in the liquid phase often controls the rate of many processes such as acid-base reactions, digestion of food and the corrosion of metals (Cussler, 1997).

The value of the diffusion coefficients may be determined experimentally or predicted using semi-empirical correlations. For diffusion in liquids, the basis for these correlations is often the Stokes-Einstein equation, which models the diffusion of a molecule as a solid spherical particle moving through a viscous liquid. However, the Stokes-Einstein equation is limited to situations where the solute is larger than the solvent and is often accurate to only 40%. Many other correlations exist which attempt to account for different situations (Cussler, 1997). Of these the correlation that is most commonly quoted is the equation of Wilke and Chang (1955), which is given below.

$$D_{AB} = 7.410^{-8} \frac{(\phi\ M_B)^{\frac{1}{2}} T}{\mu_B v_A^{0.6}} \qquad (3\text{-}4)$$

The values of coefficients in the Wilke-Chang equation are dependant on the units of the physical properties, and in the form of Equation 3-4 the units are defined as; D_{AB}, the diffusion coefficient at infinite dilution (cm^2/s); ϕ, the association parameter of the solvent; M_B, the molecular mass of the solvent (g/mol); T, the temperature (K); μ, the viscosity of the solvent (Ns/m^2), and v_A, the molar volume of the solute (cm^3/mol).

The value of the diffusion coefficient for liquid systems will often depend on the solute concentration, and those determined from Equation 3-4 would apply only to a situation where the solute was at infinite dilution. The effect of concentration on the value of the diffusion coefficient may be taken into account using correlations such as Schreiner's (Pertler *et al.*, 1995).

3.3.2.2 Mass Transfer Coefficients

Solutions of the diffusion equation (Equation 3-3) often become quite complex or impossible to deal with when mass transfer occurs in a flowing system where the flux of solute will depend on diffusional properties and the degree of turbulence. It is generally not possible to define a fundamental relationship for the effect of diffusion and turbulence on mass transfer, so they are "lumped" together as a mass transfer coefficient. The mass transfer coefficient, k, is the constant of proportionality between the flux of the solute, J_A, and the difference in concentration, which for transfer from a bulk fluid, b, to an interface, i, would be written as,

$$J_A = k\,(C_{A,b} - C_{A,i}) \qquad (3\text{-}5)$$

The magnitude of the mass transfer coefficient depends on the nature of the solute and the solvent, the flow characteristics, and on the physical geometry of the system. It can be thought of as a measure of the solute's ability to be transported, and the reciprocal of the mass transfer coefficient is the resistance to mass transfer, R,

$$R = \frac{1}{k} \tag{3-6}$$

For a few cases mass transfer coefficients can be obtained from theory, and this will be demonstrated in the next section where Fick's law of diffusion (Equation 3-3) is solved for situations relevant to experimental apparatus used in this study. However, in most cases mass transfer coefficients are obtained from experiments using a suitable concentration driving force.

3.3.3 Solutions of Fick's Law

In this section Fick's laws of diffusion will be solved for two situations: transfer across a thin film and transfer into a semi-infinite medium. These situations correspond to those that may occur in the experimental apparatus used in this study. In both of the solutions presented here it is assumed that the diffusion coefficient remains constant. When the diffusion coefficient does vary with concentration Fick's second law of mass transfer must be solved numerically using an expression that describes the change in diffusion coefficient with solute concentration.

3.3.3.1 Transfer Across a Thin Film

The film model of mass transfer is based on the observation that the concentration of the transferring solute usually changes most rapidly in the immediate vicinity of the interface and is relatively uniform in the bulk fluid away from the interface. Where the fluid is flowing near an interface the assumption is made that the concentration change occurs over a thin region that is immediately adjacent to the interface, this region is called the film. The film is considered to be stationary and so thin that steady state diffusion is immediately established across it. As there is no change in the concentration gradient across the film then Equation 3-3 reduces to,

$$\frac{\partial^2 C_A}{\partial y^2} = 0 \tag{3-7}$$

Figure 3-2 shows how a steady state concentration profile would appear for transfer of solute A across a thin film adjacent to an interface, where the boundary conditions may be described as $C_A = C_{A,i}$ at $y = 0$, and $C_A = C_{A,b}$ at $y = \delta$.

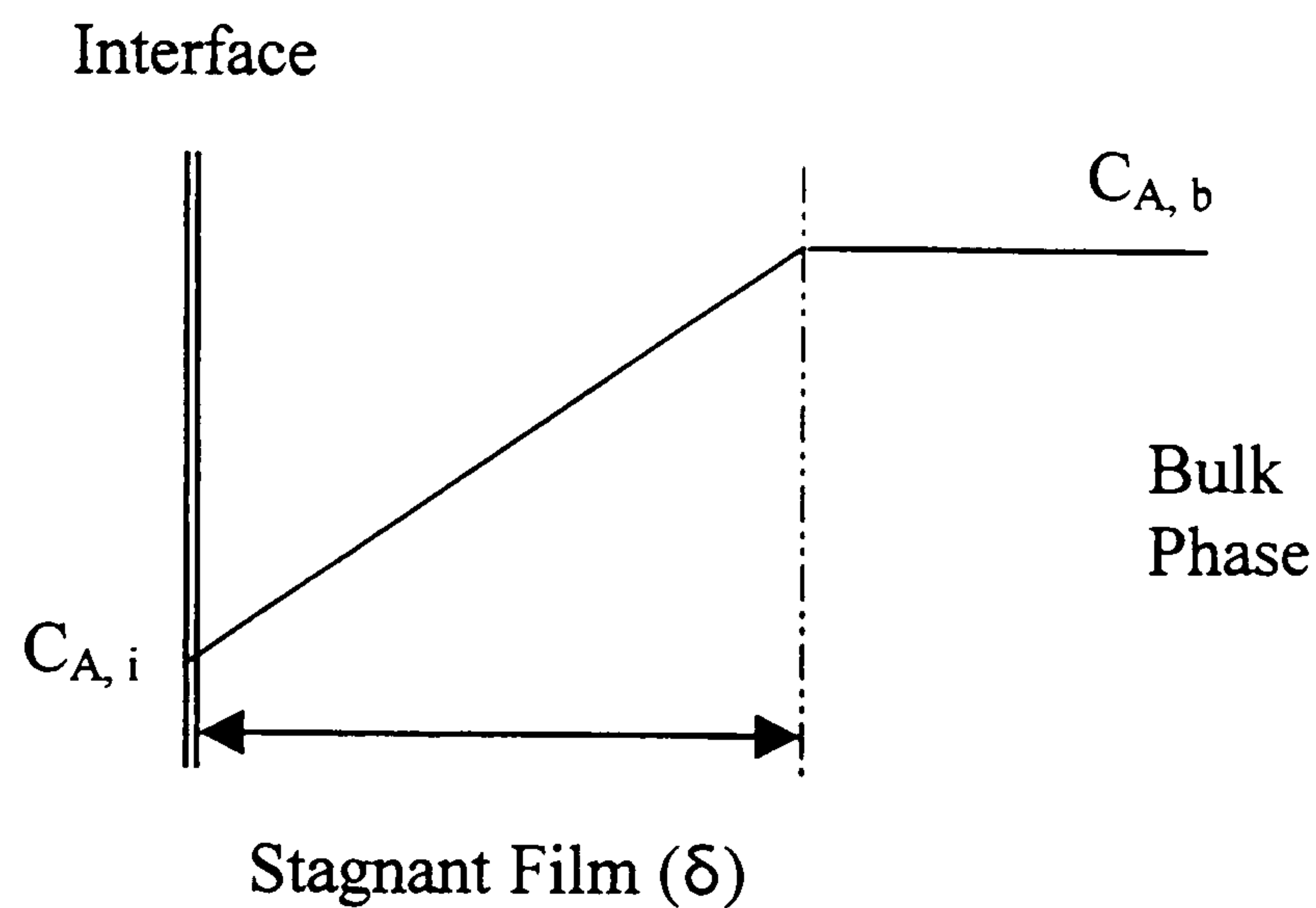


Figure 3-2 The concentration profile across a thin stagnant film between an interface and the bulk of the phase.

Using the boundary conditions described above, Equation 3-7 may be solved to give the concentration profile through the thin film and the flux across the film,

$$C_A = C_{A,i} + \frac{y}{\delta}\left(C_{A,b} - C_{A,i}\right) \tag{3-8}$$

$$J_A = \frac{D_{AB}}{\delta}\left(C_{A,b} - C_{A,i}\right) \tag{3-9}$$

From a comparison of Equation 3-9 with the definition of the mass transfer coefficient (Equation 3-5) it can be seen that,

$$k = \frac{D_{AB}}{\delta} \tag{3-10}$$

This equation could be used to predict mass transfer coefficients if δ could be predicted, or if δ were independent of liquid flow rate. However, this is never the case, and further more the film theory predicts that k varies directly with D_{AB}, which is very rarely observed. The discrepancy arises from the assumption of a discontinuity in flow conditions at the outer boundary of the film leading to a uniform resistance across the film.

Despite it shortcomings the film model is useful, due to its mathematical simplicity, to predict and analyse the effects of interfacial adsorption on mass transfer. It is a reasonable assumption for highly turbulent fluids near fixed surfaces where a thin stagnant boundary layer may exist.

3.3.3.2 Transfer Into a Semi-Infinite Medium

Diffusion of the solute into a semi-infinite medium will occur when two phases are brought into contact, and this situation will be applicable so long as the solute does not extend infinitely into the medium. When unsteady state diffusion of the solute occurs, Equation 3-3 may solved where the boundary conditions are as follows:

Initially the solute concentration in the medium has a uniform concentration (usually this is zero),

$$C_A = C_{A,0} = 0, \quad \text{for} \quad y > 0 \quad \text{at} \quad t = 0; \tag{3-11}$$

and the concentration of the feed solution is uniformly that of the interfacial concentration,

$$C_A = C_{A,i}, \quad \text{for} \quad y < 0 \quad \text{at} \quad t = 0; \tag{3-12}$$

At all times the solute never extends infinitely into the medium,

$$C_A = C_{A,0}, \quad \text{for} \quad y = \infty \quad \text{at} \quad t > 0; \tag{3-13}$$

The loss of material from the feed medium is sufficiently small that the solute concentration at the interface is always constant at the initial solute concentration,

$$C_A = C_{A,i}, \qquad \text{for} \qquad y = 0 \qquad \text{at} \qquad t > 0; \tag{3-14}$$

When the diffusion coefficient, D, is constant the method of Laplace transforms can be used to solve Equation 3-3. For $C_{A,0} = 0$ the concentration profile may be shown to be,

$$C_A = C_{A,i}\ \text{erfc}\left(\frac{y}{2\sqrt{Dt}}\right) \tag{3-15}$$

Where erfc (x) is the complimentary function of the error function, erf (x), and is the area under the Gaussian function $\frac{2e^{-x^2}}{\sqrt{\pi}}$ from x to ∞. Values of erfc (x) are tabulated in many standard texts on mass and heat transfer, e.g. Crank (1975) or Carslaw and Jaeger (1959).

The solute flux at the interface, $(J_A)_{y=0}$, may be found by differentiation of Equation 3-15,

$$(J_A)_{y=0} = -D\frac{\partial C_A}{\partial y} = \sqrt{\frac{D}{\pi t}}\left(C_{A,i} - C_{A,0}\right) \tag{3-16}$$

From a comparison of Equation 3-15 with the definition of the mass transfer coefficient (Equation 3-5) shows that,

$$k = \sqrt{\frac{D}{\pi t}} \tag{3-17}$$

This differs from the result found for the film model (Equation 3-9) where the mass transfer coefficient varied directly with the diffusion coefficient.

3.3.4 Interphase Mass Transfer

In many applications of mass transfer, material is transferred across a phase boundary such as in gas adsorption (gas-liquid interface), crystallisation (solid-liquid interface) or in the case of this study the interface between two immiscible liquids involved in a liquid-liquid extraction system.

In liquid-liquid extraction the solute will transfer through the feed solution to the two-phase interface, transfer across the interface and dissolve in the receiving solvent, and then transfer away from the interface into the bulk receiving phase. During this process the solute will experience a resistance, and each step described above will contribute to the overall mass transfer resistance, which can be described by the resistance in series model (Davis and Rideal, 1963),

$$R = R_1 + R_i + R_2 \tag{3-18}$$

When the interface is clean it is thought to offer little resistance to mass transfer (Davis and Rideal, 1963), and the main resistance will be in the liquid phases adjacent to the interface. However, when surface-active compounds adsorb to the interface they may offer resistance to solute mass transfer, and this is the subject under investigation in this study.

In interphase mass transfer the solute is assumed not to accumulate at the interface, so the solute mass transfer rates either side of the interface will be equal. The solute's concentration either side of the interface is controlled by equilibrium conditions. The rate of solute mass transfer is dependent on the physical properties of the two phases, the concentration driving force, the interfacial area, and the degree of turbulence. Therefore, mass transfer equipment is designed to maximise interfacial area and mixing. The mechanism of mass transfer close to the interface is of particular interest, and a number of models have been developed which describe the passage of the solute up to and across the interface. The basis for these models was described previously in Section 3.3.3 where solutions of Fick's law were presented for a thin film and a semi-infinite medium.

3.3.4.1 Two – Film Theory

The earliest of these theories mentioned above is the Two-Film Theory of Whitman (1923), and this theory will be used later to model mass transfer of a solute in the modified Lewis Cell, (Chapter 7 and 8). Two-film theory assumes that on either side of the interface there are stagnant films, similar to the film encountered in the film model (Section 3.3.3.1), where turbulence dies out at the edge of the interfacial film. The flux in each of the films may be described by an expression similar to Equation 3-5. As the solute does not accumulate at the interface then the flux in each film will be equal,

$$J_A = k_1\left(C_{A,b1} - C_{A,i1}\right) = k_2\left(C_{A,i2} - C_{A,b2}\right) \qquad (3\text{-}19)$$

Where k_1 and k_2 are the film mass transfer coefficients for phases 1 and 2, respectively. The interfacial concentrations of the solute ($C_{A,i1}$ and $C_{A,i2}$) are assumed to be in equilibrium and this can be described by a suitable expression for the system. The choice of expression is dependent on the method of extraction, e.g. by physical partitioning or chemical reaction.

The concentrations at the interface are generally not known, and the mass transfer coefficient is considered for the overall process, where the concentration on one side of the interface is assumed to be in equilibrium with bulk concentration of the other phase,

$$J_A = K_1\left(C_{A,b1} - C_{A,e2}\right) = K_2\left(C_{A,e1} - C_{A,b2}\right) \qquad (3\text{-}20)$$

This form of the flux equation gives rise to an overall mass transfer coefficient which is a function of the two film mass transfer coefficients, they can be shown to be related according to,

$$\frac{1}{K_1} = \frac{1}{k_1} + \frac{1}{K_P\, k_2} \qquad (3\text{-}21)$$

Similar to before (Equation 3-6), the overall mass transfer coefficient will be the reciprocal of the overall resistance to mass transfer which can be related to the resistance in each film,

$$R_{Overall} = R_1 + \frac{R_2}{K_P} \tag{3-22}$$

3.3.4.2 Other Theories of Mass Transfer

The two film theory assumes that turbulent eddies present in the bulk solution do not extend into the stagnant film. Therefore, the mass transfer coefficient, k, is expressed as the ratio of the diffusion coefficient, D, to the thickness of the stagnant film, δ (see Equation 3-10). In practice the bulk turbulence is likely to assist transfer through this hypothetically stagnant film, thus k will vary according to some power, n, of D (i.e. $k \propto D^n$, $0<n<1$). To account for this a number of other theories have been developed to describe the interaction of turbulence with mass transfer.

The Penetration Theory by Higbie (1935) describes mass transfer as an unsteady state process in which fluid eddies from the bulk travel by convection to the interface where they remain for an equal but finite period of time. During the exposure time solute transfer occurs by molecular diffusion until the eddy is replaced by a fresh element of fluid from the bulk. The physical situation of the diffusion process described by this theory is the same as solute transport in a semi-infinite medium where the mass transfer coefficient, k, is proportional to the square root of the diffusion coefficient, D (Section 3.3.3.2).

The Surface Renewal Theory by Danckwerts (1951) proposed that bulk turbulence extends up to the interface where it is continually replenished by fresh eddies of constant bulk concentration. Each eddy can have an interfacial lifetime from zero to infinity before being swept back into the bulk. Similarly to the Penetration Theory, the mass transfer coefficient, k, is proportional to the square root of the diffusion coefficient, D.

The Film Penetration Theory of Toor and Marchello (1958) combines the above three theories and describes a situation where the resistance to mass transfer is confined to the interfacial film, but the film is now composed of fluid elements which are of finite thickness and experience continual renewal. Solute transfer through the fluid elements is achieved by steady and unsteady state diffusion.

3.4 Adsorption at Interfaces

3.4.1 Introduction

Surface active compounds have the potential to adsorb to liquid-liquid interfaces where they will influence both the interface itself and the processes in which the interface may be involved in, e.g. mass transfer. In Section 3.4.2 the thermodynamics of adsorption is briefly discussed. This is then followed, in Section 3.4.3, by details of the way in which surface active compounds behave in solution, and finally an insight into the current understanding of the dynamic adsorption process is given in Section 3.4.4.

3.4.2 Adsorption to Interfaces

Properties of matter confined to phase boundaries are often profoundly different from those of the bulk. The fractional contribution of this matter at interfaces to the overall properties is so small that it is often neglected. However, there are times when the interfacial properties are significant, one such case being in colloids and emulsions where the surface to volume ratio is large. A characteristic of surfactants is that they will adsorb at interfaces in an oriented fashion. The surface activity depends on a number of factors including the structure of surfactant, the solvent type, and the temperature.

When adsorption occurs at the interface the interfacial tension will change, usually it decreases. This lowering of the interfacial tension will continue if more adsorbate is added to the bulk solution, because equilibrium exists between the adsorbate in the bulk and that at the interface. Initially the surfactant molecule may lie flat on the interface, its orientation will be governed by interactions with the phases either side of the

interface. As more adsorbate is added to the bulk solution the molecules at the interface will reorientate themselves to allow more molecules to adsorb. Adsorption to the interface will continue until the interface is saturated and a monolayer of adsorbate molecules is formed (Rosen, 1978).

The science of surface chemistry is particularly concerned with assessing the extent of this adsorption. Gibbs (1928) described the interface using thermodynamics to obtain a relationship between the surface excess concentration, the interfacial tension and the activity of the adsorbate in the bulk solution, as shown in the following equation,

$$d\gamma = -\sum \Gamma_i \, d\mu_i \qquad (3\text{-}23)$$

In the bulk phase a certain quantity of solvent will be associated with a certain concentration of adsorbate, however, due to positive adsorption at the interface the same quantity of solvent will be associated with a higher concentration of adsorbate. The difference between these two adsorbate concentrations is the surface excess concentration (Γ). The position of the interface is chosen so the surface excess concentration of the solvent is zero, this will occur at a plane parallel to the interface shown in Figure 3-1. For a two component system (comprising the solvent A and the adsorbate B), the Gibbs equation would then reduce to an expression for only the surface excess concentration of the adsorbate.

$$d\gamma = -\Gamma_B \, d\mu_B \qquad (3\text{-}24)$$

For dilute systems and by relating the chemical potential of the solute to the relative activity the excess concentration can be related to the bulk concentration,

$$\Gamma_B = -\frac{C_B \, d\gamma}{R\,T\,dC_B} \qquad (3\text{-}25)$$

The Gibbs equation in this form could be applied to a solution of non-ionic surfactant. For a solution of an ionic surfactant, in the absence of any other electrolytes, the

equation should be modified to allow for the fact that both anions and cations of the surfactant will adsorb at the interface in order to maintain electrical neutrality.

Direct determination of the amount of adsorbed surfactant at the liquid-liquid interface is not generally done due to difficulties in isolating the interfacial region from the bulk. Instead the amount of material adsorbed per unit area of interface is calculated indirectly from interfacial tension measurements. A plot of interfacial tension as a function of equilibrium concentration of the surfactant in one of the phases is generally used to describe adsorption (similar to that shown in Figure 3-3).

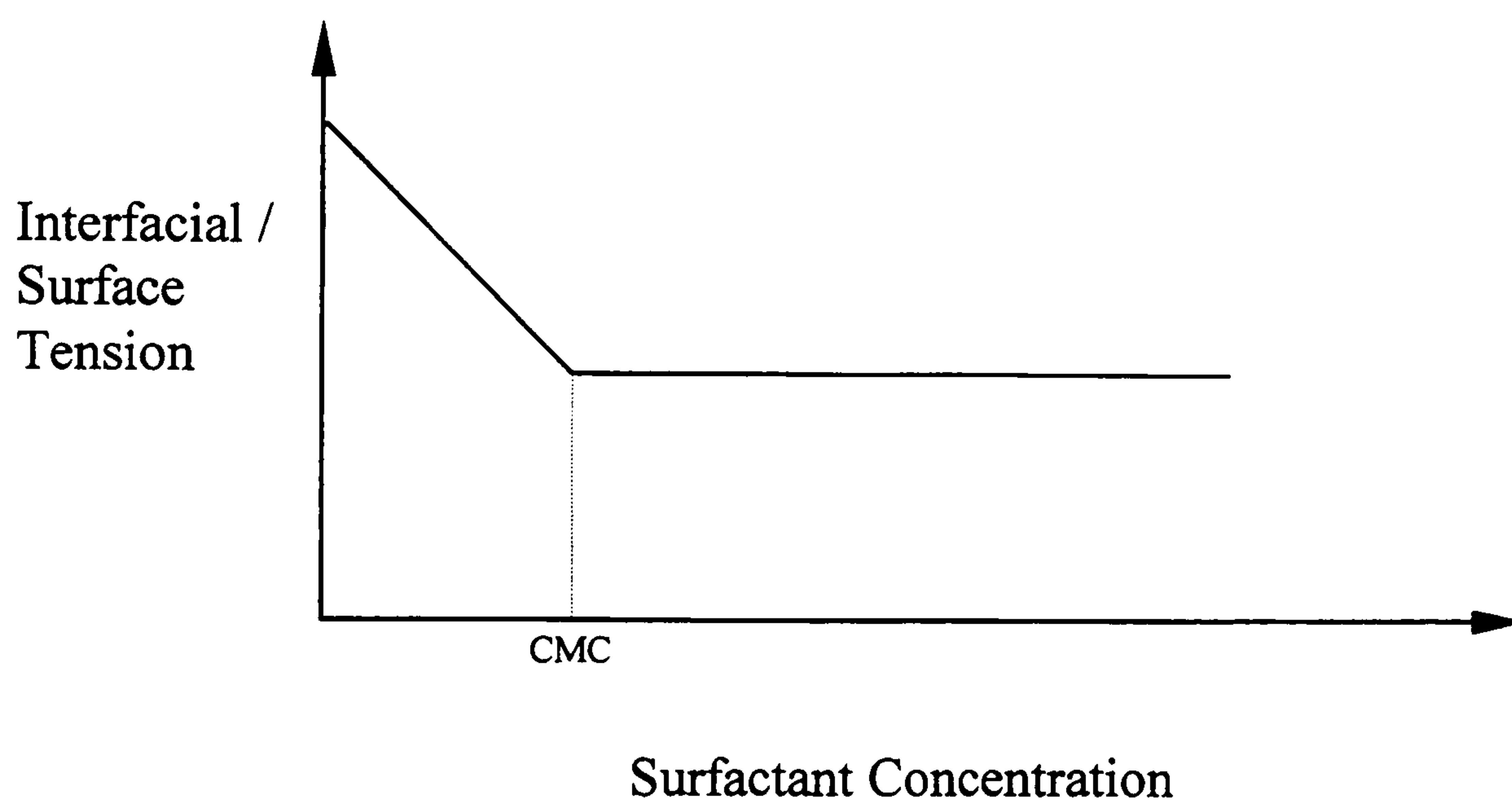


Figure 3-3 Effect of increasing surfactant concentration on interfacial/surface tension (CMC = critical micelle concentration)

3.4.3 Surfactant Behaviour in Solution

Solutions of highly surface active materials exhibit unusual physical properties. In dilute solutions surfactants act as a normal solute, at fairly well defined concentrations abrupt changes in several physical properties take place; the rate at which osmotic pressure increases with concentration becomes abnormally low, the rate of increase of turbidity with concentration becomes much enhanced. This suggests that considerable association is taking place, but the conductance of ionic surfactant solutions remains relatively high suggesting that dissociation is still occurring (Rosen, 1978).

This anomalous behaviour can be explained in terms of organised aggregates, or micelles, of surfactant in which the lipophilic hydrocarbon chains are oriented towards the interior of the micelle, leaving the hydrophilic groups in contact with the aqueous medium. The concentration above which micelle formation becomes appreciable is termed the critical micelle concentration (CMC). Surface/interfacial tension will be reduced by the addition of surfactants but only up to the CMC (Figure 3-3). Beyond this there is an additional mechanism for keeping hydrocarbon chains away from the water surface, i.e. by locating them in the middle of micelles. Since the micelles are not surface active the interfacial tension remains roughly constant beyond the CMC.

Micellar theory is still in many respects open to debate. Possible micelle structures include spherical, laminar and cylindrical arrangements, typically they tend to be approximately spherical over a wide range of concentrations above the CMC. At higher concentrations there are marked transitions to larger non-spherical liquid crystal structures. Surfactant solutions above the CMC can solubilise otherwise insoluble organic material by incorporating it in to the interior of the micelle. The balance of electrostatic and hydrophobic interaction can be such as to cause the locus of solubilisation to be anywhere in the micelle from close to the surface to the inner core.

3.4.4 Dynamic Adsorption

Upon forming a new interface the mechanical and thermal properties of the interface will almost immediately come to equilibrium and there will be an interfacial tension. All this will occur before any adsorption has occurred and the value of the interfacial tension is at a maximum. Adsorption at interfaces is a slow process and after a period of time the interfacial tension will decrease to the equilibrium value due to the adsorption of surfactants. The Gibbs adsorption equation presented in Section 3.4.2 (Equation 3-23) considered only the equilibrium state of adsorption, the actual process of reaching this state can take from milliseconds to many hours depending on the nature of the surfactant and the solvent.

The dynamic process of adsorption will consist of diffusion of the surfactant to the interface where it will readily adsorb and desorb to the interface. A fundamental

relationship that is often used to describe the adsorption process is the model of Ward and Tordai (1946).

$$\Gamma = 2\sqrt{\frac{D}{\pi}}\left[C_b\sqrt{t} - \int_0^{\sqrt{t}}(C_{s,t} - C_{s,\tau})d\sqrt{\tau}\right] \quad (3\text{-}26)$$

The first term inside the square bracket accounts for adsorption and the second term accounts for desorption. At equilibrium the rates of adsorption and desorption will be equal, and Γ will reach an equilibrium value. In this model diffusion of the surfactant is assumed to control the adsorption process, with adsorption and desorption at the interface occurring relatively quickly. However, consistently reported deviations of the model from experimental results has lead to hypotheses that the adsorption-desorption process and events occurring at the interface may also be rate limiting. Ideas presented include the existence of an energy barrier or that molecules reorientate themselves at the interface, however much debate still exists on the rate controlling mechanisms during dynamic adsorption (Rosen, 1978).

3.5 Interfacial Convection

3.5.1 Introduction

In certain liquid-liquid systems higher than expected rates of mass transfer have been recorded, and these increased rates are due to convection currents very close to the interface that aid mass transfer through a surface renewal mechanism. This section is concerned with examining the occurrence of interfacial convection in liquid systems. In Section 3.5.2 a description of Marangoni phenomena is presented, and the basis for predicting the occurrence is discussed. Marangoni convection arises due to changes in the interfacial tension, however, interfacial convection may also be initiated by density differences, and this is briefly discussed in Section 3.5.3

3.5.2 Marangoni Phenomena

Marangoni phenomena is the name given to spontaneous interfacial flows driven by interfacial tension gradients. These gradients may be initiated by localised changes in variables that affect the value of the interfacial tension, such as solute concentration, temperature, or the interfacial electrical potential. Interfacial convection generated by this movement provides an additional component to the interfacial flux that is not included in the general theories of mass transfer. Additionally the interfacial movement may alter the hydrodynamics of fluid flow close to the interface which affects drop coalescence, jet break up, and drop drag coefficients, thus affecting the performance of contacting equipment.

In multicomponent systems changes in interfacial tension are primarily due to changes in solute concentration and temperature, and to a lesser extent to the interfacial electrical potential. Prior to the onset of interfacial convection the interfacial tension would be uniform across the surface. A mechanical vibration, or similar, may cause an element of fluid from the bulk to reach the interface where it can cause a localised change in the interfacial tension due to a change in the solute concentration or temperature.

The appearance of Marangoni convection may be envisaged by considering a two-phase system where mass transfer occurs and the interfacial tension decreases with solute concentration. If a bulk element reaches a point on the two-phase interface of this system then the increase in solute concentration will cause a positive interfacial tension gradient and the interface will expand (flow) away from this point, as illustrated in Figure 3-4a. The interfacial flow will cause liquid to be drawn towards the interface from the bulk of the phase, this will occur on both sides of the interface. As the interfacial flow drags the adjacent fluid layers, mass transfer across the interface will occur, and the extent of mass transfer will dictate whether the interfacial movement is sustained. If the original interfacial tension gradient is maintained the interface will continue to flow. Fresh liquid will continue to be drawn towards the interface, and circulation patterns (or roll cells) will occur as liquid layers adjacent to the interface are dragged with the interface, as shown in Figure 3-4b. The sustained interfacial convection will enhance the flux of solute across the interface, and the system is said to

have stationary instabilities. If, however, mass transfer causes the direction of the interfacial tension gradient to be reversed then the initial disturbance will be damped out and the system will return to stability. Or if the gradient is sufficiently large then the interface will flow in the opposite direction to the original flow and the system is then said to have oscillatory instabilities (Perez de Ortiz (1992).

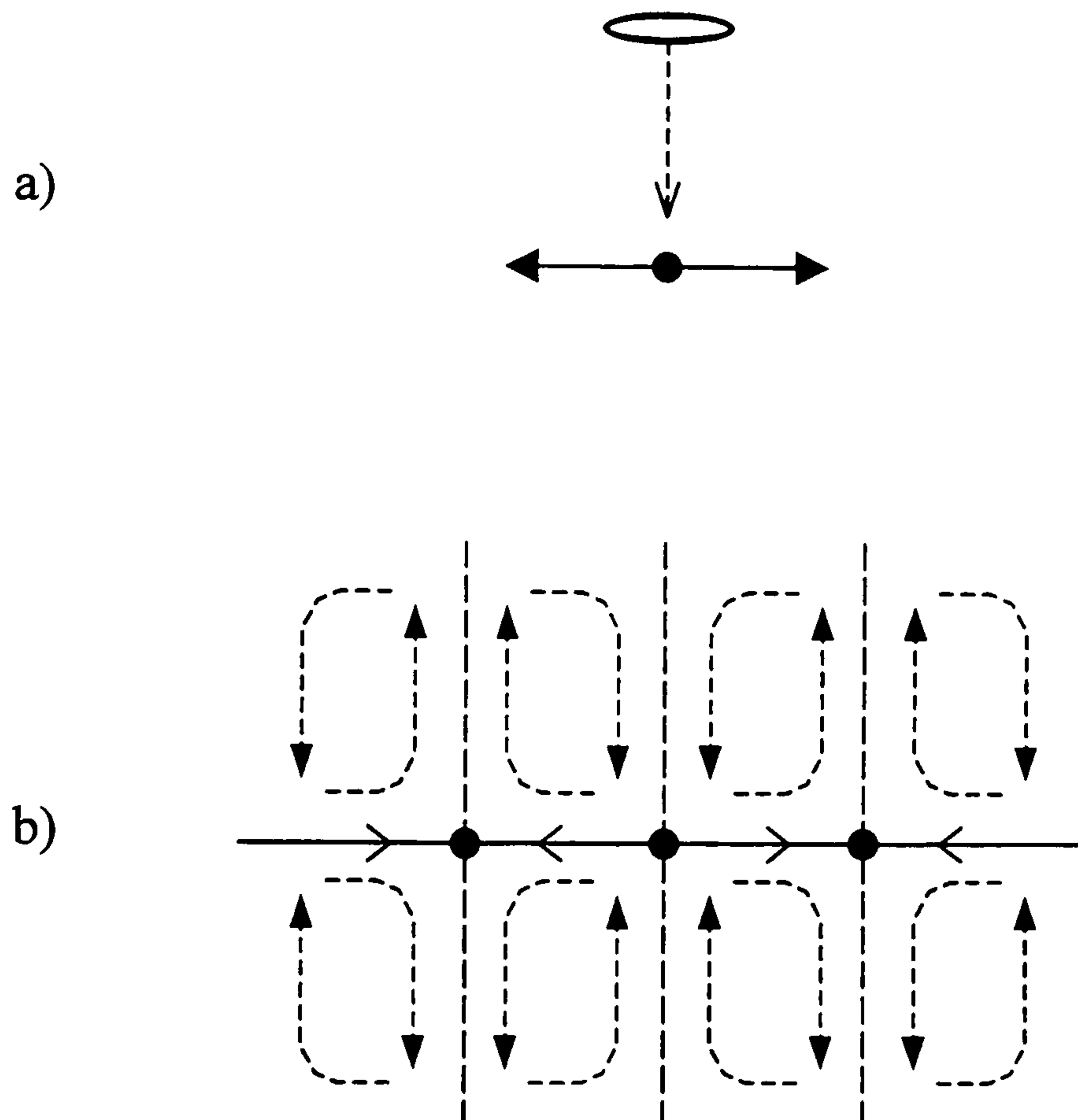


Figure 3-4 The effect of the interfacial tension gradient on flow at the interface for a) a single element reaching the interface and b) sustained interfacial convection.

Based on its origins and the relative depth of penetration to the bulk liquid phase, interfacial convection can be classified into three cases (Perez de Ortiz,1992):

- ◊ Marangoni instabilities.
- ◊ Marangoni disturbances.
- ◊ Thin film phenomena.

The first two cases occur when the depth of the penetration is small compared to the bulk liquid depth. Marangoni instabilities arise due to molecular diffusion, and Marangoni disturbances arise from surface renewal caused by external convection in systems that would otherwise be stable. In thin films, such as during drop coalescence, the depth of the interfacial flow is sufficient to change or break the flow between two drops, resulting in increased rates of drop coalescence. The presence of Marangoni instabilities/disturbances may give rise to interfacial convection that will increase interfacial mass transfer fluxes, however, thin film phenomena can cause drop coalescence and reduce the specific interfacial area. Thus the presence of Marangoni phenomena in liquid-liquid extraction can have important implications for contactor design and performance.

Adsorption of surfactants at the interface can affect the phenomena in two ways. Surfactants can lower the interfacial tension to a level below that which a localised perturbation in the solute concentration could cause, thereby eliminating the possibility of Marangoni convection. However, if Marangoni convection does occur the presence of surfactants may alter the rate of mass transfer, or the fluid flow properties causing a previously stable system to become unstable (Perez de Ortiz, 1992). At present there is no theory that can be used to predict the effect of interfacial convection on mass transfer rates. In order to allow such predictions it is necessary to identify the physical conditions that give rise to Marangoni instabilities during solute mass transfer. The onset of interfacial flows requires that localised changes in interfacial tension can occur, however, the development of this initial flow into sustained convection depends on other conditions. Two physical properties that play an important role in sustaining interfacial convection are the solute diffusivity and the kinematic viscosity in both phases.

Sternling and Scriven (1959) developed stability criteria based on a model mass transfer system. Using linear stability analysis the effect of small changes in the physical variables that describe the flow was investigated, and criteria for the onset of instabilities were defined as:

- ◊ Solute transfer out of the phase of higher viscosity.
- ◊ Solute transfer out of the phase in which the diffusion coefficient is lower.
- ◊ Large differences in viscosity and diffusivity between the phases.
- ◊ Steep concentration gradients near the interface.
- ◊ Interfacial tension sensitive to solute concentration.
- ◊ Low viscosity and diffusivity in both phases.
- ◊ The absence of surface-active agents.

The intensity of interfacial convection depends strongly on the magnitude of the interfacial tension gradient and the relative rates of mass and momentum transfer in the two phases. A notable feature of the stability analysis conducted to date is the discrepancies with experimental results; to allow stability criteria to lead to a description of mass transfer the reliability of predictions must be improved. Analysis is required that will reflect the varying intensity of Marangoni convection and allow the interfacial flux of solute to be predicted. Further information on interfacial stability criteria can be found in the review by Perez de Ortiz (1992), and the recent study of Agble and Mendes-Tatsis (2000).

3.5.3 Gravitational Convection

Interfacial flows driven by interfacial tension gradients are not the only cause of interfacial convection. The effect of the solute on the density gradient close to the interface may also cause interfacial convection. If solute transfer causes a negative density gradient (i.e. a fluid layer becomes heavier than that below it) then gravity will cause the fluid layers to displace one another. The density gradient may be caused by the movement of the solute, the contraction of volume due to mixing, or the heat of solution. In situations where interfacial convection is present it is possible that both the Marangoni effect and gravity contribute to the enhanced mass transfer rates, with a greater contribution due to gravitational convection (Sawistowski, 1971).

Chapter 4

Liquid-Liquid Extraction

4.1	Introduction	80
4.2	Solute Partitioning	81
4.3	Enhancing Partitioning	83
	4.3.1 Solute Dissociation	83
	4.3.2 Ion Pair Extraction	84
4.4	Solvent Selection	85

4.1 Introduction

Liquid -liquid extraction processes are used primarily to separate similar components in a liquid mixture, and to concentrate the desired compound in the receiving phase. The separation is achieved through differences in the equilibrium partitioning of components with a second liquid phase. In its most basic form the process would consist of the following steps:

- ◊ The two liquid phases (usually an aqueous feed solution and an organic receiving solution) are brought into contact.
- ◊ Mass transfer of compounds into the solvent phase occurs until the equilibrium partition conditions are attained.
- ◊ The two phases are then separated, and then further purification and concentration steps may be employed.

In practice, one phase is dispersed into the other phase as droplets to give the largest possible interfacial area for mass transfer, where the dispersed phase can be either of the phases. In biotechnology the first stage in most liquid-liquid extraction processes is to remove the residual micro-organisms by filtration. Commonly the desired compound is produced extracellularly by the micro-organism and released into the growth medium. However, the desired compound may be retained within the cell, and in such cases the cell would have to be disrupted to release the product into the aqueous medium.

In this Chapter chemical processes associated with liquid-liquid extraction are described. In Section 4.2 the equilibrium partition coefficient is defined and a thermodynamic description of partitioning is given. In Section 4.3 two methods that can be used to enhance the partitioning behaviour of a solute are described. These two methods will be used later in the experimental study of liquid-liquid extraction. Finally in Section 4.4 factors that influence the selection of the extracting solvent are discussed.

4.2 Solute Partitioning

The separation of a solute between the two liquid phases involved in the extraction process is described by the partition coefficient, K_P. The partition coefficient is the ratio of the solute concentration in the receiving (solvent) phase to that in the feed (aqueous) phase at equilibrium (Hariri, 1989),

$$K_P = \frac{C_{Sol}}{C_{Aq}} \tag{4-1}$$

The partition coefficient will ideally be constant and independent of the solute concentration. Often this is not the case due to thermodynamic non-idealities of the phases involved, and the partition coefficient will vary with solute concentration. However, in biochemical processes the concentration of the desired solute is usually quite low, and the partition coefficient often remains effectively constant over the concentration ranges encountered. The value of the partition coefficient should be determined experimentally, and details of how this has been done for this study are given in Section 6.2. The partitioning of a solute may also be described by considering

the thermodynamic properties of the two-phase system at equilibrium, this description allows an estimation of partitioning to be made. At equilibrium the chemical potential (μ) of the solute in each phase will be equal, as given by,

$$\mu_{Aq} = \mu_{Sol} \tag{4-2}$$

The chemical potential of the solute may be related to the chemical potential at standard reference states (μ^0) (Smith and Van Ness, 1987),

$$\mu^0_{Aq} + \mathrm{RT} \ln \mathrm{C_{Aq}} = \mu^0_{Sol} + \mathrm{RT} \ln \mathrm{C_{Sol}} \tag{4-3}$$

Using Equation 4-1, C_{Aq} and C_{Sol} may be expressed in terms of the partition coefficient, which can then be related to the solute's standard chemical potential by rearranging Equation 4-3,

$$\ln \mathrm{K_P} = \frac{\mu^0(\mathrm{Aq}) - \mu^0(\mathrm{Sol})}{\mathrm{RT}} \tag{4-4}$$

Equation 4-4 may be used to estimate the partition coefficient of a solute between an aqueous phase and a solvent phase if the standard chemical potential of the solute in those phases is known. Often this may not be possible and Equation 4-4 may be modified by relating the chemical potential to the solute's solubility in a given phase (Belter *et al.*, 1996),

$$\ln \mathrm{K_P} = \frac{\overline{V}_{\mathrm{Aq}}(\delta_\mathrm{i} - \delta_{\mathrm{Aq}})^2 - \overline{V}_{\mathrm{Sol}}(\delta_\mathrm{i} - \delta_{\mathrm{Sol}})^2}{\mathrm{R\,T}\overline{V}_\mathrm{i}} \tag{4-5}$$

where $\overline{V}$ is the partial molar volume, and δ is the solubility parameter for either the aqueous feed (Aq), the receiving solvent (Sol), or the solute (i). From this relationship it can be seen that changing the thermodynamic properties of the solvent will alter the partition coefficient. The value of the solubility parameter is available for a number of common solvents (Hildebrand *et al.*, 1970), and the solubility parameter for the solute can be determined by experiment with solvents of known properties. With knowledge

of the solubility parameter for the solute, estimates of the partition coefficient can be made for other solvent pairs where the solubility parameters are known. Partition coefficients estimated in this way are unlikely to be accurate, however, they will provide a qualitative guide to an experimental investigation.

4.3 Enhancing the Partition Coefficient

In many cases a solute will not be thermodynamically compatible with any solvent suitable for a liquid-liquid extraction process, i.e. the partition coefficient will be too low (or non-existent) for the process to be a viable option. In such cases it may still be possible to use a liquid-liquid extraction process by altering the chemical structure of the compound to make it more soluble in the receiving phase and thus enhance the partition coefficient. This can be achieved in two ways,

◊ If the solute is a weak acid or base, a change in the pH of the solution will produce a change in the degree of dissociation and allow the compound to exist in a form that is soluble in the solvent.

◊ If the solute is ionic it may be associated with a counter ion that will make it more soluble in the solvent.

In both these methods the alteration of the structure is not permanent and the compound may be returned to its original state. These two methods will be discussed in the following two sections.

4.3.1 Solute Dissociation

Many solutes produced by biochemical pathways will be either weak acids or bases, and in aqueous solutions these compounds will be partially ionised. At the intermediate pH values required for cell culture (pH 5-8) these compounds will exist predominately in a dissociated form. However, for a compound to dissolve in an organic solvent it will need to be undissociated. Using the dissociative properties of weak acids and bases a change in the pH of the aqueous solution will alter the extent of ionisation; this can be described according to the Henderson-Hasselbalch equation.

$$pH = pK_a + \log\frac{[A^-]}{[HA]} \tag{4-6}$$

where, pK_a is the dissociation constant for the weak acid, and HA and A^- are undissociated and dissociated forms of the acid, respectively. If the solution pH is below the pK_a of the acid then more acid will exist in the undissociated form. In the undissociated form the acid may dissolve and be extracted by the solvent. When considering the total acid concentration, decreasing the extent of dissociation will cause an increase in the apparent partition coefficient because more acid will be available to partition into the organic solvent phase.

4.3.2 Ion Pair Extraction

If a solute has ionic characteristics it will ionise, either partially or completely, in an aqueous solution. However, as mentioned above, for a compound to exist in an organic solvent phase it will have to form an ion pair with no net charge. In some instances the combined ion pair may still have a low solubility in the organic solvent phase, and thus the equilibrium partitioning between two phases will be low. To enhance extraction the counter ion of the solute may be changed to make the ion pair more soluble. In this way extraction will be enhanced without changing the solute itself. An example of this method is the extraction of tetrabutylammonium chloride by chloroform (Belter *et al.*, 1996). The partition coefficient for tetrabutylammonium is 13, however, when sodium acetate is added to the aqueous phase, the partition coefficient for tetrabutylammonium is 132. The increase in the partition coefficient occurs because the tetrabutylammonium cation is extracted with the acetate anion instead of the chloride anion. The acetate anion makes the tetrabutylammonium cation more soluble in chloroform because it is less polar than the chloride anion.

4.4 Solvent Selection

The choice of the solvent is a basic consideration for any liquid-liquid extraction process. Previously (Section 4-2) it has been described how the solvent can influence the partition coefficient for the extraction system. Obviously it is desirable to have a high partition coefficient so that the solute is concentrated in the receiving phase; however, many other factors will influence the choice of solvent to allow the overall process to be viable. In biotechnology the choice of solvent for the liquid-liquid system is often influenced by interactions that the solvent may have with the micro-organism or the solute. In processes where the continuous removal of the product or of a growth inhibitor is required then contacting directly and saturating the growth medium with an organic solvent may cause cell lysis or denaturation of valuable proteins. The effect of a number of organic solvents on microbial activity was assessed by Laane *et al.* (1985), and the results were correlated in terms of the Hansch parameter for solvent polarity (log P). It was found that solvents with a log P value below 2 were unsuitable for use with micro-organisms; these are the most polar solvents, and hence, they will displace the essential water layer surrounding the cell. Where the value of log P was between 2 and 4 there was variable biological activity, and above 4 the activity was generally high. Examples of common solvents with log P values above 4 include alcohols higher than decanol and alkanes higher than heptane.

In addition to issues of biocompatibility the choice of the solvent will also be influenced by a number of other issues:

◊ ***Physical Properties.*** The physical properties of the chosen solvent will affect many aspects of the overall separation process. Viscosity will influence the energy requirements of pumping and mixing, and together with density, the phase separation characteristics of the two-phase system.

◊ ***Two-Phase Properties.*** The properties of the combined phases will also affect the separation process. Interfacial tension will effect phase separation through emulsion formation, and the miscibility of the two phases will influence solvent losses and phase volume requirements.

◊ ***Solute Recovery.*** The solute should be easily recovered from the solvent to allow it to be further purified and the solvent recycled.

◊ ***Toxicity.*** Over time, quantities of the solvent will be discharged into the environment along with spent feed solutions; the effect that such discharges on the surrounding environment should be considered and minimised.

◊ ***Economics.*** Although the solvent should be recovered and reused in the process, solvent losses will still occur. If the solvent is expensive this may have a detrimental effect on the process economics.

Chapter 5

Experimental Liquid-Liquid Extraction Systems

5.1	Introduction	88
5.2	Experimental Extraction Methods	88
5.3	System Selection	90
	5.3.1 Criteria for Solute Selection	91
	5.3.2 Physical Extraction	92
	5.3.3 Reactive Extraction	93
5.4	Physical Extraction System	94
5.5	Reactive Extraction System	95
5.6	Fermentation of Solutes	98
	5.6.1 Chloramphenicol Production	99
	5.6.2 Phenylalanine Production	100
5.7	Overview of Experimental Study	101

5.1 Introduction

In this chapter the specific details of the extraction systems used in this study will be presented. Previously (Chapter 4) the chemical basis of liquid-liquid extraction was presented, together with methods available to enhance it. In this study two different liquid-liquid extraction processes were used, and in Section 5.2 a general outline of each extraction method is presented and the reasoning behind the use of each is detailed. Before giving the technical details of these two processes the criteria that formed the basis for the selection of the solute and solvent systems are presented in Section 5.3. In Sections 5.4 and 5.5 the technical aspects of each extraction process are detailed, for the reactive extraction system further constants are defined which describe the extraction process. In this work the effect of fermentation broth constituents on the extraction process has been examined, and for both of the extractions the solutes have been produced by fermentation. In Section 5.6 details of the bacterium, culture conditions, and media formulations used to produce the solutes by fermentation are presented. Finally in Section 5.7 an overview of the experimental work conducted using these two extraction processes is given.

5.2 Experimental Extraction Processes

In this study the effect of fermentation broth components on two different liquid-liquid extraction processes was examined. These two processes, which will be called physical extraction and reactive extraction, encompass the methods previously described in Sections 4.2 and 4.3. Physical extraction has been used to recover a non–polar solute that will partition into a non-polar solvent, and reactive extraction has been used to recover a polar solute that does not readily partition into a non-polar solvent.

Physical extraction is the name that will be used to describe an extraction process that is based purely on the solute's solubility in the solvent phase. In this extraction process no other compounds are added to the system to improve the solute partitioning, i.e. no alteration of the solution pH is required or any counter ions added to enhance the solute solubility in the solvent phase. The solute will be extracted into the solvent phase and the extent of extraction will be governed by the equilibrium partition coefficient.

Reactive extraction is the name that will be used to describe an extraction process where the solute is altered to improve its solubility in the solvent phase. In this case the aqueous phase pH will be altered and also an added counter ion will be used to enhance the partitioning into the solvent phase. In contrast to the method described in Section 4.3.1, the solution pH is altered so that the solute will exist in its dissociated state, and due to this dissociation the solute will then not be in a form that will allow it to partition into the solvent. To achieve extraction an ion pair method is then employed. However, again in contrast to the method described in Section 4.3.2 the counter ion will be present in the solvent phase (not the aqueous phase as in Section 4.3.2). An ion exchange reaction will occur at the two-phase interface between the dissociated solute (X^-) and the solvent phase ion exchange reagent (Q^+Cl^-), which is shown schematically in Figure 5-1. The counter ion of the ion exchange reagent (Cl^-) is replaced by the solute and released into the aqueous phase, and this allows the solute to combine with the ion exchange reagent and dissolve in the solvent phase.

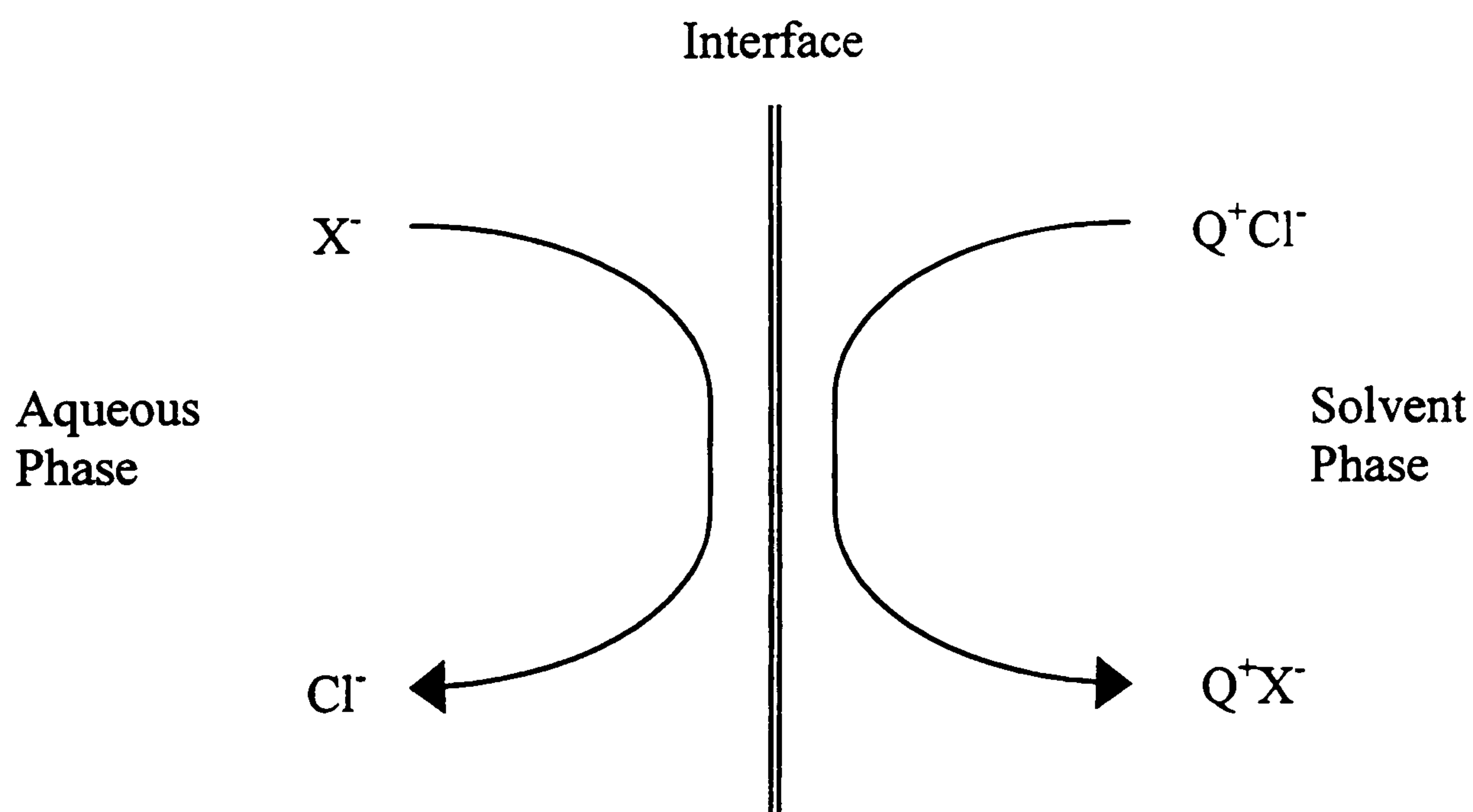


Figure 5-1 Schematic of the interfacial ion exchange reaction occurring during reactive extraction; where X^- is the dissociated solute, Q^+ the ion exchange reagent, and Cl^- the counter ion of the ion exchange reagent.

Two different types of extraction processes have been investigated because of the increasing use of novel techniques to achieve extraction and recovery of novel compounds. Through the first process, physical extraction, the effect of fermentation broth constituents on a simple extraction mechanism will be investigated, and in this process fermentation broth constituents will primarily affect the extraction by altering the mass transfer process occurring at the interface. It is hypothesised that through this simple system the effects of surface-active compounds present in the fermentation broth, and others that may be added, will be clearly identifiable without having to take account of any additional factors associated with the enhancement techniques described in Section 4.3. In the second process, reactive extraction, compounds present in the fermentation broth may also effect the mass transfer characteristics of the system, however, they may also affect the extraction reaction mechanism and alter the equilibrium conditions that are generally believed to prevail at the interface (Section 3.3.4). Therefore the reactive extraction process will be used to study the effects of fermentation broth components, and added compounds, on the extraction reaction mechanism.

5.3 System Selection

During the formulation of the experimental liquid-liquid extraction systems a number of criteria were defined for choosing a suitable solute and solvent for each extraction process. The criteria were developed based on either practical aspects relevant to a laboratory based experimental study, or on generic aspects relevant to industrial liquid-liquid extraction processes. The criteria for the selection of a suitable solute are given in Section 5.3.1, and the criteria for the selection of a suitable solvent have been given previously in Section 4.4 for industrial processes, and similar arguments are applied here for a laboratory study. In Section 5.3.2 a brief summary of the selection process for the physical extraction system is given where a number of possible solutes were assessed, and in Section 5.3.3 a brief summary of the selection of the reactive extraction system is given.

5.3.1 Solute Selection Criteria

The criteria considered in choosing a solute suitable for this experimental investigation are given below.

◊ ***Analysis:*** An accurate and routine method of analysis is required, ideally this would be based on UV absorbency, and be capable of analysis when using complex solutions such as fermentation broth.

◊ ***Solubility:*** The compound should have appreciable solubility in the aqueous phase to allow there to be a sufficiently large concentration difference between the two phases. This will allow shorter experiments as the time required to achieve a measurable concentration change will be shorter. Also the compound should be quick dissolving in the aqueous phase. This will allow fresh solutions to be prepared immediately before an experiment, thus avoiding any problems associated with the degradation of the compound in solution, e.g. penicillin in acidic solutions.

◊ ***Partitioning:*** The solute should partition well into the chosen solvent, this will allow the effects of a low partition coefficient to be de-coupled from effects on the mass transfer process.

◊ ***Production:*** The compound should be produced extracellularly by a micro-organism, since extraction of an intracellular compound would lead to a number of problems outside the scope of this investigation. The micro-organism should also be available from a UK culture collection, since this will avoid any problems with the importation of micro-organisms from a foreign country. The micro-organisms must also be compliant with the available laboratory facilities, which has a biohazard containment rating of 2.

◊ ***Cost:*** The pure form of the solute must be relatively inexpensive due to the large number of experiments that are anticipated where the non-fermentation system will be investigated. Also where the product yield from the fermentation is low the broth will be spiked so that all the mass transfer experiments are performed under similar conditions.

◊ ***Industry:*** The solute should be of relevance to a current industrial process.

5.3.2 Physical Extraction System

The number of compounds produced biochemically is extensive and initially solutes were chosen based on solubility, cost and availability of both the pure form and of a micro-organism capable of producing it. The studies of Weiss *et al.* (1957), and Andrew and Weiss (1959) on the solubility of antibiotics in water and various solvents were used as an initial guide in identifying a suitable compound.

In deciding upon a suitable compound for the study of physical extraction a number of compounds have been examined, and two of these and the reason behind their rejection are given below. The third compound detailed below was chosen for use in this experimental study as it fulfilled the selection criteria.

◊ ***Erythromycin:*** Previously used in similar studies (Lye and Stuckey, 2000; Habaki *et al.*, 1998, Kawasaki *et al.*, 1996) this compound was found to have a low solubility in the aqueous phase and was also slow to dissolve, taking 12-24 hours to completely dissolve. Furthermore, upon the addition of antifoaming agents to the aqueous phase the solute was observed to come out of solution. Analysis of the compound involved the use of a colourimetric assay that had a large experimental error; this meant results were often inconclusive. Successful work conducted during this study using erythromycin was presented at the International Solvent Extraction Conference (Pursell *et al.*, 1999), and is included in Appendix C.

◊ ***Novobiocin:*** This compound fulfilled many of the solute selection criteria. However, it would only partition appreciably with polar solvents. Therefore the

solvents were partially miscible with the aqueous phase, which caused considerable problems with phase volume changes and solvent losses. Due to these problems only a brief investigation was conducted before the compound was rejected.

◊ ***Chloramphenicol:*** This compound was chosen as a model solute for the experimental study as it adequately fulfilled the criteria described in Section 5.3.1. Background details of this compound and the extraction process are described in the remainder of this section.

Chloramphenicol is a broad-based antibiotic that has been used extensively since its discovery in the 1940's (Vining and Westlake, 1984). It is a neutral compound that is stable at neutral and acidic pH, and it may be produced via fermentation of the bacterium *Streptomyces venezuelae* that is available from the NCIMB in Aberdeen.

The solvent that was chosen to extract chloramphenicol from aqueous solutions was the alcohol 1-octanol. Octanol fulfilled may of the criteria outlined in Section 4.4, in particular that it has a low solubility in aqueous solutions, has a low toxicity and chloramphenicol partitions into it strongly. Its log P value is 3.1 which means that it may be possible to contact the solvent directly with the growth medium without significantly affecting the micro-organisms.

5.3.3 Reactive Extraction System

The choice of a suitable solute for an experimental study of reactive extraction was more complicated than the choice for physical extraction due to the complex nature of the required component interactions. Given the complexities involved in finding a suitable compound for use in the study of physical extraction it was decided to find an extraction system that had been previously defined in the literature. A suitable extraction system involving the recovery of phenylalanine, an amino acid, had been studied previously by a number of researchers, e.g. Haensal *et al.* (1986), Uddin *et al.* (1990), Chan and Wang (1993), Coelhoso *et al.* (1997), Scarpello and Stuckey (2000). Phenylalanine, is one of the 21 "essential" amino acids that form the basis for all protein structures. Where a particular species cannot internally synthesise the amino acid it is

essential that it be obtained externally through its diet. For this reason phenylalanine is produced industrially as an additive to some animal feeds. It is also used as an intermediate in the production of the artificial sweetener aspartame (Uddin *et al.*, 1992).

Phenylalanine is a weak acid that will not significantly partition into non-polar solvents; however, it may be extracted using a reactive extraction process. The extraction process involves the recovery of phenylalanine from an aqueous solution using the ion exchange reagent Aliquat 336, which will be present in the receiving/solvent phase as it is insoluble in aqueous solution. To allow phenylalanine to be extracted by a reactive extraction mechanism it must be in its dissociated form, this is achieved by raising the solution pH using sodium hydroxide. The ion exchange reagent, Aliquat 336, is a quaternary ammonium salt and is commonly known as trioctylmethylammonium chloride. It is a very viscous liquid, so dilution with a suitable solvent is required, 1-octanol was chosen for the purpose because phenylalanine does not partition into it (Scarpello, 1998), and it does not interact with the ion exchange reagent. It also fulfils the criteria for solvent selection (Section 4.4 and 5.3.2).

5.4 Physical Extraction

The antibiotic chloramphenicol (Sigma, M_{Wt} = 323.1) was used as the model solute to investigate the extraction of a non-polar solute through physical extraction. Its chemical structure is show in Figure 5-2. The solvent used was 1-octanol (99%, Aldrich, M_{Wt} = 130.29 g/mol, ρ = 0.799 g/ml), which may extract chloramphenicol from an aqueous phase without the addition of any further compounds, i.e. buffers or ion exchange reagents. The concentration range studied was from 0 to 1000 μM, which corresponds to typical productivities for chloramphenicol production by fermentation (Gottlieb and Diamond, 1951),

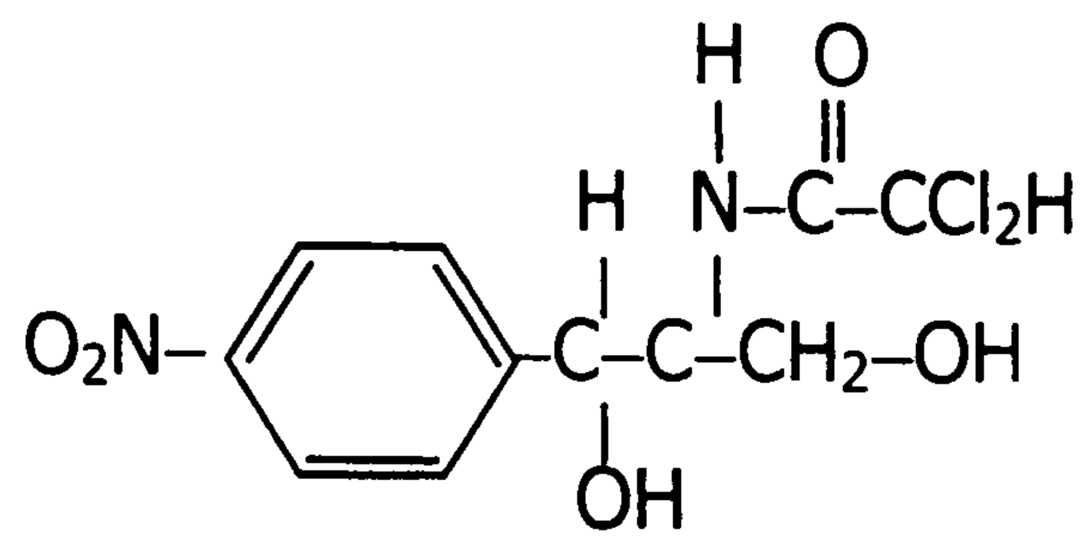
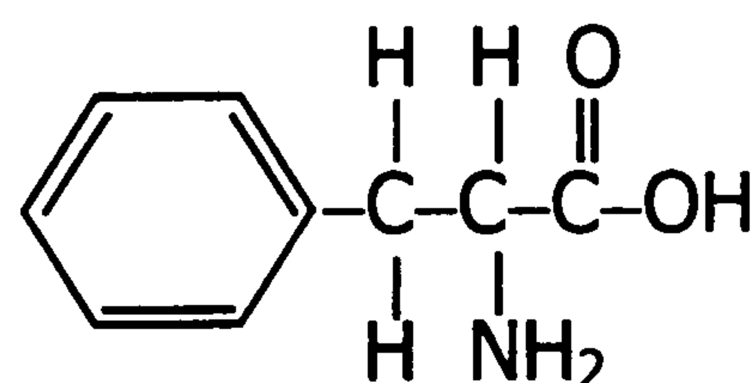


Figure 5-2 Chemical structure of chloramphenicol.

5.5 Reactive Extraction

The amino acid *l*-phenylalanine (>99%, Aldrich, M_{Wt} = 165.19 g/mol) was used as the model solute to investigate the extraction of a polar solute through reactive (ion exchange) extraction. The ion exchange reagent used was the quaternary ammonium salt Aliquat 336 (Industrial Grade, Aldrich, M_{Wt} = 404, ρ = 0.882 g/ml), which was dissolved in 1-octanol (99%, Aldrich, M_{Wt} = 130.29 g/mol, ρ = 0.799 g/ml). The chemical structures of phenylalanine and Aliquat 336 are shown in Figure 5-3.

(a)



(b)

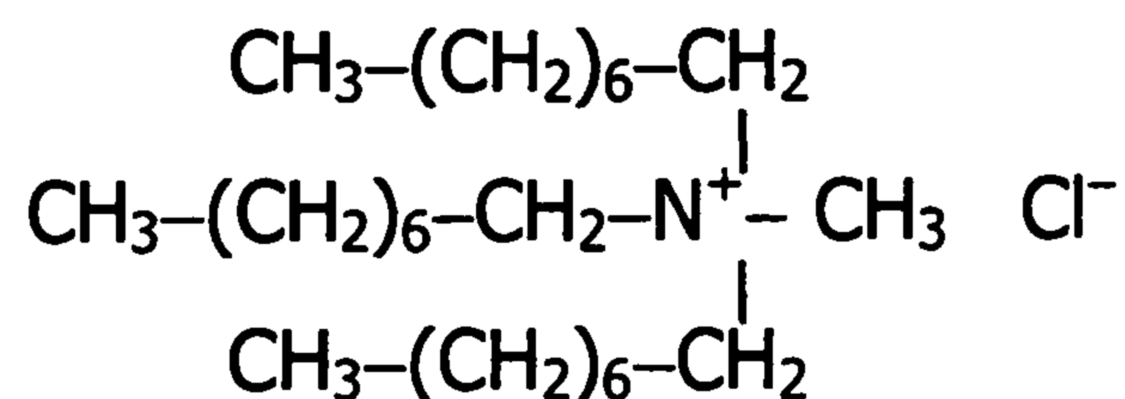


Figure 5-3 The chemical structure of (a) phenylalanine, and (b) Aliquat 336.

Sodium hydroxide (>98%, Sigma, M_{Wt} = 40 g/mol) was used to alter the aqueous phase pH to allow the ion exchange reaction to occur. The initial phenylalanine concentration used in this work was in the range 10 to 100mM, with an initial sodium hydroxide concentrations of 0 to 100mM

The ion exchange reaction between phenylalanine and Aliquat 336 occurs with a 1:1 stoichometry. The reaction takes place at the interface as the ion exchanger is initially present in the solvent phase and has a low solubility in the aqueous phase. The ion exchange reaction is described by the following reversible reaction:

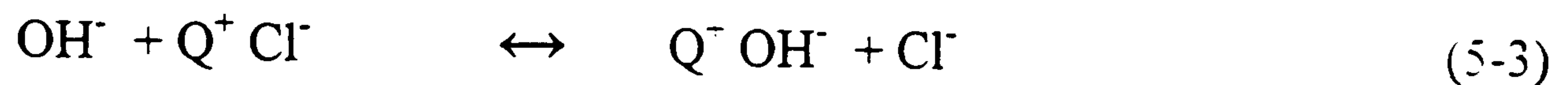
$$Phe^- + Q^+ Cl^- \leftrightarrow Q^+ Phe^- + Cl^- \qquad (5\text{-}1)$$

where Phe^- is the phenylalanine anion, and Q^+ and Cl^- are the cation and anion of Aliquat 336, respectively. At the interface the phenylalanine anion replaces the chloride anion associated with the long hydrocarbon cation chain of the ion exchange molecule. The chloride anion is released into the aqueous phase and the phenylalanine anion is extracted into the solvent phase.

To allow phenylalanine to take part in the reaction it must be in its dissociated form, and this may be achieved by altering the aqueous pH to above the dissociation constant for phenylalanine. Phenylalanine is zwitterionic with two dissociation constants, pK_{a1} = 1.93 and pK_{a2} = 9.27, therefore at pH values above 9.27 the anionic form of phenylalanine will predominate. The extent of this dissociation can be described by rearranging the Henderson-Hasselbalch equation,

$$\frac{[Phe^-]}{[Phe_{Total}]} = \frac{1}{1+10^{(pK_a - pH)}} \qquad (5\text{-}2)$$

Sodium hydroxide was used to alter the aqueous phase pH; and at a pH above 11.3 greater than 99% of the phenylalanine will exist in its anionic form. The hydroxyl anions present are also liable to extraction by the ion exchanger; the co-extraction reaction is described in a similar manner to the phenylalanine extraction reaction,



$$OH^- + Q^+ Cl^- \leftrightarrow Q^+ OH^- + Cl^- \qquad (5\text{-}3)$$

where OH^- represents the hydroxide anion.

The extraction reactions (Equations 5-1 and 5-3) are reversible reactions that will not go to completion. The extent of each reaction at equilibrium can be described using the Law of Mass Action. This describes an equilibrium constant for each reaction as the ratio of concentrations of the products to the reactants, for Equations 5-1 and 5-3 the equilibrium constant may be written as,

$$K^*_{Phe} = \frac{C_{QPhe,e} C_{Cl,e}}{C_{Phe,e} C_{QCl,e}} \qquad (5\text{-}4)$$

$$K^*_{OH} = \frac{C_{QOH,e} C_{Cl,e}}{C_{OH,e} C_{QCl,e}} \qquad (5\text{-}5)$$

where K^* is the equilibrium constant for the extraction of either phenylalanine (Phe) or hydroxide (OH), and the subscript e denotes concentrations at equilibrium.

The value of the equilibrium constant, K^*, may be determined from the gradient of a plot of the numerator of the quotient in Equation 5-4 or 5-5 against the denominator, resulting from variation in one of the reactants. Haensel *et al.* (1986) determined the equilibrium constant for phenylalanine extraction by Aliquat 366 assuming equimolar chloride to phenylalanine extraction. However, Scarpello and Stuckey (2000) showed that there was considerable co-extraction, and that ignoring the effect of co-extraction in the evaluation of the chloride concentration would result in the incorrect determination of the equilibrium constant. In their study Scarpello and Stuckey (2000) introduced the co-extraction constant, α, which describes the ratio of chloride evolution to phenylalanine depletion in the aqueous phase,

$$\alpha = \frac{\Delta C_{Cl,e}}{\Delta C_{Phe,e}} \qquad (5\text{-}6)$$

Values of the co-extraction constant between –1 and –2 indicate that more phenylalanine has been extracted over all other possible extractable solutes. Below –2 the amount of co-extracted material is greater than the amount of phenylalanine extracted. In their work, values of the co-extraction constant from –1.5 to –9.5 were reported as a result of changing initial phenylalanine concentration. In the study of Haensel *et al.* (1986) it was reported that the value of the equilibrium constant was affected by the type of aqueous phase buffering used, where a borate buffer was tested as well as using sodium hydroxide. However, the equilibrium constant should only vary with temperature, but not as a result of adding different compounds to the aqueous phase. The change in the equilibrium constant Haensel *et al.* (1986) observed would have been caused by the assumption that there was no co-extraction and so equal amounts of phenylalanine and chloride anions were exchanged. In fact phenylalanine extraction was affected by the co-extraction of another compound (in this case either the hydroxide or the borate anion).

The incorrect interpretation of results by Haensel *et al.* (1986) and the observed changes in the co-extraction constant made by Scarpello and Stuckey (2000) highlight the fact that the levels of extraction and co-extraction do vary and are dependant on the nature of the anion species present in solution. Therefore anionic compounds present in fermentation broths will affect the extraction reaction mechanism.

5.6 Fermentation of Solutes

Fermentations of chloramphenicol and phenylalanine producing bacteria were conducted in order to obtain fresh fermentation broth to allow an investigation of the effect of compounds and biomass produced during the fermentation process. Details of the bacterial strains, the media compositions and growth conditions used in this study are given in Section 5.6.1, and 5.6.2.

All media and growth flasks were sterilised prior to inoculation in an autoclave (Astell Hearson) at 121°C for 20 minutes. In some cases it was necessary to sterilise some media components separately. Glucose and phosphate buffer salts react to produce a compound toxic to bacteria, therefore, these compounds were always autoclaved

separately. Autoclaving magnesium sulphate and phosphate together salts gave rise to a precipitate; these compounds were also autoclaved in separate containers. After sterilisation the media components were then combined. This was done using sterile techniques i.e. within close proximity of a hot Bunsen burner flame, and all flask bottle necks were flamed. The presence of microbial contamination was visually assessed by growing the bacteria on nutrient agar plates, in all cases a monoculture was observed.

5.6.1 Chloramphenicol Production

Chloramphenicol was produced by fermentation with the bacteria *Streptomyces venezuelae* (NCIMB 8231). Batch fermentations where conducted only on a small scale (≈ 100ml) in 250ml conical shake flasks incubated in an orbital shaker (Gallenkamp), with temperature control, rotating at 150 rpm. The composition of media and the growth conditions were based on the work of Gottlieb and Diamond (1951); the growth conditions were; temperature = 30°C, pH = 7.4 and the media composition is given in Table 5-1.

Table 5-1 Composition of the media used for small-scale (≈ 100ml) fermentation for chloramphenicol production.

Component	Concentration (g/l)
Glycerol (Aldrich, ACS Reagent)	10
DL-serine (Sigma, >98%)	11
Sodium lactate (Sigma, sodium salt >98%)	5
di-potassium hydrogen phosphate (BDH, GPR Grade)	2.39
Potassium di-hydrogen phosphate (BDH, GPR Grade)	1.39
Magnesium sulphate – 7 hydrate (BDH, AnalR Grade)	1
Sodium chloride (BDH, AnalR Grade)	3

5.6.2 Phenylalanine Production

Phenylalanine was produced by fermentation of the bacteria *Corynebacterium glutamicum* (NCIMB 10337). Two scales of batch fermentation where used, small scale (≈ 100ml) in 250ml conical shake flasks, incubated in an orbital shaker (Gallenkamp), with temperature control, rotating at 150 rpm, and large scale (≈ 20 litre) in the fermentation unit of the Department of Biochemistry at Imperial College. Culture fluid from the small-scale fermentations was used as the inoculum for the large-scale fermentation. The compositions of media used and the growth conditions are based on the US Patent of Nakayama *et al.* (1973), the growth conditions were: temperature = 30°C, pH = 7.4 and the media composition are given in Tables 5-2 and 5-3.

Table 5-2 Composition of the media used for small-scale (≈ 100ml) fermentation for phenylalanine production.

Component	Concentration (g/l)
Glucose (BDH, GPR Grade)	20
Soybean Peptone (BDH)	10
Yeast Extract (Merck)	10
Sodium chloride (BDH, GPR Grade)	3

Table 5-3 Composition of the media used for large-scale (≈ 20 litre) fermentation for phenylalanine production.

Component	Concentration (g/l)
Glucose (BDH, GPR Grade)	70
Corn Steep Liquor (Sigma)	1
di-potassium hydrogen phosphate (BDH, GPR Grade)	1
Potassium di-hydrogen phosphate (BDH, GPR Grade)	1
Magnesium sulphate – 7 hydrate (BDH, AnalR Grade)	0.5
Soybean Peptone (Sigma)	9

5.7 Overview of the Experimental Study

The broad aim of this study was to study the effects of the fermentation broth components on solute recovery by liquid-liquid extraction, and the specific aims where previously detailed in Section 2.5. In this section an outline is presented of how each extraction process has been used to meet the aims of the study.

The physical extraction system was used as a primary investigation system to assess effects on the mass transfer process during liquid-liquid extraction. The effect that soluble and insoluble components of the fermentation broth had on mass transfer has been studied. In addition, the effect of biological macromolecules (protein and lipid compounds) was also studied in an attempt to correlate observed effects with molecular properties. Ionic and non-ionic surface active compounds may also be added before and during the extraction process, and the effect that such compounds had was also investigated using the physical extraction process.

The reactive extraction process was used primarily to investigate the effect that ionic fermentation broth and media components had on the extraction reaction mechanism. The interactions between the competing anions during extraction reactions and their effect on mass transfer and equilibrium conditions have been characterised. Also, as with the physical extraction process, the effect of surface-active compounds that may be added during processing was also investigated.

Chapter 6

Experimental Methods

6.1	Introduction		103
6.2	Extraction Equilibrium		103
6.3	The Lewis Cell		104
6.4	The Schlieren Optical System		105
	6.4.1	Schlieren Mass Transfer Cell	107
	6.4.2	Combined Optical Set Up	109
6.5	Analytical Methods		111
	6.5.1	Physical Extraction System	111
	6.5.2	Reactive Extraction System	112
		6.5.2.1 Phenylalanine	112
		6.5.2.2 Chloride	112
		6.5.2.3 Other Anions	113
		6.5.2.4 pH	114
	6.5.3	Other Techniques	114
		6.5.3.1 Biomass Concentration	114
		6.5.3.2 Surface and Interfacial Tension	115
		6.5.3.3 Total Carbon Content	115
		6.5.3.4 Ultrafiltration	115
		6.5.3.5 UV absorbence Spectrum	115
		6.5.3.6 Viscosity	116

6.1 Introduction

This chapter presents the materials and methods used in the experimental work to investigate the effect of fermentation broth components on solute extraction during liquid-liquid extraction. Equilibrium constants for both extraction processes were determined using a mixer-settler technique which is described in Section 6.2. In Section 6.3 the Lewis cell, which was used to obtain mass transfer coefficients for the liquid-liquid extraction process is described. In some cases increased mass transfer coefficients were recorded, and in order to ascertain whether this effect was due to interfacial turbulence the Schlieren optical system was used, which is described in Section 6.4. The analytical techniques used to define the solute concentrations, and other parameters relevant to liquid-liquid systems are detailed in Section 6.5.

6.2 Extraction Equilibrium

The determination of the equilibrium extraction parameters (partition coefficient for physical extraction, and the reaction constant for reactive extraction) are required to allow the extraction efficiency and mass transfer coefficients to be determined. The extraction equilibrium experiments were performed using a mixer-settler technique conducted in 25 cm^3 test tubes with screw top lids.

The test tubes were typically filled with 10 cm^3 of each phase, although volumes were varied depending on the required phase volume ratio. To reduce the time required to reach equilibrium the phases were mixed using a vortex mixer. The tubes were then placed in a constant temperature water bath (Grant, LTD 6), in all cases at 25°C, and allowed to equilibrate for at least one hour. It was found from a number of mixer-settler experiments conducted with varying contact times that equilibrium was usually attained after, at most, 30 minutes. A one-hour contact time was chosen to allow for any error associated with the mixing process or reduced dynamics due to the presence of added compounds. To separate the phases the test tubes were placed in a temperature controlled centrifuge (Heraeus, Biofuge Stratos) at 2500 rpm and 25°C for 5 minutes.

Immediately after phase separation samples were taken from the aqueous and solvent phases for the measurement of solute concentrations; the analytical methods used are detailed in Section 6.4.1 for the physical extraction system and Section 6.4.2 for the reactive extraction system.

6.3 The Lewis Cell

To investigate mass transfer, liquid-liquid extraction experiments were performed in a Lewis cell, which was constructed in the departmental workshop and is similar to designs used in previous studies of liquid-liquid extraction (Haensel *et al.*, 1986; Scarpello and Stuckey, 2000). A schematic diagram of the Lewis cell is shown in Figure 6-1. It consisted of a baffled cylindrical glass jacketed vessel with a cross-sectional area of 43.6cm^2 and a total volume of 640cm^3 (320cm^3 per phase). The temperature in the glass jacket was maintained to $^+/_-$ 0.1°C using an external heating-cooling facility (Grant, LTD 6), in all cases the temperature used was 25°C. Each phase was stirred using a four bladed counter rotating impeller, positioned at equal distances from the interface, and each impeller was driven independently by an AC motor, which allowed the speed to be controlled to between 0 and 500 RPM. To prevent the interface from being disturbed wire gauze was positioned on either side of the it. Before the start of an experiment the cell was filled with both phases. To avoid wetting the top half of the cell, the aqueous phase was added through a port in the base of the cell. The solvent phase was then added through a port in the top of the cell, and this was done slowly to avoid the formation of an emulsion at the interface.

To enable the effect of added compounds on the mass transfer coefficient to be assessed measurement of the change in solute concentration in one of the phases is necessary. For the physical extraction process the concentration was measured using samples taken from the solvent phase and for the reactive extraction process the concentration was determined using aqueous phase samples. However, in order to maintain the phase volume ratio during an experiment samples were taken simultaneously from both phases. Aqueous phases sampling was conducted through a Teflon septum in the base of the cell, using a graduated 1 ml syringe and needle. Solvent phase samples were removed through the port at the top of the cell, using a 1ml pipette (Gilson, P1000

Pipetteman). Typically ten pairs of 0.75 ml samples were taken during the course of an experiment at 15-minute intervals; the analytical methods used to determine concentrations are detailed in Section 6.4.1 for the physical extraction system, and Section 6.4.2 for the reactive extraction system.

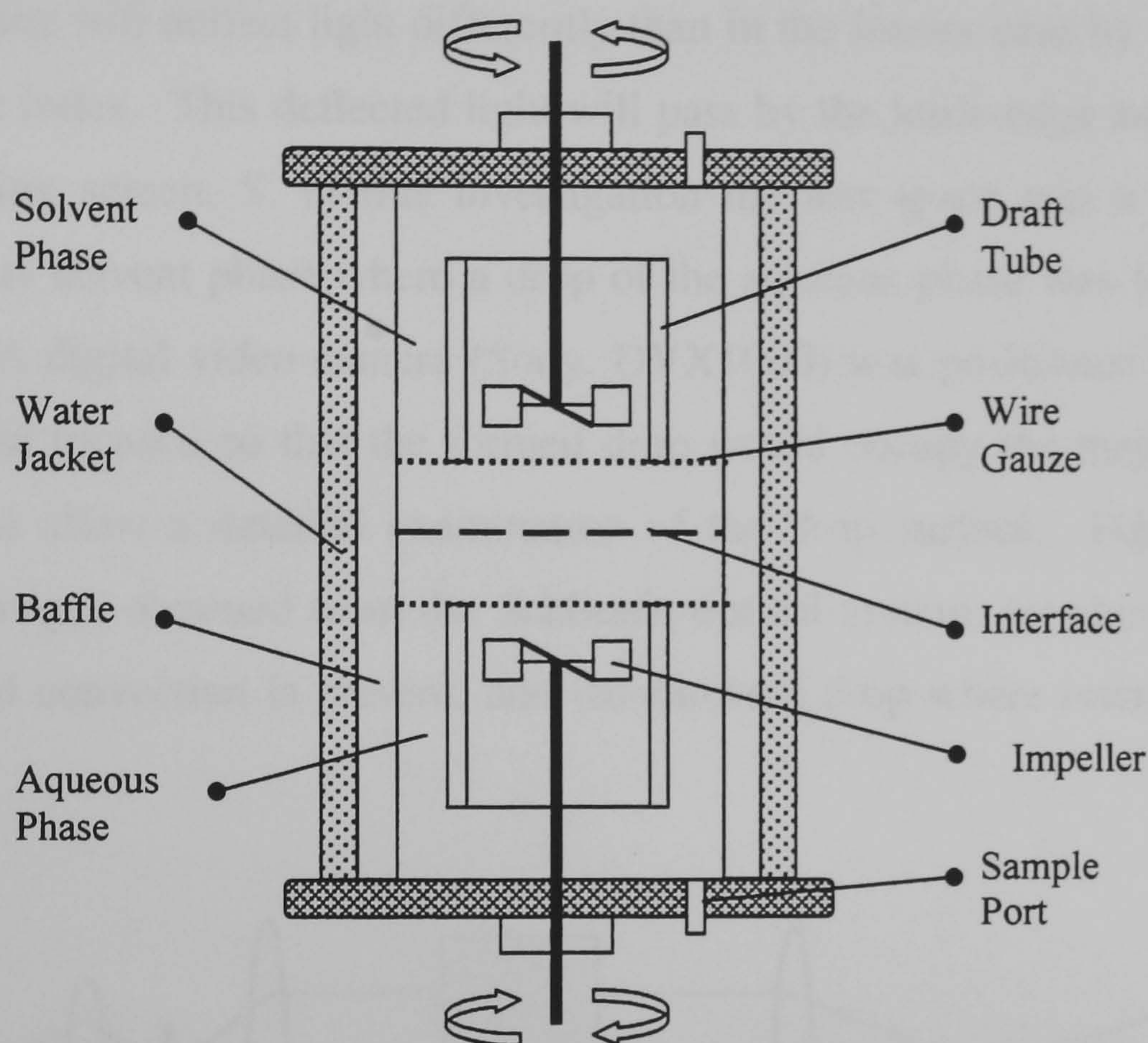


Figure 6-1 Schematic diagram of the Lewis cell.

6.4 Schlieren Optical System

The Schlieren optical system has been used widely as a visualisation technique to detect the presence of interfacial convection (Orell and Westwater, 1962; Agble and Mendes-Tatsis, 2000). In this study the Schlieren optical system was used to observe instabilities at the liquid-liquid interface caused by solute transfer from an aqueous drop to a continuous solvent phase. A schematic of a Schlieren optical system is shown in Figure 6-2. Light from a laser is focused by a microscopic lens, L_1, on a pinhole, P, to create a point source of light. Light from point P is collimated by lens L_2 that then passes through the test space, T. The light beam is then focused on a knife-edge, K, by

a second collimating lens, L_3, Any light that passes the knife edge is projected onto a viewing screen, S, for observation and recording.

The system is configured so that in a homogeneous test space all the light is focused on the knife-edge and none is transmitted to the viewing screen. When inhomogenities are present they will deflect light differently than in the former case by virtue of a differing refractive index. This deflected light will pass by the knife-edge and be projected onto the viewing screen, S. In this investigation the test space was a cell containing the continuous solvent phase where a drop of the aqueous phase was formed on the tip of needle. A digital video camera (Sony, DVX1000) was positioned behind the viewing screen and focused so that the formed drop would occupy the majority of the field of view, and allow a detailed examination of the drop surface. Figure 6-3 shows two typical images obtained from the Schlieren optical system; (a) shows a drop where no interfacial convection is present, and (b) shows a drop where interfacial convection is present.

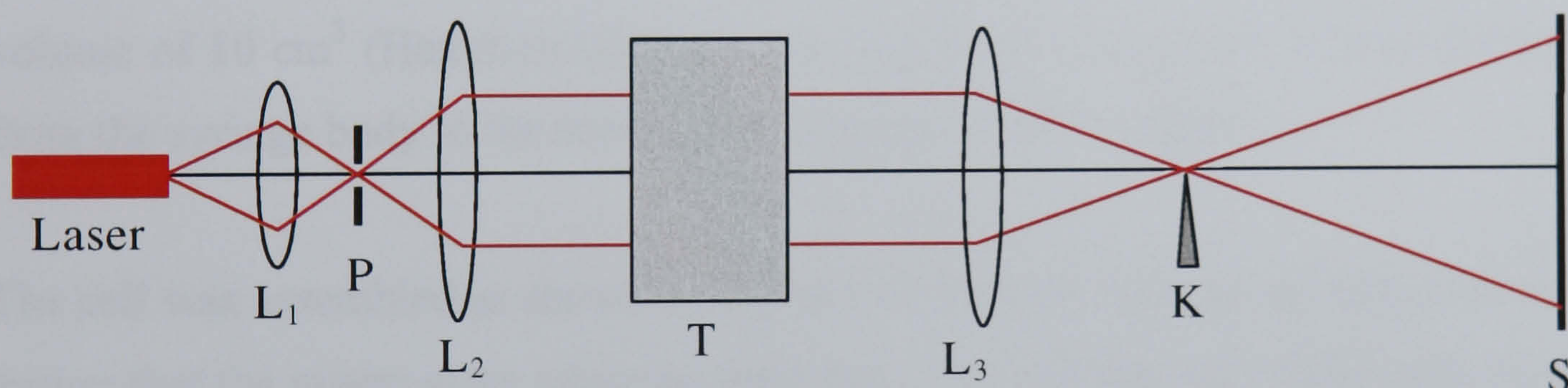


Figure 6-2 Schematic of the Schlieren optical system.

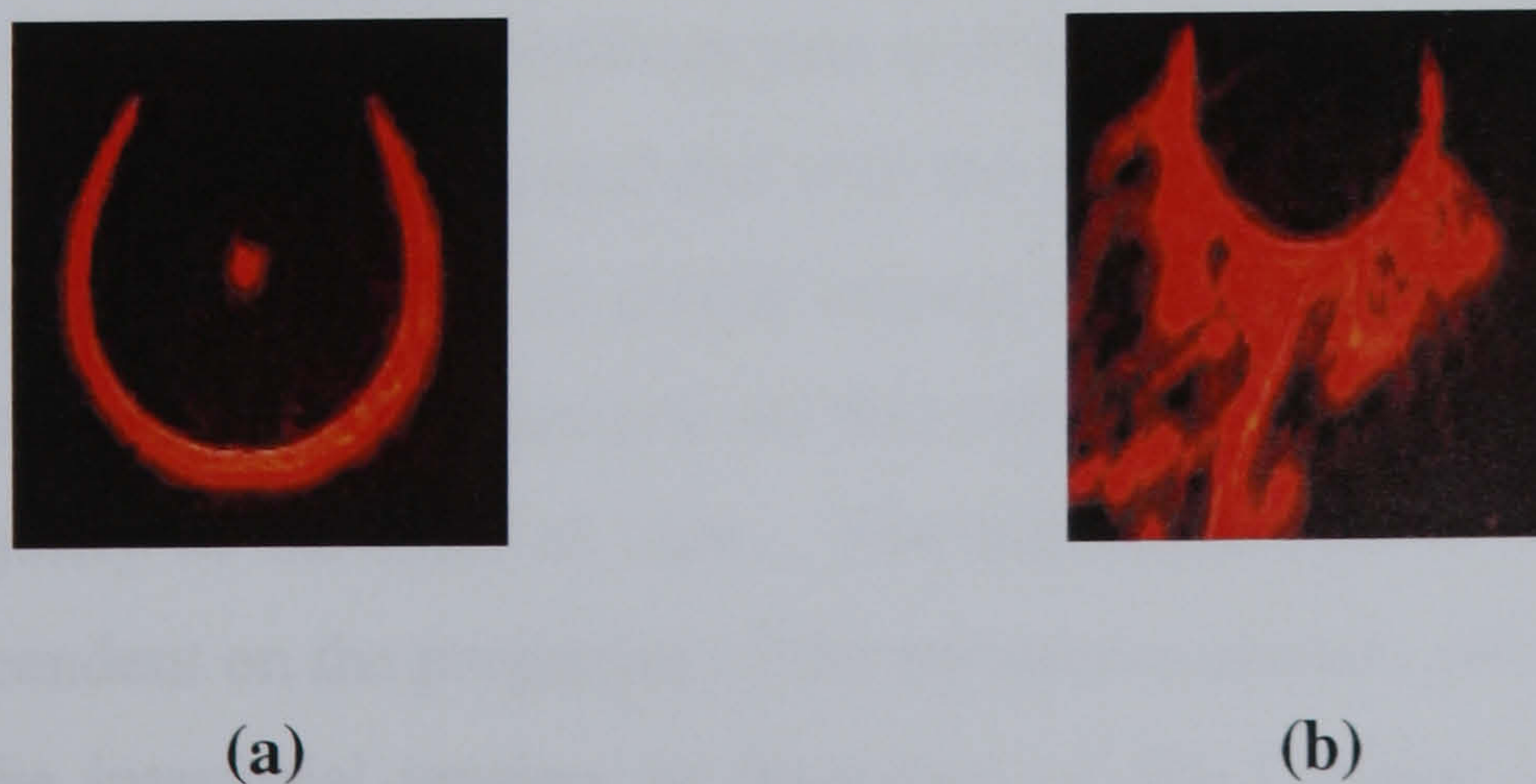


Figure 6-3 Two images of drops taken using the Schlieren optical system, (a) without the presence of interfacial turbulence and (b) with interfacial turbulence present.

6.4.1 Schlieren Mass Transfer Cell

In this study the test space used in the Schlieren optical system was a cell which contained the solvent phase, and in which a drop of the aqueous phase was formed on the end of needle. The cell was the same design as that used by Agble and Mendes-Tatsis (2000).

An exploded view of the cell is shown in Figure 6-4. The cell consists of a PTFE spacer of internal dimension 50mm (L) x 75mm (H) x 18mm (W), and externally 80mm (L) x 75mm (H) x 28mm (W). Quartz glass windows, 5mm thick, were fitted into recesses on both sides of the spacer. The windows were held in place by a PTFE gasket and aluminium frame plate on either side. The frame was secured by eight bolts that passed through the gaskets and the body of the spacer. An entry port at the top of the PTFE spacer allowed the needle of a syringe to be fitted into the cell via a tapered plug. The syringe capillary (Hamilton GB Ltd) was a flat-ended needle of internal diameter 1.5mm, external diameter 2.1mm, 16 gauge and point style 3. The syringe used had a volume of 10 cm^3 (Hamilton GB Ltd), and was fitted with a lock to stop fluid flowing from the syringe body to the needle after a drop had been formed.

The cell was assembled as shown in Figure 6-4 with the bolts being tightened evenly to ensure that the quartz glass windows were flat. The cell was then filled with the solvent (continuous) phase and left to equilibrate (≈ 2 hours) to the temperature of the laboratory, which was kept at 22°C. Meanwhile the syringe was filled with the aqueous (drop) phase, and similarly left to equilibrate to the temperature of the laboratory. During this time the optical system was adjusted to ensure that all the light was being focused on the knife-edge. The capillary and syringe were mounted onto the test cell, which was then clamped to the optical rail with the capillary tip in the path of the light beam, as shown in Figure 6-5. A digital camera (Sony, DVX1000) was positioned behind the viewing screen and focused so that when the drop was formed it would occupy the majority of the field of view. The maximum size of drop that could be formed was dependent on the properties of the two-phase system, particularly the phase densities and the interfacial tension, as described by the Laplace equation (Hua and Rosen, 1988). A number of drops were allowed to form, detach and fall to the base of

the cell so that the liquid in the needle which had been in partial contact with the solvent phase was discarded, and the drop used was a fresh sample from the barrel of the syringe. Once a drop of sufficient size on the camera viewer was formed the capillary was locked to prevent any further flow into the drop from the syringe.

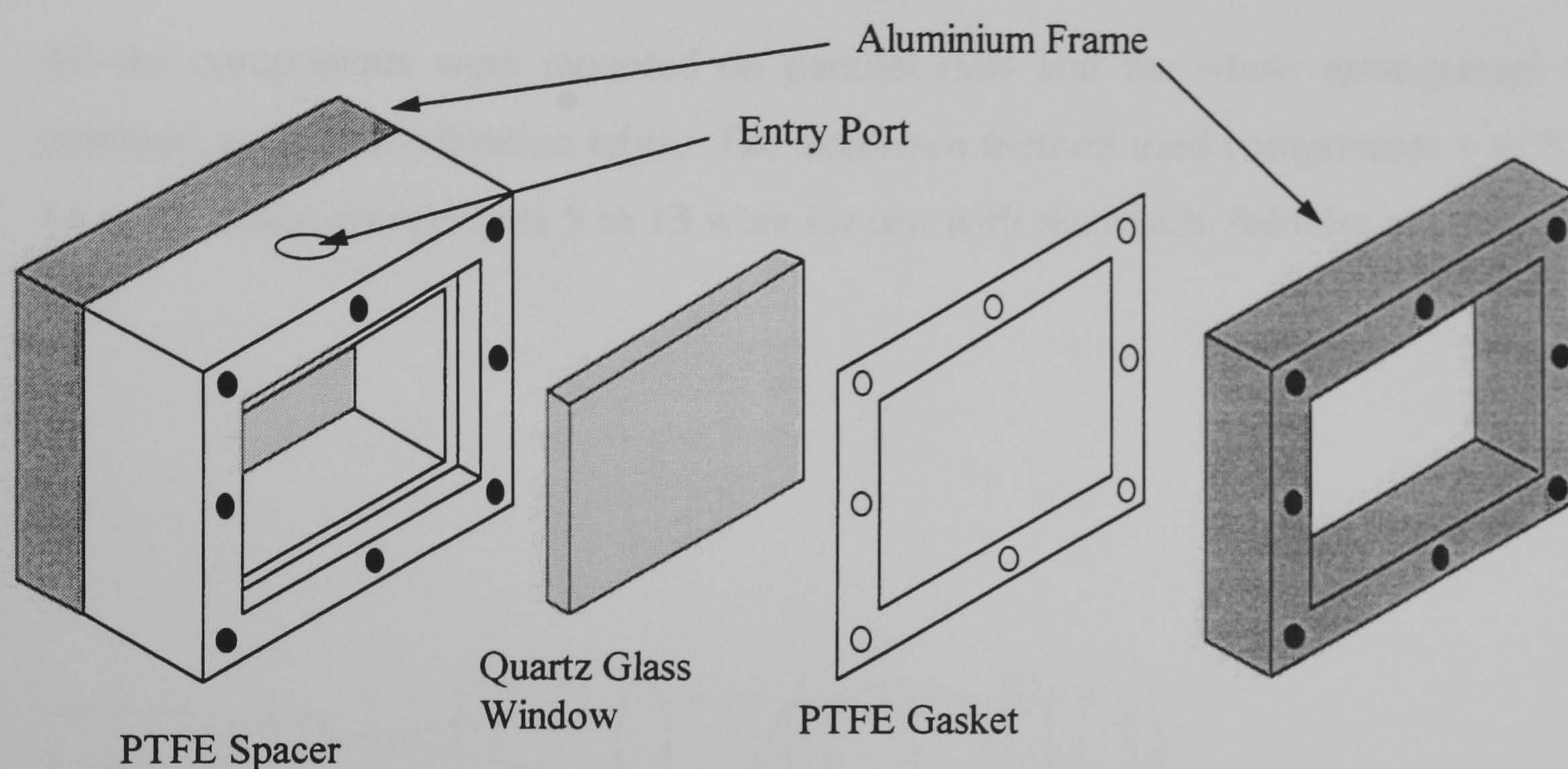


Figure 6-4 Exploded view of Schlieren cell.

After the experiment the contents of the cell were drained through the entry port and the cell was completely dismantled. All the components were then washed with a mild detergent (Teepol). The quartz glass windows and the PTFE sections were further cleaned by placing them for 2 hours in a warm water bath of Decon 90. Finally these parts were rinsed thoroughly with water before being placed in a hot ultrasonic bath of deionised water for 30 minutes to remove any traces of surfactants or solvents. Upon removal from the water bath the quartz glass windows were dried using compressed air before being polished using a lens cleaning cloth.

6.4.2 Combined Optical Set Up

The Schlieren optical system used in this investigation was the same apparatus used in a previous study by Agble (1998). In that study the Schlieren optical system was constructed in conjunction with a Mach-Zehnder interferometer as the two optical systems share some common components. A schematic diagram of the combined set-up and details of each component are given in Figure 6-5 and Table 6-1 respectively.

All the components were mounted on parallel rails and the whole arrangement was mounted on an anti-vibration table. The Schlieren method used components 1 to 5 and 14 to 20, while components 5 to 13 were for use with the Mach-Zehnder interferometer only.

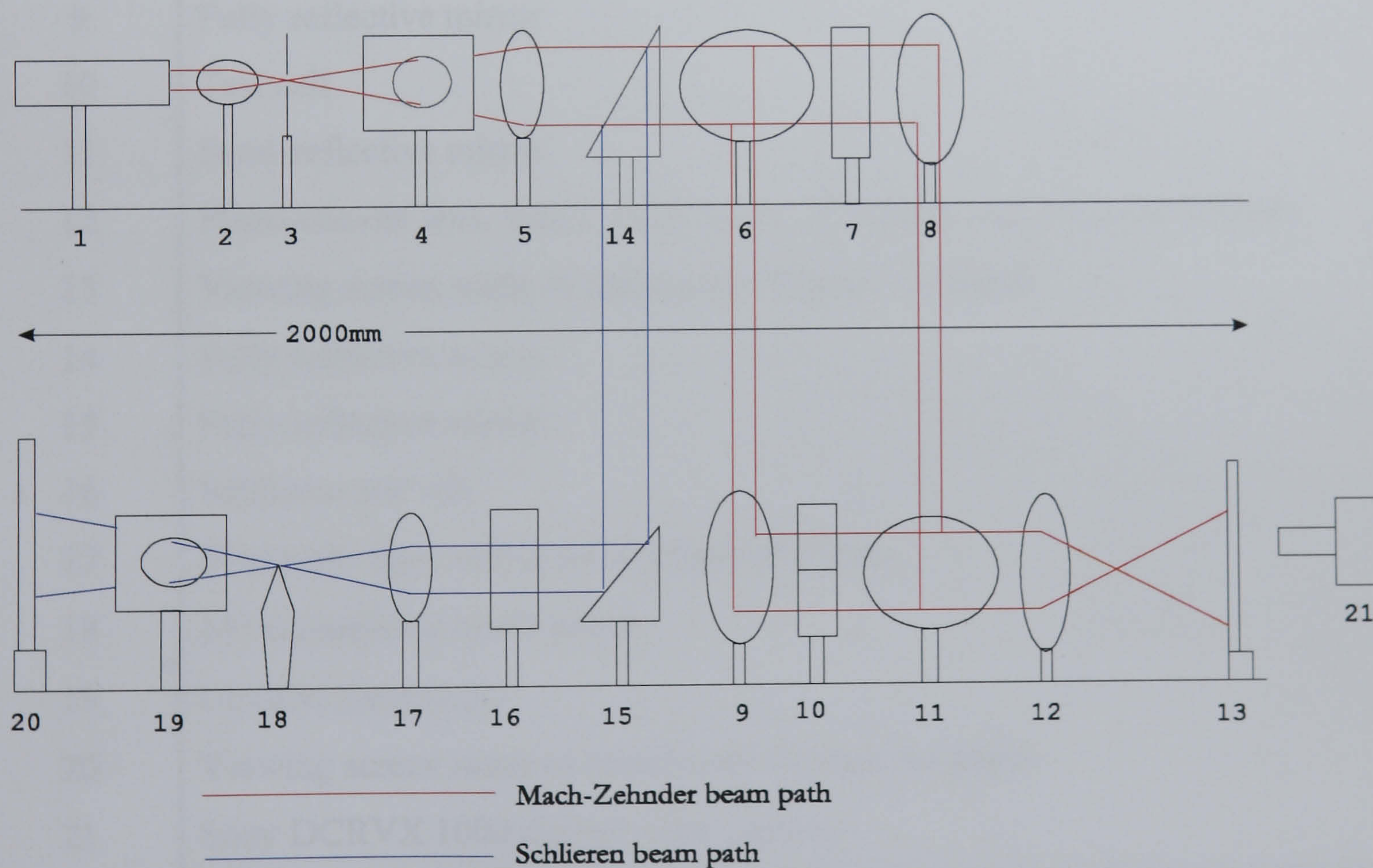


Figure 6-5 Schematic of the combined optical set up (Agble, 1998).

Table 6-1 Details of the components used in the combined Mach – Zehnder interferometer / Schlieren optical method set-up. Component index numbers relate to those designated in Figure 6-5.

Index	Component Description
1	Helium-Neon Laser, of wavelength 632.8nm, maximum power = 25mW.
2	Microscope objective lens, with a focal length of 1.4mm
3	25 micron pinhole
4	Circular diaphragm
5	Plano-convex lens, with a focal length of 500mm and diameter 100mm
6	Semi-reflective mirror
7	Reference cell
8	Fully reflective mirror
9	Fully reflective mirror
10	Test cell
11	Semi-reflective mirror
12	Plano-convex lens, with a focal length of 125mm and diameter 100mm
13	Viewing screen made of translucent 50gram A4 paper
14	Fully reflective mirror
15	Fully reflective mirror
16	Schlieren test cell
17	Bi-convex lens, with a focal length of 75mm
18	15° tapered rod (knife edge)
19	Circular diaphragm
20	Viewing screen made of translucent 50gram A4 paper
21	Sony DCRVX 1000 digital video camera.

6.5 Analytical Methods

To investigate the effect of added compounds on the mass transfer system it was necessary to determine the change in solute concentration, and the concentration change of any co-extracted compounds. Details of the analytical techniques and methods for the two extraction systems are given in Sections 6.5.1 and 6.5.2 for physical extraction and reactive extraction, respectively. In Section 6.5.3 details are given for other analytical techniques that have been used in this study to characterise the two-phase systems.

The primary analytical methods used in this study were based on the UV-visible light absorbance of a solution at a wavelength of light particular to the compound under investigation. Using a UV-visible spectrometer it is possible to select a wavelength of light and then measure the absorbance of light by the test solution compared to a reference solution where the compound of interest is absent. To relate absorbance to solute concentration a calibration curve using solutions of known concentration is constructed; this calibration is only valid when all the measurements are made relative to the same reference solution. A linear dependence between concentration and absorbance is observed, and this was the case for all compounds examined in this study. The wavelength of light at which a compound will absorb light is determined by the bond structure of the compound, and the wavelength at which maximum absorbance occurs can be determined by scanning across a range of wavelengths to find the peak absorbance. Where a compound has a low UV absorbance due to its structure it is possible to make it visible to spectrometric examination by reaction with a compound that does have an appreciable UV-visible light absorbance.

6.5.1 Physical Extraction System

The solute used in the physical liquid-liquid extraction system was the antibiotic chloramphenicol. The concentration of chloramphenicol in the aqueous and solvent phase was determined using an UV spectrometer (Cecil, CE1021). In the aqueous phase chloramphenicol had a peak absorbence at a wavelength of 272nm, in the solvent phase the peak absorbence was at 278nm. Generally the concentration was measured in

the solvent phase because compounds produced during fermentation interfered with absorbence readings. Based on UV absorbence measurements, the compounds that interfered with aqueous phase readings were found not to partition into the solvent phase, and therefore did not interfere with UV absorbence measurements of the solvent phase. The absorbance-concentration calibration curve for solvent phase readings was constructed by mass balance, using measurements of the aqueous and solvent phase absorbence from equilibrium experiments.

6.5.2 Reactive Extraction System

The solute used in the reactive liquid-liquid extraction system was the amino acid phenylalanine, which was extracted using the ion exchange reagent Aliquat 336. Due to the non-specific nature of the ion exchange reagent other anions present in the aqueous feed solution were liable to extraction. Therefore, it was necessary to determine the concentration change of both the target solute and any other compounds that may be co-extracted.

6.5.2.1. Phenylalanine

Phenylalanine concentration was determined directly using a UV spectrometer (Cecil, 1021). In the aqueous phases used phenylalanine had a peak absorbance at a wavelength of 257nm. In the solvent phase used (1-octanol and Aliquat 336) it was not possible to detect changes in the UV absorbance around 257nm due to interference caused by the ion exchange reagent, Aliquat 336. Aliquat 336 absorbs light in a similar wavelength region to phenylalanine; thus changes in the absorbance due to changes in phenylalanine concentration are masked by the presence of Aliquat 336. Therefore, measurements of the phenylalanine concentration were only performed from the aqueous phase.

6.5.2.2 Chloride

During the ion exchange reaction chloride was released into the aqueous phase, and determination of the chloride concentration would allow the involvement of the ion

exchange reagent in all extraction reactions to be determined. It was not possible to directly determine chloride concentration using an UV spectrometer; however, it was possible to measure chloride concentration using a reaction that gave rise to a coloured product. The light absorbance of the coloured product could be detected by a UV spectrometer, and the colour intensity was directly related to the concentration of chloride present in solution. The chloride concentration was determined using the colourimetric assay method of Iwasaki *et al.* (1956). The assay was able to detect chloride concentrations of up to 3 mM; concentrations encountered were often in the 10 to 50 mM range so samples were diluted accordingly. The assay involved the addition of two reagents,

(a) 3 g/l mercuric thiocyanate (Aldrich, 98%) in ethanol (Fluka, 99%).

(b) 80 g/l ammonium iron (III) sulphate – 12 hydrate (BDH, AnalR Grade) in 6M nitric acid (BDH).

To 0.5 ml of test solution, 0.6 ml of reagent (a) and then 0.2 ml of reagent (b) were added in a stoppered test tube, which was then well mixed using a vortex mixer and the mixture was left to react for fifteen minutes. The samples were analysed for colour intensity on an UV spectrometer (Cecil, CE1021) at 460nm. For each set of samples a new calibration curve was determined as ambient conditions (laboratory temperature and reaction time) affected the colour development.

It was found that other halide compounds, e.g. bromide, also contributed to the colour development in the chloride assay. Therefore, when any other anions were present in the system their concentration and that of chloride were determined by ion chromatography, and is described in the next section.

6.5.2.3 Other Anions

Using ion chromatography it was possible to determine the aqueous concentration of a range of anionic compounds, including chloride. The ion chromatography system used was a Dionex DX120 that is based on a HPLC system. The column used to separate the anions was an Ion PAC AS14 (Dionex) that used electrostatic interaction to separate anions, the column had dimensions of 4mm (D) x 250mm (L). The method of detection

was the solution conductivity measured using an ATT detector (Dionex); for all anions examined a linear calibration between concentration and conductivity was obtained. Ion chromatography was performed at ambient temperature using an AS40 automated sampler that injected a sample volume of 5 ml. The eluent used was a solution of 3.5 mM Na_2CO_3 and 1 mM $NaHCO_3$; the flow rate used was constant at 1.1 ml/min. For each particular anion the accurate range was 0 to 500 mg/l, therefore samples were diluted accordingly.

When all the other anion concentrations were determined the hydroxide ion concentration was determined by mass balance.

6.5.2.4 pH

Phenylalanine is a weak acid that has dissociation constants of 3.23 and 9.27. Above pH 9.27 phenylalanine will exist predominately in its extractable anionic form. To determine the extent of phenylalanine dissociation (Section 5.5), the solution pH was measured using a Corning 240-pH meter, which was accurate to $^{+}/_{-}$ 0.1 pH units and calibrated using Colourkey buffers (BDH).

6.5.3 Other Techniques

The other analytical techniques that were used to provide information about the two-phase extraction system are detailed in the sub-sections below.

6.5.3.1 Biomass Concentration

The concentration of bacterial cells present in the fermentation broth was determined gravimetrically. Membrane filter papers (Whatman, cellulose nitrate) with a nominal cut-off of 0.2 μm where dried to constant weight in an oven. Using a Buchner funnel a known volume of fermentation broth was passed through the papers, which were then returned to the oven and dried to constant weight. The biomass weights presented are the result of at least 5 repetitions; the spread amongst individual readings was $^{+}/_{-}$ 1.2 %

6.5.3.2 Surface and Interfacial Tension

Liquid surface and liquid-liquid interfacial tensions were measured using a digital tensiometer (Krüss, K10) with a platinum Du Nouy ring. The tensiometer was capable of measuring liquid surface and interfacial tensions over 1-200 mN/m, and was accurate to $^{+}/_{-}$ 0.1 mN/m. All measurements were performed at a constant temperature of 25°C. which was maintained using an external heating facility (Grant, LTD6).

6.5.3.3 Total Carbon Content

The carbon distribution of the weight fractions obtained from the ultrafiltration process was determined using a total organic carbon analyser (Shimazdu, TOC-5050). Samples were analysed three times, and if the coefficient of variance was above 2% additional measurements were made. The accurate range was from 1 to 1000ppm for total carbon.

6.5.3.4 Ultrafiltration

The effect that different molecular weight fractions of the fermentation broth had on the mass transfer process was investigated. Filtered fermentation broth was fractionated into molecular weight fractions using an ultrafiltration cell (Amicon, 8200). The nominal molecular weight cut-offs of the membranes used, and the manufacturers (Diaflo) product codes are given in Table 6-2. The test solution was filtered in series, starting with the membrane that had the highest cut-off; the retentate was reconstituted to the original volume of the test solution using de-ionised water.

6.5.3.5 UV Absorbance Spectrum

When determining the concentration of a solute using the UV-visible absorbance method, the best results were obtained when measurements were made at the wavelength of peak absorbance. A scanning UV-Visible spectrometer (Shimazdu, UV 2101PC), with a range of 200-800nm, was used to detect UV-visible absorbance peaks.

Other compounds present in the aqueous phase, e.g. other fermentation broth components, may also be extracted into the solvent phase. If a co-extracted compound absorbs light then its partitioning behaviour may be qualitatively assessed by scanning the UV-visible absorbence spectrum of the extracting solvent phase. Scanning the organic phase in this way also allowed for an assessment of whether other compounds would interference with (added to) solute absorbance measurements; if interference occurred then mass transfer would be over predicted.

6.5.3.6 Viscosity

The kinematic viscosity of the aqueous phase was measured using a U-tube viscometer (BDH), size O, with a range of 0.2 - 1 centistokes. The viscometer was pre-calibrated by the manufacturer to BS ISO 9001; the constant was reported as 0.001023 cS/s. Measurement times were repeatable to within 1 second or 0.001023 centistokes. The kinematic viscosity of the solvent phase was measured using a U-tube viscometer (BDH), size B, with a range of 2 - 15 centistokes. The viscometer was calibrated using pure 1-Octanol, and the constant was found to be 0.0115 cS/s. Measurement times were repeatable to within 1 second or 0.0115 centistokes. All viscosity measurements were performed in a water bath (Grant, LTD 6) to ensure a constant temperature of 25°C was maintained.

Table 6-2 Nominal molecular weight cut-off of membranes used for ultrafiltration of fermentation broth.

Membrane	Molecular weight cut-off (Daltons)
YM1	1000
YM3	3000
YM10	10000
YM30	30000
XM50	50000
YM100	100000
XM300	300000

Chapter 7

Physical Extraction: Results and Discussion

7.1	Introduction	118
7.2	Equilibrium Extraction	119
	7.2.1 The Effect of pH on the Partition Coefficient	119
	7.2.2 The Effect of Concentration on the Partition Coefficient	120
	7.2.3 The Effect of Added Compounds on Extraction Equilibrium	122
7.3	Analysis of Dynamic Mass Transfer Using Two-Film Theory	122
7.4	Mass Transfer Control in the Dynamic System	123
7.5	The Effect of Concentration Driving Force on Dynamic Mass Transfer	126
7.6	The Effect of Media Components on Solute Extraction	127
7.7	The Effect of Fermentation Broth on Solute Extraction	128
	7.7.1 Filtered Fermentation Broth	129
	7.7.2 Weight Fractionated Fermentation Broth	130
	7.7.3 Biomass	135
7.8	The Effect of Biosurfactants on Solute Extraction	138
7.9	The Effect of Antifoam Agents on Solute Extraction	143
7.10	The Effect of Surfactants on Solute Extraction	147
	7.10.1 SDS	147
	7.10.2 DTAB	151
	7.10.3 Surfactant and Biomass	154
	7.10.4 Surfactant and Protein	159
7.11	Summary of Results and Discussion	160
	7.11.1 Effects of Fermentation Broth Components On Mass Transfer	160
	7.11.2 Effect of Biosurfactants on Mass Transfer	161
	7.11.3 Effect of Surfactants on Mass Transfer	162

7.1 Introduction

In this chapter experimental results are presented from the study of liquid-liquid extraction using a physical extraction process where the model system employed was the extraction of the antibiotic chloramphenicol using the organic solvent 1-octanol. The main experimental tool used was the Lewis cell, from which overall mass transfer coefficients have been obtained using the model described in Section 2.4, and experimental concentration measurements. The overall mass transfer coefficient has been used to compare the effects of compounds on the mass transfer process during liquid-liquid extraction. The equilibrium partition coefficient has also been determined to assess whether added compounds will influence the final extraction distribution of the solute. Surface and interfacial tensions have been measured to investigate the adsorption of compounds to the two-phase interface. In some systems enhanced mass transfer coefficients have been measured, and the occurrence of interfacial turbulence in these systems was investigated using the Schlieren optical method.

Initially in Section 7.2 the effect of process conditions on the extraction equilibrium is examined. In Section 7.3 the use and applicability of the model of mass transfer, which was detailed in Section 2.4, is assessed. The controlling resistance in the mass transfer process for the clean system (no surface-active compounds present) is examined and discussed in Section 7.4, and in Section 7.5 the effect of the initial concentration driving force is considered. Section 7.6 presents experiments conducted to assess whether the media components used for the production of chloramphenicol by fermentation had an effect on mass transfer. In Section 7.7 the effect of whole, filtered and weight fractionated fermentation broth, and the biomass produced during the fermentation, is examined. In Section 7.8 the effect of adding known biosurfactant compounds on mass transfer is investigated. Antifoaming agents, which are surface active, are often added during the fermentation process, and in Section 7.9 the effect of two laboratory antifoaming agents are examined. Novel liquid-liquid extraction techniques often involve the use of surface-active compounds, so in Section 7.10 the effect of two ionic surfactants applicable to such processes for chloramphenicol extraction from water, biomass and protein solutions are presented. Finally in Section 7.11 a brief summary of the significant experimental results is presented.

7.2 Extraction Equilibrium

To achieve solute recovery, equilibrium staged extraction processes are often employed. and the extent of recovery for each stage is determined by the extraction equilibrium constants for the system. For physical extraction the equilibrium partition coefficient (Section 4.3), describes the distribution of the solute between the two liquid phases at equilibrium. In Sections 7.2.1 and 7.2.2 the effect of two process conditions, feed solution pH and solute concentration, on the equilibrium partitioning of the solute are examined.

The experimental error associated with the determination of the equilibrium partition coefficient was assessed from 6 experimental sets of 6 determinations, and it was found that the average standard error was $^{+}/_{-}$ 3.7% of the mean value obtained from an experimental set.

7.2.1 The Effect of pH on the Partition Coefficient

Microbial fermentations are often conducted at solution pH that will allow the bacteria to maximise production of the solute, and generally this is in the pH range 5-8. However, downstream of the fermenter the pH may be altered to improve processing, such as to increase cell flocculation to aid separation of the biomass from the broth. Therefore, the separation process may encounter a range of pH conditions, and in Figure 7-1 the effect of the feed solution pH on the equilibrium partition coefficient of chloramphenicol between aqueous buffer solutions and 1-octanol is presented.

It is seen that the partition coefficient is constant, within experimental error, over the lower pH range of 3 to 7 with an average value of 15.9. However, at the basic pH tested the partition coefficient drops considerably to a value of 3.0. When extraction was performed from de-ionised water only the value of the partition coefficient was 16.2. From this it is concluded that the presence of the buffering compounds had no effect on the extraction behaviour of chloramphenicol.

Chloramphenicol is a neutral compound and changes in the solution pH will not cause it to ionise, so the observed reduction of the partition coefficient cannot be attributed to a reduction in its solubility due to dissociation, as discussed in Section 4.1. However. many compounds produced biologically may be affected by pH in other ways, such as proteins that lose their activity, or penicillin that degrades. It is reported that chloramphenicol degrades in basic solutions (Smith and Hinman, 1963). Therefore, the reduction in the partition coefficient seen for pH's above 7 may be attributed to degradation of the compound, resulting in a compound that does not partition, or a compound with different analytical characteristics to chloramphenicol.

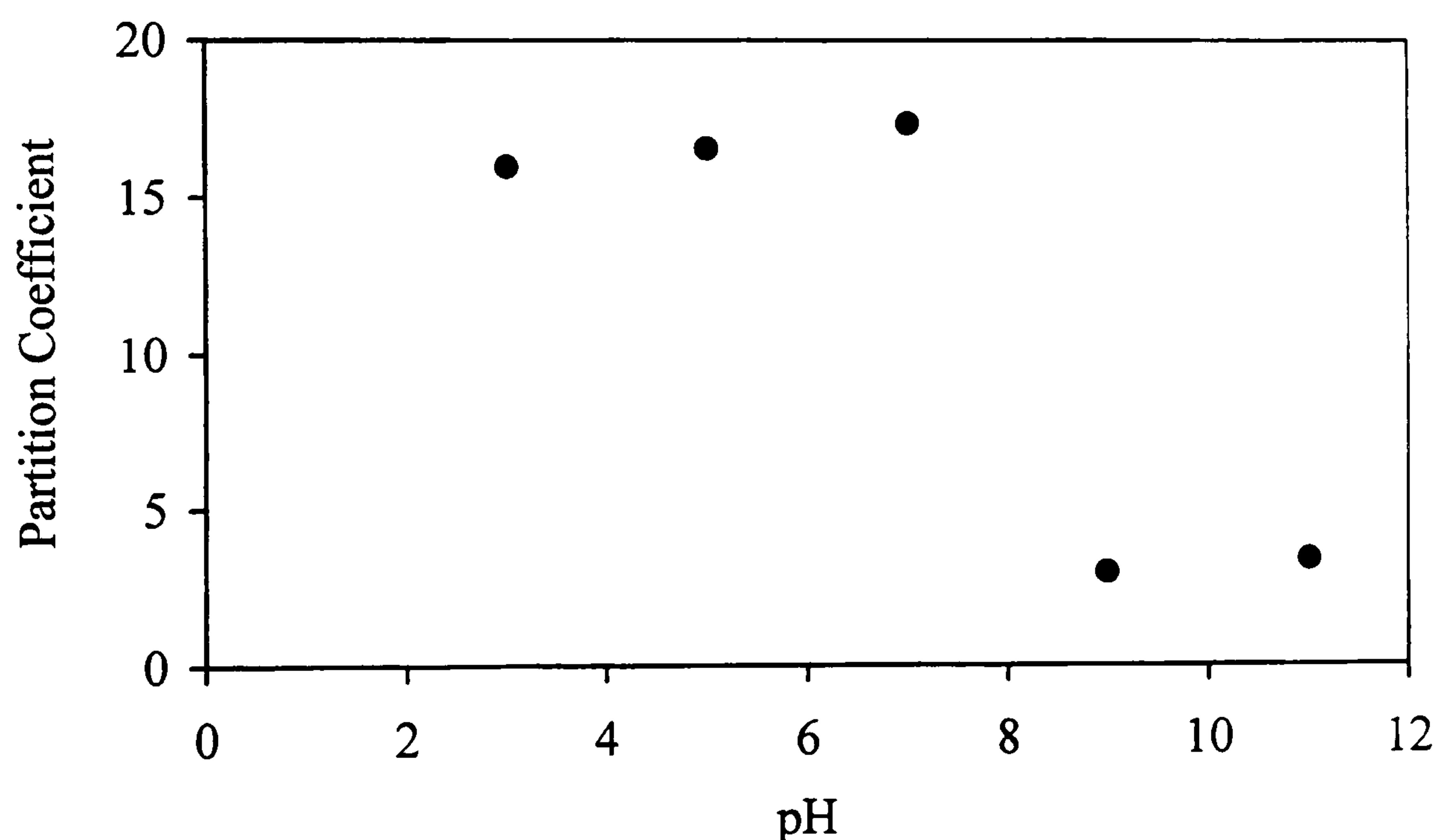


Figure 7-1 The effect of aqueous phase pH on the partition coefficient of chloramphenicol between de-ionised water and 1-octanol. Initial chloramphenicol concentration of 250μm, temperature = 25°C, and PVR = 1.

7.2.2 The Effect of Concentration on the Partition Coefficient

Due to the thermodynamic non-ideality of solutions the equilibrium partition coefficient may vary with concentration, and this variation would also have an effect on equilibrium staged separations. In Figure 7-2 an extraction isotherm for chloramphenicol extraction with 1-octanol is presented, where the initial solute concentration in the aqueous phase was varied from 0 to 250 μM.

It is seen that over the range considered the isotherm had a constant gradient, as indicated by the linear trendline shown in Figure 7-2. The gradient of the trendline and thus the value of the equilibrium partition coefficient was 16.02. In biological process the concentrations of solutes produced in microbial fermentation are often quite low, and over low concentration ranges it is common for extraction isotherms to be linear (Belter *et al.*, 1996), with non-idealities becoming more apparent at higher concentrations which are closer to the saturation concentrations.

The modelling of the mass transfer process, which is discussed in the next section, assumes that solute concentrations either side of the interface are in equilibrium. The equilibrium is described by the partition coefficient, and for the purpose of obtaining overall mass transfer coefficients it is assumed that the partition coefficient will remain constant. Thus, the linear isotherm supports the use of chloramphenicol as a model solute to investigate mass transfer based on the proposed mechanism.

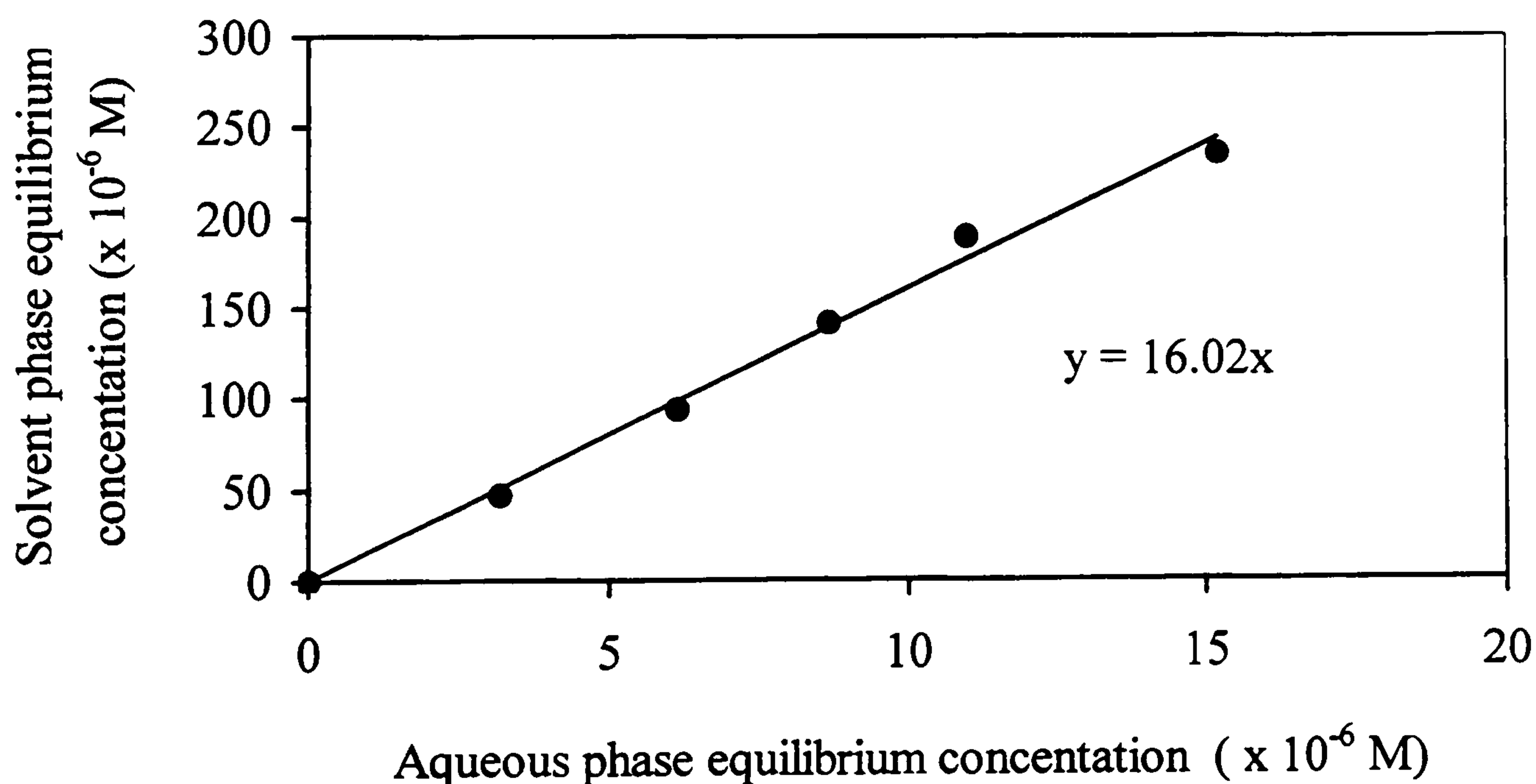


Figure 7-2 Equilibrium extraction isotherm for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 0 to 250μm, pH = 5.5, temperature = 25°C, and PVR = 1.

7.2.3 The Effect of Added Compounds on Extraction Equilibrium

In the remainder of this chapter the results of mass transfer experiments are reported for extraction from fermentation broth, and where compounds have been added to the extraction medium. On no occasion was the partition coefficient affected by broth components or added compounds. Therefore, the yield from an equilibrium extraction operation will be unaffected by the composition of the extraction medium. and if equilibrium is assumed to prevail at the interface during mass transfer the driving force will also be unaffected.

7.3 Analysis of Mass Transfer Using Two-Film Theory

In the remainder of this chapter results are presented for liquid-liquid extraction experiments using the Lewis cell, as described in Section 6.3. To allow mass transfer experiments to be compared, concentration measurements from the Lewis cell have been analysed using a two-film model of mass transfer to obtain an overall mass transfer coefficient for the extraction process (Section 2.4). The model identifies a concentration function (Equation 2-10) which is a linear function of time, where the constant of proportionality is the overall mass transfer coefficient. Thus, a plot of the concentration function against time should yield a straight line, the gradient of which will be the overall mass transfer coefficient.

In Figure 7-3 three plots of the concentration function are given for experiments where the solvent side stirrer speed in the Lewis cell was varied. It can be seen that in all cases the plot for each experiment yields a good straight line, which supports the use of the proposed mass transfer model in the analysis of extraction experiments performed in the Lewis cell. The overall mass transfer coefficient may be influenced by various physico-chemical properties of the liquid-liquid system, and in this study it will be used primarily to investigate the effect of surface-active compounds on the solute extraction process.

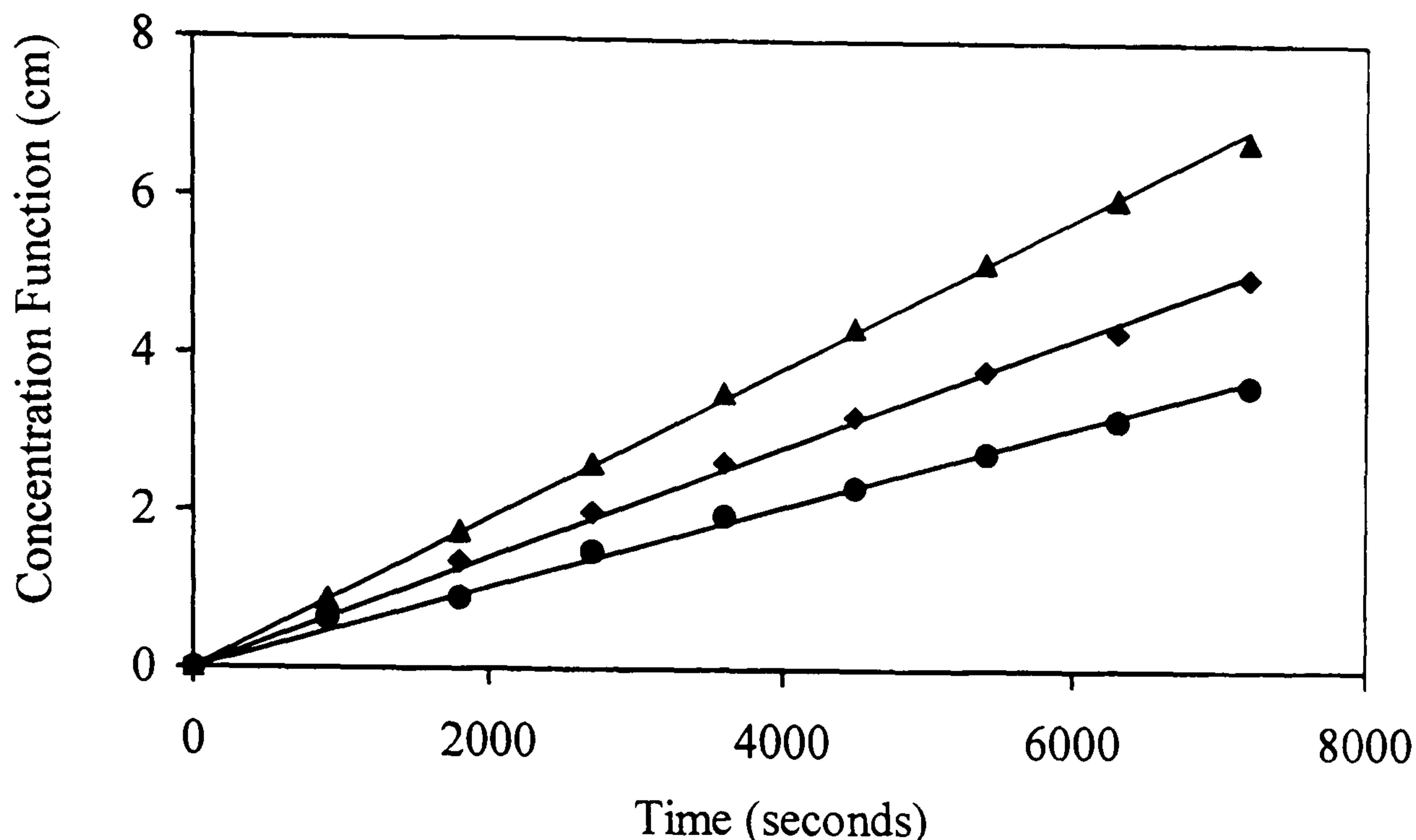


Figure 7-3 Plots of the concentration function used to determine overall mass transfer coefficients, for different solvent side stirrer speeds (N_{Sol}): 160 rpm (●), 220 rpm (◆) and 280 rpm (▲), for extraction of chloramphenicol from de-ionised water to 1-octanol. Initial chloramphenicol concentration of 250μm, N_{Aq} = 90 rpm, temperature = 25°C, and PVR = 1.

The experimental error associated with the determination of the overall mass transfer coefficient was assessed from 6 mass transfer experiments conducted under the same conditions, and it was found that the standard error was 4.7% of the mean value.

7.4 Mass Transfer Control in the Dynamic System

In Section 3.3.4 the mechanism of interphase mass transfer was examined and the resistance in series model of mass transfer was discussed (Equation 3-17). In this model the resistance to mass transfer was proposed to be a summation of the individual resistances associated with transfer through the interfacial films, and the interface itself. It is generally assumed that for a clean interface the resistance to mass transfer is very low, and can be neglected (Davis and Rideal, 1963). Thus, for a clean system the two stagnant films will control the mass transfer process. In this section the magnitude of the resistance in each film is examined to ascertain if one phase predominately controls the flux of the solute.

The overall mass transfer coefficient is a function of the physico-chemical properties of the two-phase system. In Section 3.3.3.1 the film model solution for Fick's Law showed that the individual mass transfer coefficient is directly proportional to the diffusion coefficient and inversely proportional to the film thickness (Equation 3-10). Thus, by independently varying the hydrodynamics for each phase it is possible to determine where the main resistance to mass transfer lies. Increasing the stirrer speed will give rise to a reduction in the thickness of the stagnant film adjacent to the interface, which according to the film model is the only place where a resistance to mass transfer exists, and so reducing the film thickness will reduce the resistance to mass transfer.

Figure 7-4 shows the effect of independently varying the stirrer speed of each phase in the Lewis cell on the overall mass transfer coefficient for chloramphenicol extraction using 1-octanol. It can be seen that changing the stirrer speed in either phase has an effect on the overall mass transfer coefficient, indicating that the thin films on both sides of the interface offer a significant resistance to mass transfer. The overall mass transfer coefficient is affected more by changes in the solvent side hydrodynamics with the gradient of the slope being an order of magnitude greater (0.0098 compared to 0.00094). This compares with the viscosity (ν) of the phases, which are also an order of magnitude different (86.9 x 10^{-7} m^2/s compared to 8.9 x 10^{-7} m^2/s). The Wilke-Chang correlation (Equation 3-4) and other equations for estimating diffusion coefficients show an inverse dependence on the viscosity of solvent in which diffusion occurs. Thus, in the case examined here it is suggested that the diffusion coefficient in the solvent phase is an order of magnitude smaller than in the aqueous phase.

In two film theory the overall mass transfer coefficient is assumed to be directly proportional to the diffusion coefficient, therefore a ten fold increase in the viscosity will lead to a ten fold decrease in the diffusion coefficient and in turn the overall mass transfer coefficient. Thus, it is to be expected that changing the stagnant film thickness in the organic phase would have a more pronounced effect on the overall mass transfer coefficient than a similar change in the aqueous phase. If changing the hydrodynamics only has an effect on the mass transfer by changing the thickness of the stagnant film adjacent to the interface, then, from Figure 7-4, it is possible to say the thickness of the stagnant film is directly proportional to the hydrodynamics (Reynolds number) of the

phase. Indeed, it can be seen for the solvent side that doubling the Reynolds number leads to a doubling of the overall mass transfer coefficient, however, the same is not true for the aqueous phase. Furthermore, the overall mass transfer coefficient tends to zero as the Reynolds number on the solvent side is reduced but again this is not true for the aqueous side. This demonstrates that the hydrodynamics in the solvent phase strongly controls the mass transfer process where transport is by diffusion only. The effect of changing the aqueous side film thickness does not appear to be as significant, and the diffusion process may be aided by eddy penetration in to the interfacial film.

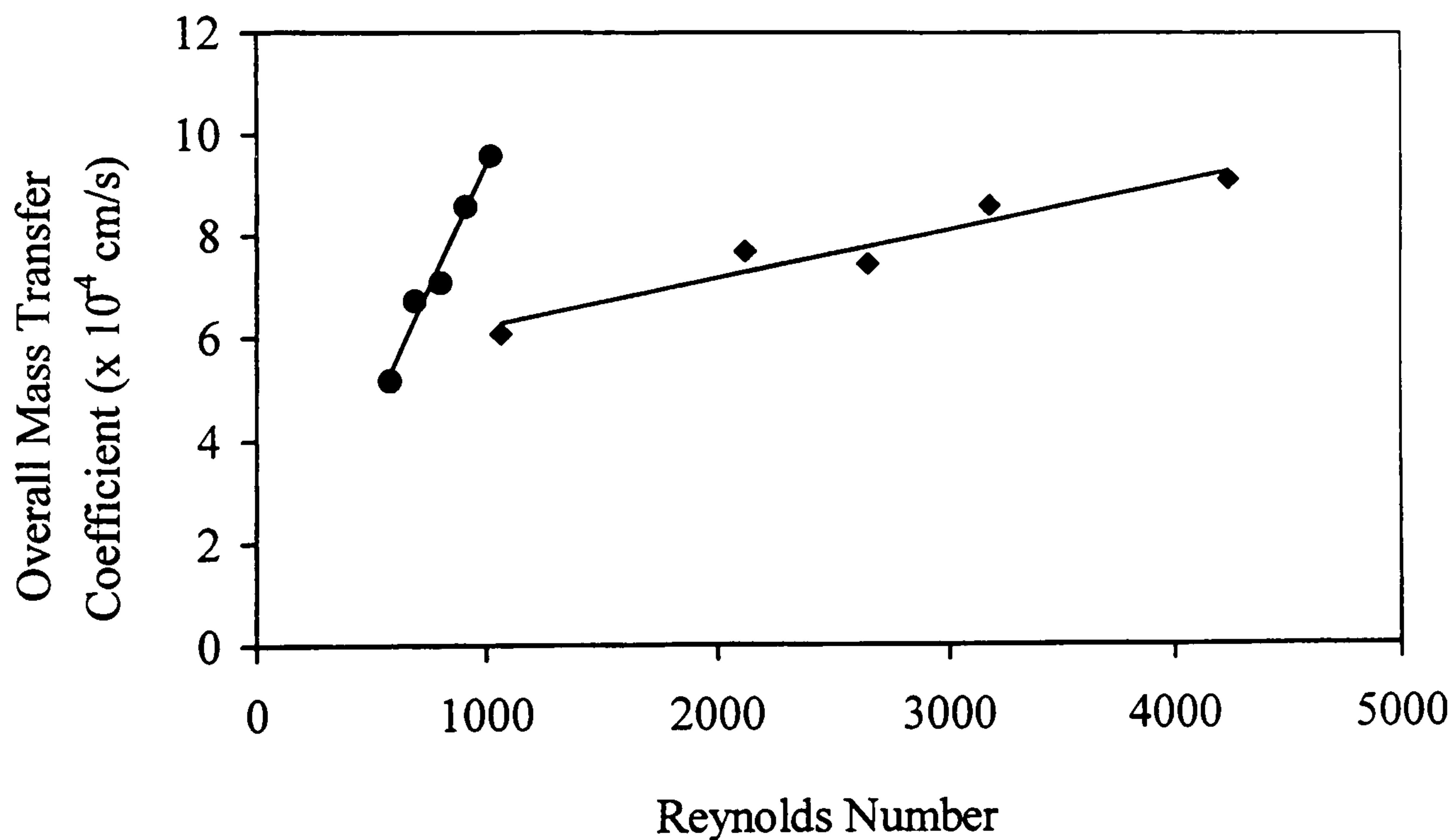


Figure 7-4 The effect of phase hydrodynamics on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. For organic phase variation, ●, N_{Sol} = 160 to 280 rpm while N_{Aq} = 90 rpm. For aqueous phase variation, ◆, N_{Aq} = 30 to 120 rpm while N_{Sol} = 250 rpm. Initial chloramphenicol concentration of 0 to 250μm, pH = 5.5, temperature = 25°C, and PVR = 1.

7.5 The Effect of Concentration Driving Force on Dynamic Mass Transfer

In the two-film theory it is assumed that the partition coefficient is not a function of concentration, and it was shown in Section 7.2 that this assumption is satisfied for the system under study. It is often assumed that the overall mass transfer coefficient does not vary with concentration, however, there are reports of this effect occurring (Austin and Sawistowski, 1967; Olander and Reddy, 1964), but the nature of the effect is unclear. A possible explanation, when the system is diffusion controlled, is that the diffusion coefficient varies with concentration.

Figure 7-5 shows the effect of increasing initial chloramphenicol concentration on the overall mass transfer coefficient. As can be seen this has no effect on the value of the overall mass transfer coefficient over the range of concentrations examined. Therefore, the effect of variable transport properties within the film can be discounted when considering effects on mass transfer in this system.

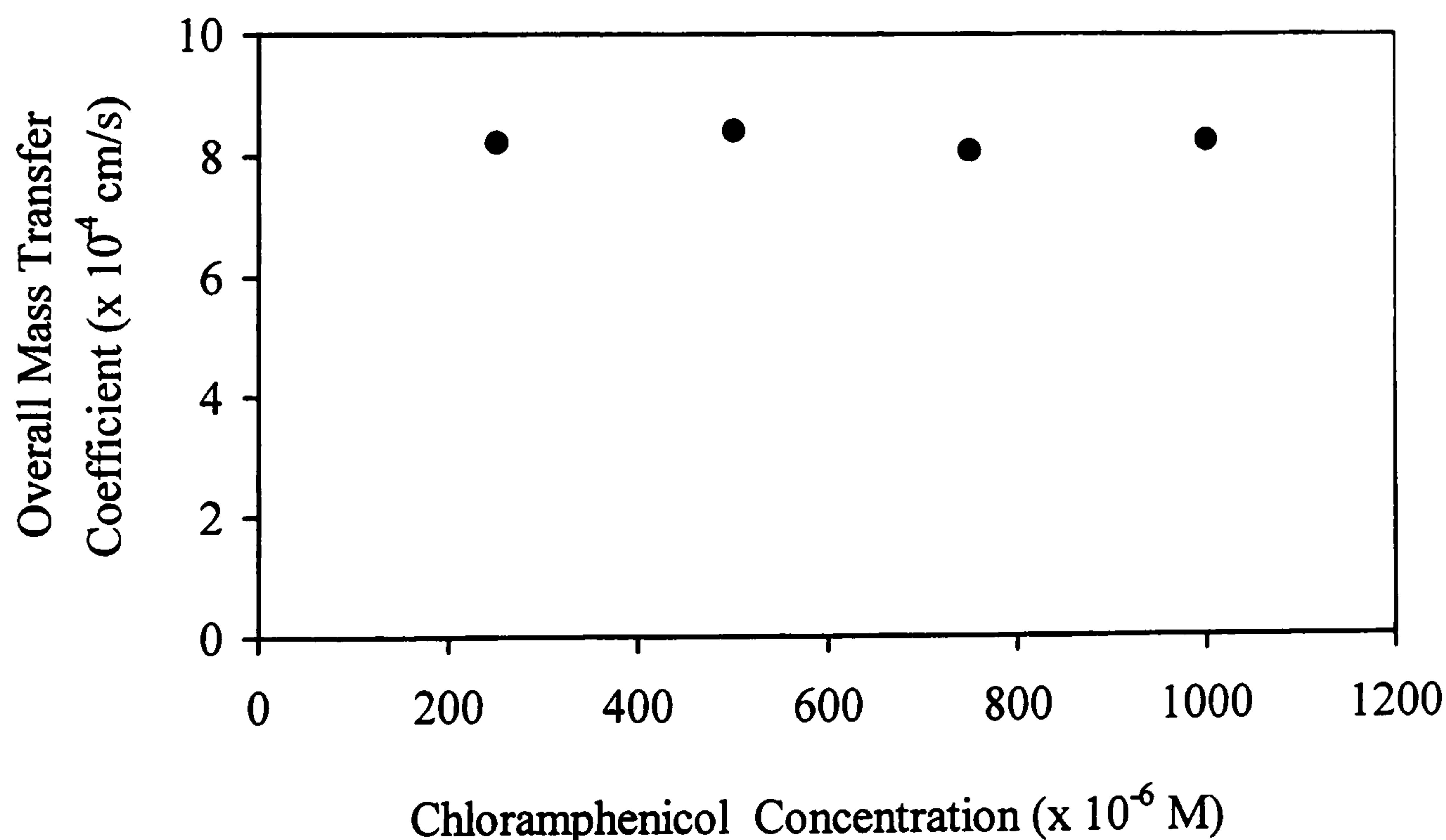


Figure 7-5 The effect of the initial concentration of chloramphenicol on the overall mass transfer coefficient for extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 0 to 1000μm, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

7.6 The Effect of Media Components on Solute Extraction

To obtain samples of fermentation broth for use in this investigation, small-scale fermentations of a chloramphenicol producing bacterium *Streptomyces venezuelae* were conducted. Further details of the fermentation procedure, including the media composition, are given in Section 5.6.1. However, before investigating the effect of the post-fermentation culture fluid it was necessary to ascertain whether any of the original components of the media had any effect on the mass transfer process. This is necessary because during the fermentation not all of the components will be utilised, and so a quantity of each component will be present in the liquid-liquid extraction system. The effect of each of the main components, and the entire formulation was examined, and also the effect of phosphate buffered saline (PBS), as this formulation is used later when studying the effects of proteins.

The results of mass transfer experiments are presented in Figure 7-6, where it can been seen that, within the limits of the experimental error, the components of the growth medium, either individually or together, have no effect on mass transfer. The effect of these components on the equilibrium partition coefficient and the interfacial tension were also investigated, however, again no effect was observed. The components used in the media formulation for chloramphenicol production had no effect on the equilibrium partitioning, the mass transfer, or the interfacial properties of the two-phase system. Therefore, results presented later for the effect of fermentation broth components can be attributed to compounds produced by the bacteria during the fermentation, and not to any compounds originally present.

The media components examined here were based on the medium formulations developed by Gottlieb and Diamond (1957). However, many other compounds are used in media formulations, and often these compounds can be quite ill defined, and may possibly contain compounds that are surface active and will affect the extraction process. In addition to the original media components many other types of compounds may be added to improve the process. For example, antifoaming agents are added to aid process conditions, or surfactants can be used in novel extraction techniques. Later in this chapter the effect of such compounds on liquid-liquid extraction are examined.

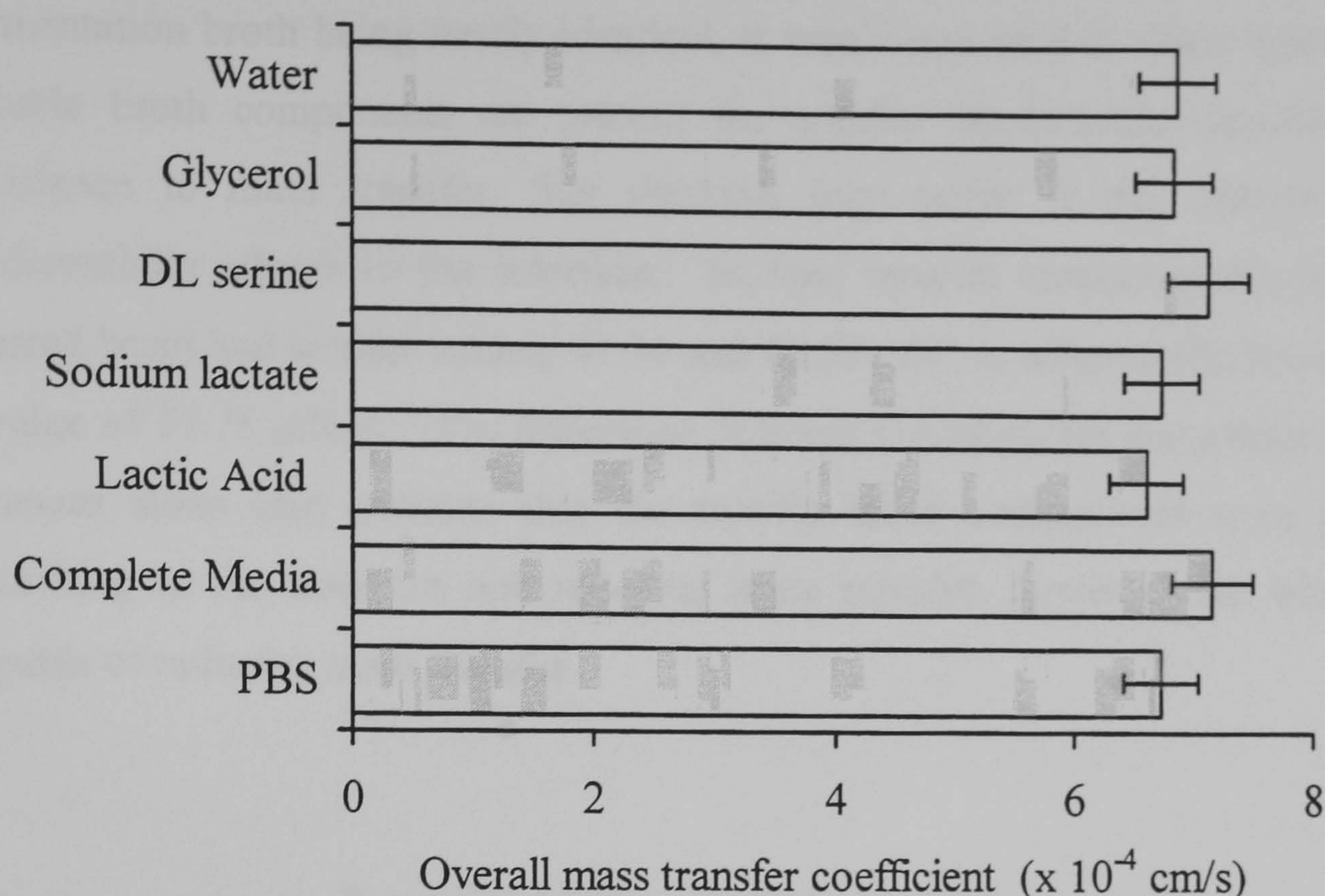


Figure 7-6 The effect of media components on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

7.7 The Effect of Fermentation Broth on Solute Extraction

In Chapter 2 a review of the surface-active effects of compounds produced by bacteria was given. From this information it is apparent that micro-organisms may produce a wide range of compounds that, even at very low concentrations, can have a marked influence on interfacial processes. In this section the effect of whole and filtered fermentation broth, and also the biomass alone, on the overall mass transfer coefficient was investigated

Figure 7-7 shows the effect of the three different grades of fermentation broth on the overall mass transfer coefficient. It is seen that extraction of chloramphenicol from any form of fermentation broth shows a considerable reduction in the overall mass transfer coefficient. The concentration of the biomass was 1g/l, and was found to reduce the overall mass transfer coefficient by 50% compared to extraction from de-ionised water. When soluble fermentation broth components were present, either with or without the biomass, the reduction was over 70%. With the values for whole and filtered

fermentation broth being nearly identical, it would appear that when both biomass and soluble broth components are present the soluble components constitute the main resistance to mass transfer, this situation may occur if the soluble components preferentially adsorb to the interface. Surface tension measurements for whole and filtered broth had similar values, 44.34 and 45.85 mN/m, whereas the biomass recorded a value of 57.75 mN/m. The difference between the value for the whole broth and the biomass alone may indicate that the soluble broth components were preferentially adsorbing to the interface and reducing mass transfer, however, the biomass is also capable of reducing mass transfer.

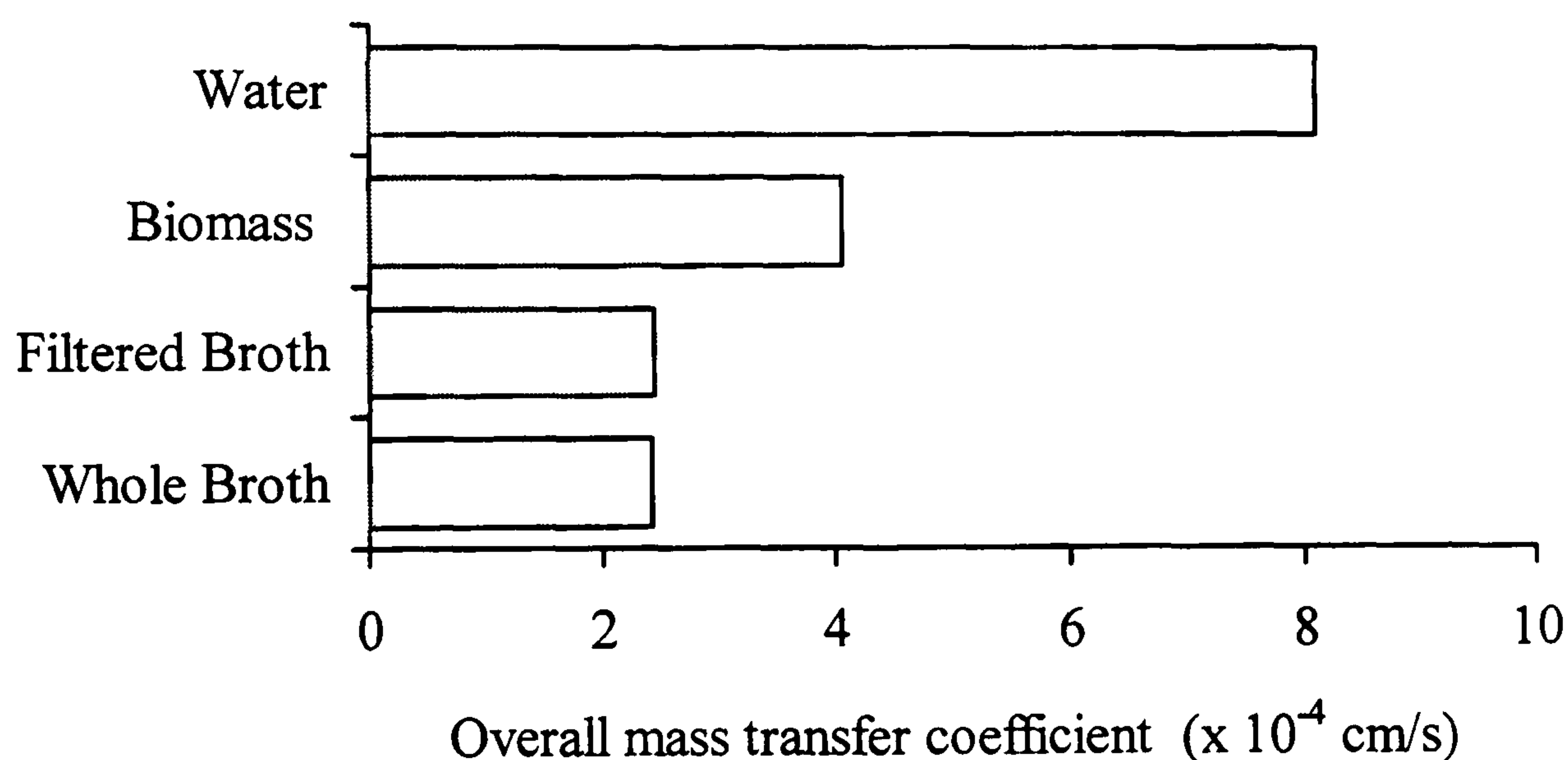


Figure 7-7 The effect of different grades of fermentation broth on the overall mass transfer coefficient for chloramphenicol extraction into 1-octanol. Initial chloramphenicol concentration of 250µm, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

7.7.1 Filtered Fermentation Broth

Fermentation broth may contain many soluble components besides the compound to be recover. This section will attempt to shed some light on the nature of these compounds and the effect that they have on mass transfer. From a series of dilutions of the filtered broth it is possible to examine the effect of the biosurfactant concentration on the mass transfer and surface properties of the liquid-liquid system.

In Figure 7-8 the effect of dilution of the filtered fermentation broth on surface tension is presented. It can be seen that an increase in the concentration of broth components caused a reduction in the surface tension of the solution. This behaviour is typical of surface-active compounds, and that by diluting the bulk solution the concentration of surface-active compounds at the interface is reduced. The trend of the reduction suggests that the undiluted solution had not reached its critical micelle concentration. Thus the surface was not completely saturated with surface-active molecules, however, the trend does appear to be close to a minimum.

In Figure 7-9 the effect of a series of dilutions of the filtered fermentation broth on the overall mass transfer coefficient is presented. It can be seen that the overall mass transfer coefficient decreases as the concentration of filtered broth increases, and the trend is similar to the effect of dilution on surface tension seen in Figure 7-8. It is concluded from this that surface active compounds in the filtered fermentation broth are adsorbing to the liquid-liquid interface, and that they are affecting mass transfer. Similar to the trend for the surface tension, the reduction in the overall mass transfer coefficient did not reached a minimum. Thus, if more surface active compounds were present then it is probable that a further reduction in the overall mass transfer coefficient would occur. However, this information tells us nothing about the quantity, size, or effect of any individual compounds, therefore, in the next section the filtered broth was characterised using ultrafiltration and total carbon analysis.

7.7.2 Weight Fractionated Fermentation Broth

In order to characterise the filtered fermentation broth an ultrafiltration membrane cell was used to separate the broth into molecular weight fractions. Using this procedure it was anticipated that the size range of compounds which had the largest influence on the interfacial process would be apparent. The ultrafiltration membrane cell, which is described in Section 6.5.3.4, allowed the filtered fermentation broth to be separated into eight molecular weight fractions between 500 and 300,000 Daltons. Each weight fraction was reconstituted to the original broth volume so as to preserve the original concentration of the compounds.

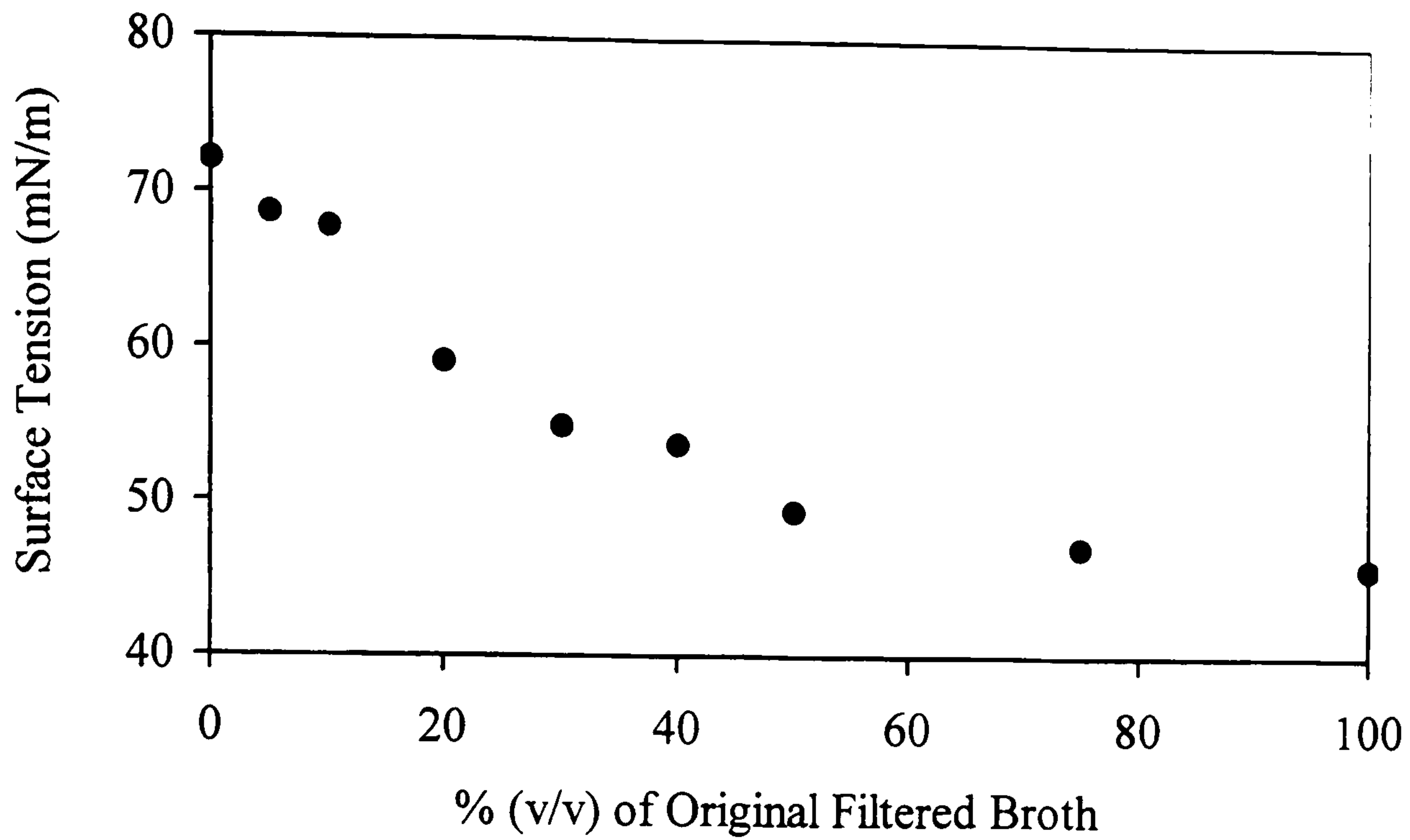


Figure 7-8 The effect of diluting the filtered fermentation broth on the solution surface tension. Initial chloramphenicol concentration of 250μm, pH = 4.7-5.5, temperature = 25°C.

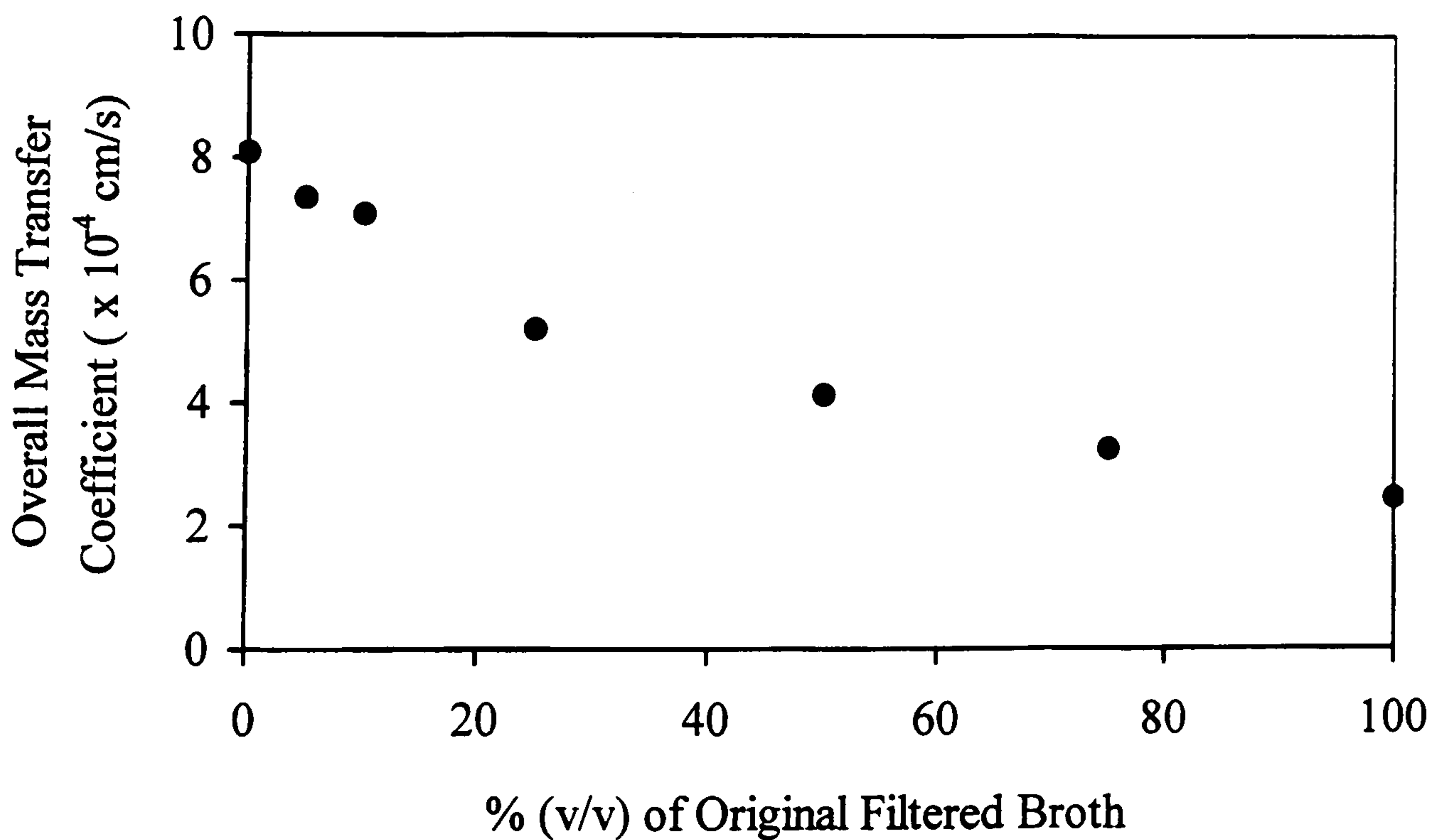


Figure 7-9 The effect of diluting the filtered fermentation broth on the overall mass transfer coefficient for chloramphenicol extraction by 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 4.7-5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR=1.

In Figure 7-10 the effect of each molecular weight fraction on the surface tension is presented. All the weight fractions and the complete filtered broth had surface tensions lower than that of water, indicating the presence of surface active compounds in every fraction. The surface tension decreased as the weight fractions decreased down to the fraction of 10,000 to 30,000 Daltons, where a minimum surface tension of 53.99 mN/m was recorded. Thereafter the surface tension increased with decreasing molecular weight except for the lowest fraction, under 500 Daltons, where the surface tension reaches a new minimum of 53.76 mN/m. The surface tension for the complete filtered fermentation broth (45.84 mN/m) was lower than any of the individual fractions. Therefore, it appears that in combination the broth components exert a greater effect on the interface than they do individually. No CMC was observed for the filtered broth (Figure 7-8) indicating that the interface was not completely occupied by molecules. Thus all the surface active components may adsorb to the interface, and their combined surface activity would reduce the surface tension. However, for a particular weight fraction there would be a smaller amount of material at the interface and the effect on the interface would be lower.

The effect of each weight fraction on the overall mass transfer coefficient obtained from Lewis cell experiments is presented in Figure 7-11. The main effect of the fermentation broth components would appear to be in the four middle fractions over the range 1,000 to 100,000 Daltons, and particularly the range 10,000 to 30,000 Daltons where the overall mass transfer coefficient was reduced by 50% compared to water. This was also the weight range where the greatest reduction in surface tension was observed. Outside these fractions the overall mass transfer coefficient was, within experimental errors, unaffected by components in the broth. None of the fractions were able to reduce the overall mass transfer coefficient to the same extent as the complete filtered fermentation broth which caused a reduction of over 70% compared to extraction from de-ionised water. This may occur for a similar reason to that described above, where the amount of adsorbed material at the interface in the fractionated systems is lower than for the complete system, therefore, the resistance to mass transfer is reduced.

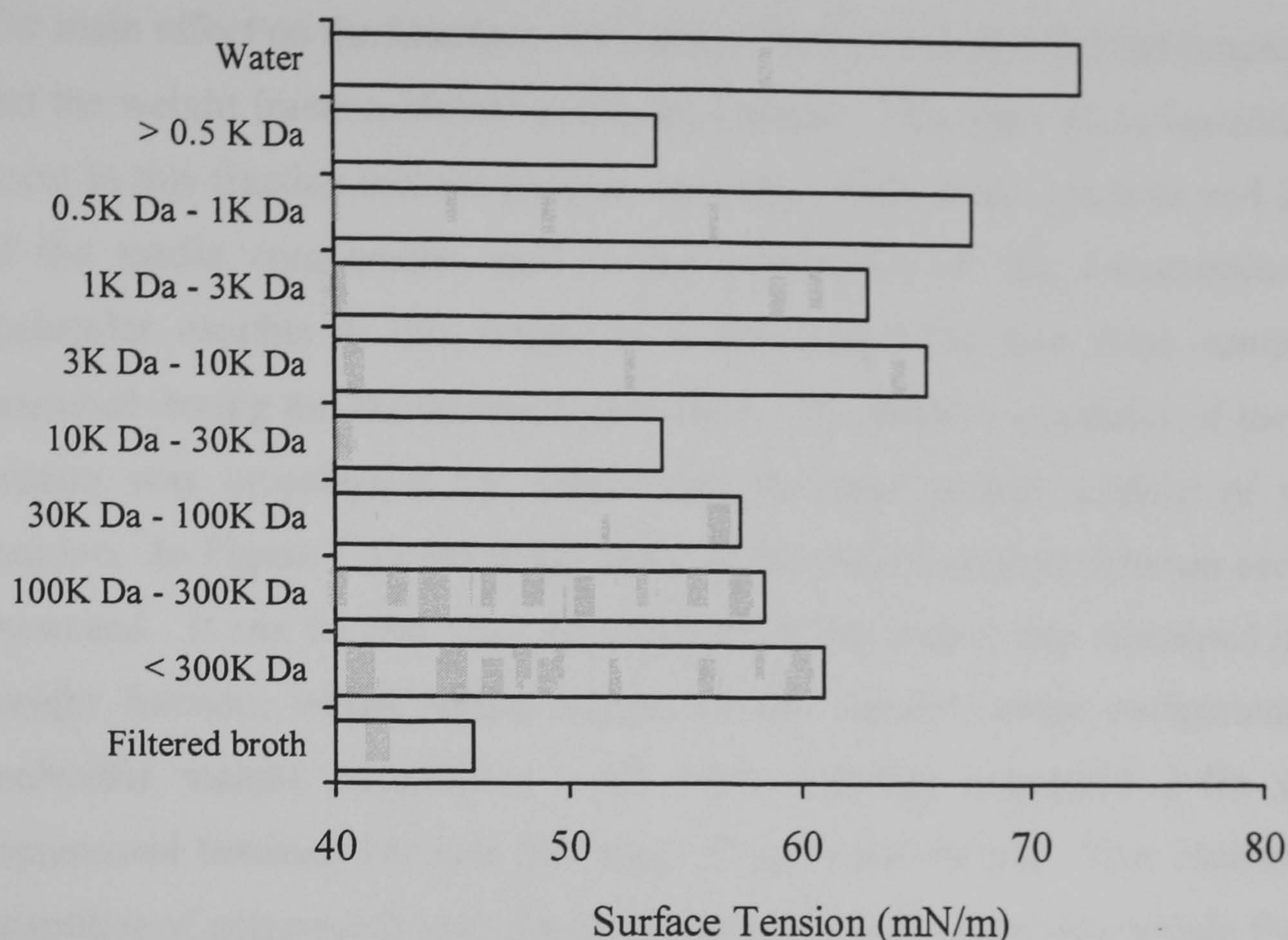


Figure 7-10 The effect of molecular weight fractions on the solution surface tension. Initial chloramphenicol concentration of 250μm, pH = 5.5, temperature = 25°C.

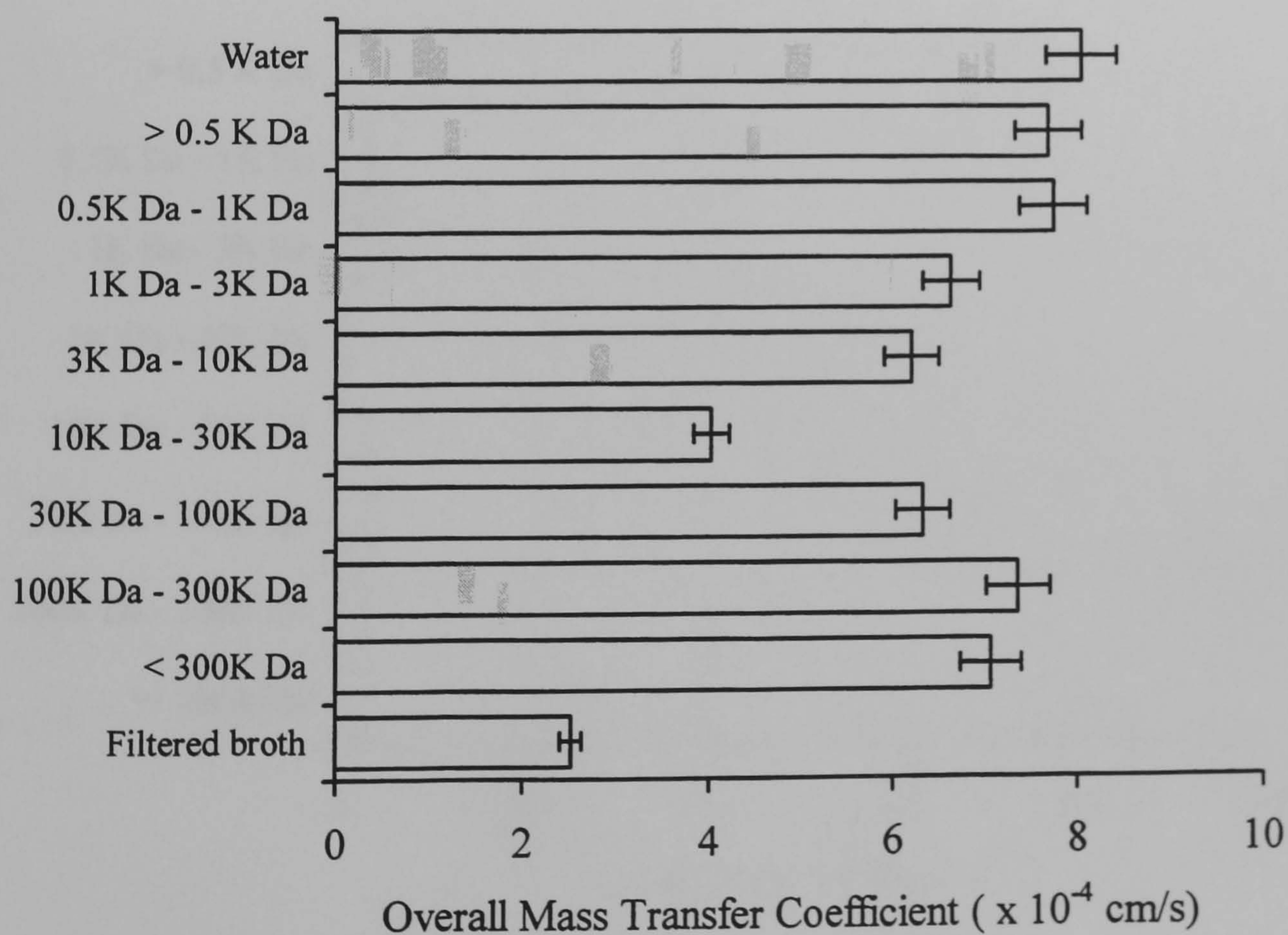


Figure 7-11 The effect of molecular weight fractions on the overall mass transfer coefficient for chloramphenicol extraction into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR=1.

The main effect on the interface was caused by the complete filtered fermentation broth and the weight fraction 10,000 to 30,000 Daltons. The types of compounds that would occur in this fraction include polymer or polypeptide chains, proteins and lipids. None of the media components used in the production of this fermentation broth had molecular weights in this range, so it was concluded that these compounds were produced during the fermentation processes. The relative quantities of the compounds present was investigated by determining the total carbon content of each weight fraction. In Figure 7-12 the distribution of the carbon content between each fraction is presented. It can be seen that the majority of the carbon was contained in the lowest weight fraction, which would consist of any unused media components and low-molecular weight metabolites. All other fractions contained 2-4% each, which represented between 150 and 300 mg/l of elemental carbon. This indicates the small quantities of compounds that may be present, and with increasing weight fraction would represent a decreasing number of moles of compound.

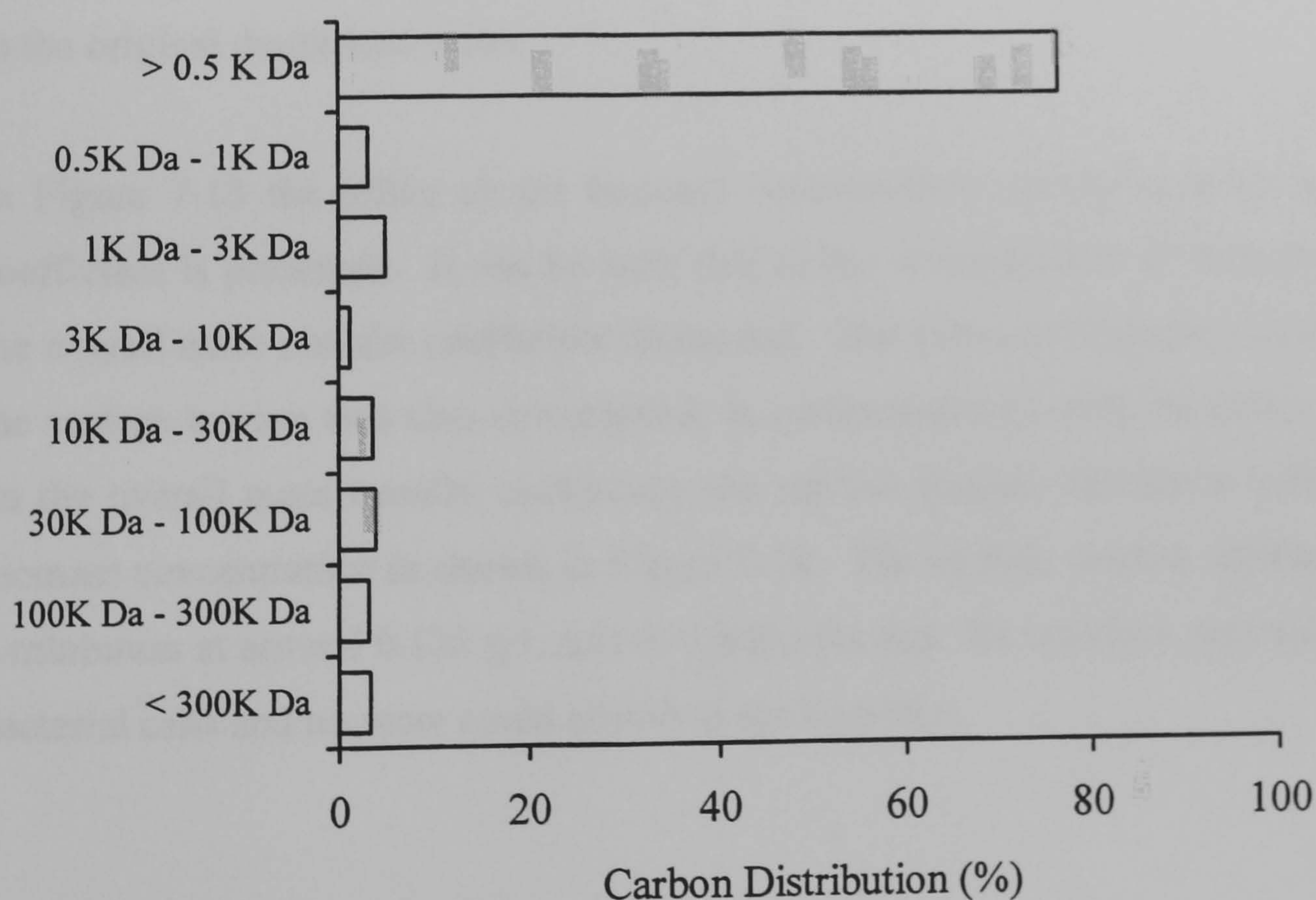


Figure 7-12 The distribution of carbon across the molecular weight fractions.

7.7.3 Biomass

Bacterial cell walls are derived from carbohydrates and contain many polymeric compounds. The main function of the wall is to provide mechanical strength for the cell membrane and allow the cell to cope with osmotic stress and other environmental shocks. The surface properties of a cell are due to the cell walls, which have a variable structure that is dependent on environmental conditions. Typically cell membrames are composed of carbohydrates, proteins, polymeric and lipid compounds, of which many have surface-active and electrostatic properties that they impart to the cell wall, and generally cell walls are negatively charged. The surface-active nature of biomass will cause it to concentrate at the two-phase interface, where, in this case, it may affect the mass transfer process.

Biomass was obtained by centrifuging the whole fermentation broth. To remove any traces of soluble broth components the biomass was then washed by re-suspending it in de-ionised water, and then recovered again by centrifuging the suspension. The washing procedure was repeated until the surface tension of the wash solution was equal to the original de-ionised water.

In Figure 7-13 the effect of the biomass concentration on the overall mass transfer coefficient is presented. It can be seen that as the concentration of biomass increased the overall mass transfer coefficient decreased. The effect of biomass concentration on the surface tension was also investigated; in correspondence with the effect of biomass on the overall mass transfer coefficient, the surface tension decreased with increasing biomass concentration as shown in Figure 7-14. The surface tension appeared to reach a minimum at around 0.125 g/l, and this indicated that the interface was saturated with bacterial cells and no more could adsorb to the interface.

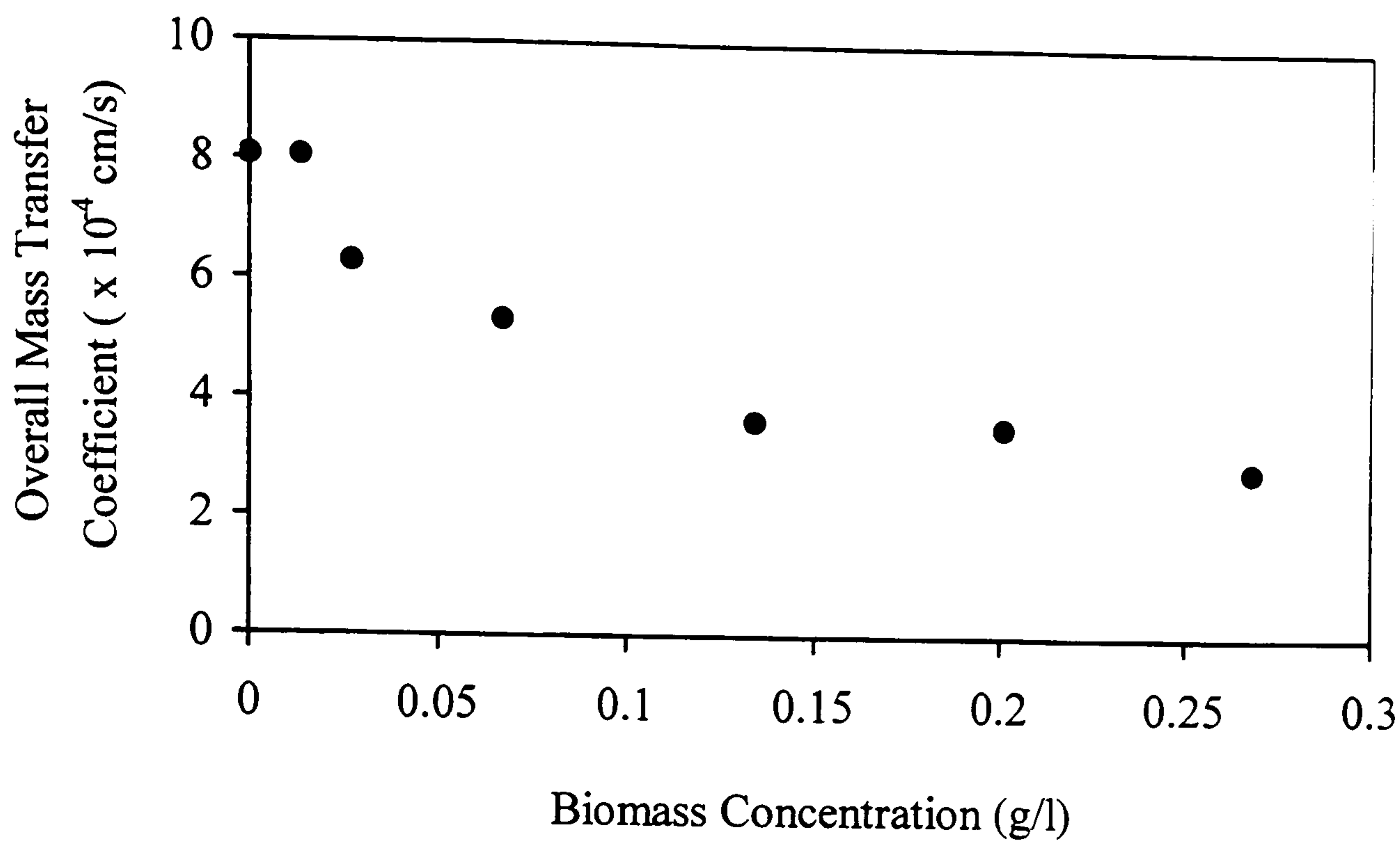


Figure 7-13 The effect of biomass concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR=1.

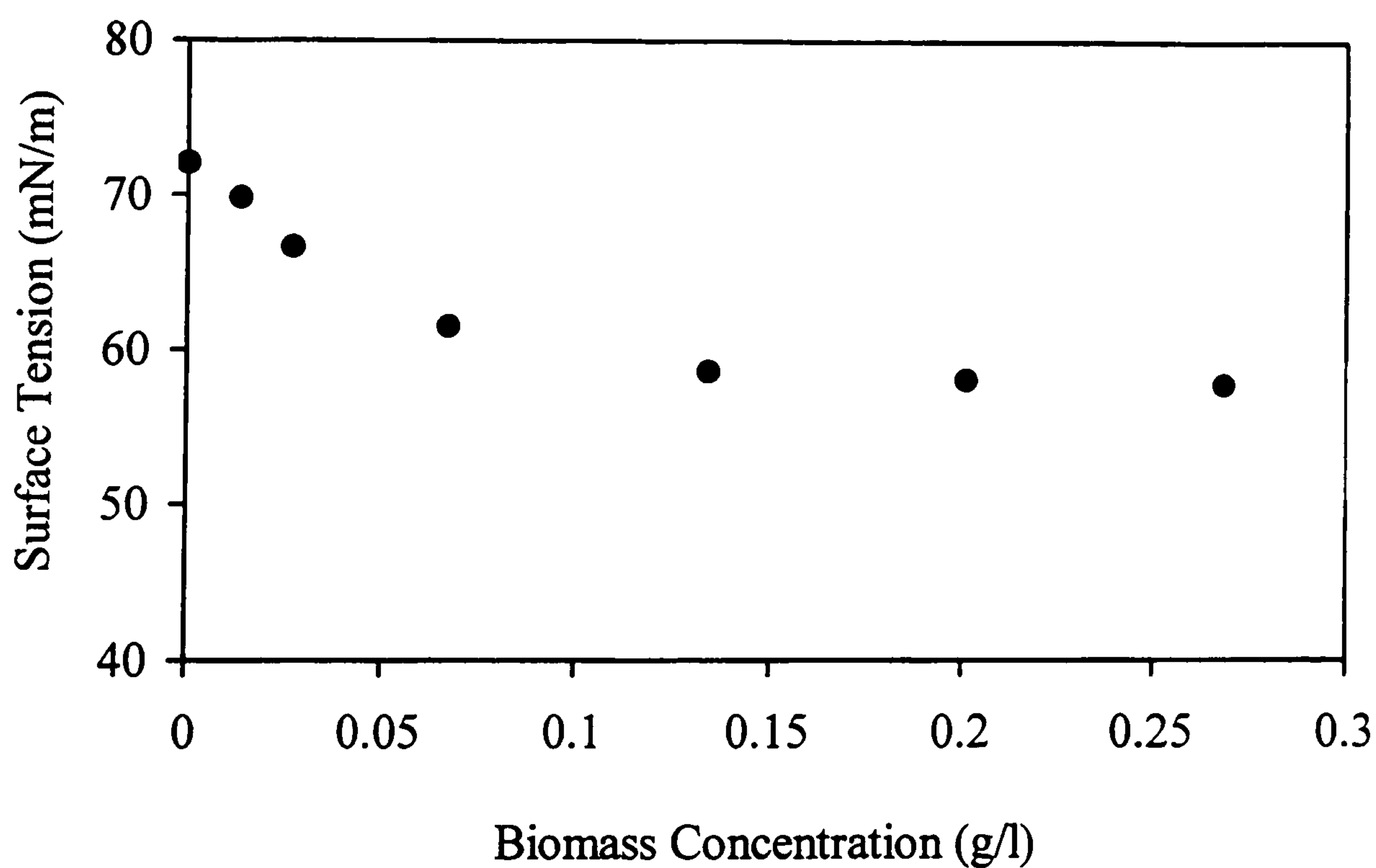


Figure 7-14 The effect of biomass concentration on the surface tension of chloramphenicol solutions. Initial chloramphenicol concentration of 250μm, pH = 5.5, temperature = 25°C.

Although the surface tension reached a minimum at 0.125 g/l, the overall mass transfer coefficient continued to decrease with increasing biomass concentration. This effect may be explained by the formation of multi-layers of biomass, which would not affect the interface but may affect transport through the interfacial region. In a previous study (Crabbe *et al.*, 1986) the effect of yeast cells on extraction of ethanol was investigated. They found that the biomass caused a reduction in mass transfer at similar concentrations to those studied here. From visualisation of the two-phase interface they reported that multi-layers of biomass formed at the interface.

The effect of biomass on the mass transfer process may not be due to an effect on the mass transfer mechanism, but more a physical process blocking the available interfacial area. The mass transfer model may be re-analysed where the overall mass transfer coefficient is taken as a constant (at the value for zero biomass) and the interfacial area becomes a variable. This would then result in a prediction of the interfacial area available for mass transfer, and would follow an identical trend to that seen for the mass transfer coefficient in Figure 7-13.

The maximum reduction in either the overall mass transfer coefficient, or the interfacial area caused by the addition of biomass was 66%. For particles of equal size the value of surface coverage caused by closely (hexagonal) packed discs is 91%, or for spheres the volume occupied is 74%. Both of these values are above the reduction seen here, however the interfacial configuration and size distribution of the cells at the interface is unknown. The effect on mass transport could either be due to resistance in a two-dimensional plane, which may not pass through the equator of each cell, or a three dimensional volume. A volume model seems more likely as the biomass will be present not only at the interface but also in the adjacent thin film where they will hinder free diffusion of the solute.

7.8 The Effect of Biosurfactants on Solute Extraction

Investigation of the effects of fermentation broth on solute extraction revealed that a significant reduction in the overall mass transfer coefficient was due to compounds present in the weight fraction 10,000 to 30,000 Daltons. This fraction would correspond to compounds such as proteins and lipids. Therefore, in an attempt to correlate molecular properties to observed effects an investigation of the effect of macromolecular compounds was conducted. For this investigation three proteins and a phospholipid compound were used as model compounds. These different types of compounds may all be classified as biosurfactants, as they all are of biological origin and possess the ability to adsorb to interfaces. The biosurfactants examined were the proteins: bovine serum albumin (BSA), β-casein and cytochrome c; and the phospholipid phosphatidylcholine (PC)

Initially to ascertain whether the biosurfactant compounds would adsorb to the interface and affect the liquid-liquid extraction process a Lewis cell experiment was conducted where a protein was added to the aqueous phase after the phases had been contacted. A small quantity (2 cm^3) of a concentrated solution of the protein BSA was injected into the aqueous phase to give a total protein concentration of 0.01g/l. In Figure 7-15 a plot of the concentration function used to determine the overall mass transfer coefficient is presented. The injection of protein, which occurred immediately after the cell was sampled at 2700 seconds. It can be seen that the protein injection had a dramatic effect on the slope of the plot, which changed from 7.04×10^{-4} to 2.54×10^{-4}, and thus on the overall mass transfer coefficient for the system. This result highlights the effect that biosurfactants can have on the interface and the mass transfer process, and furthermore, that in a forced convection system such as the Lewis cell, the slow adsorption kinetics associated with macromolecules is aided significantly by the bulk mixing.

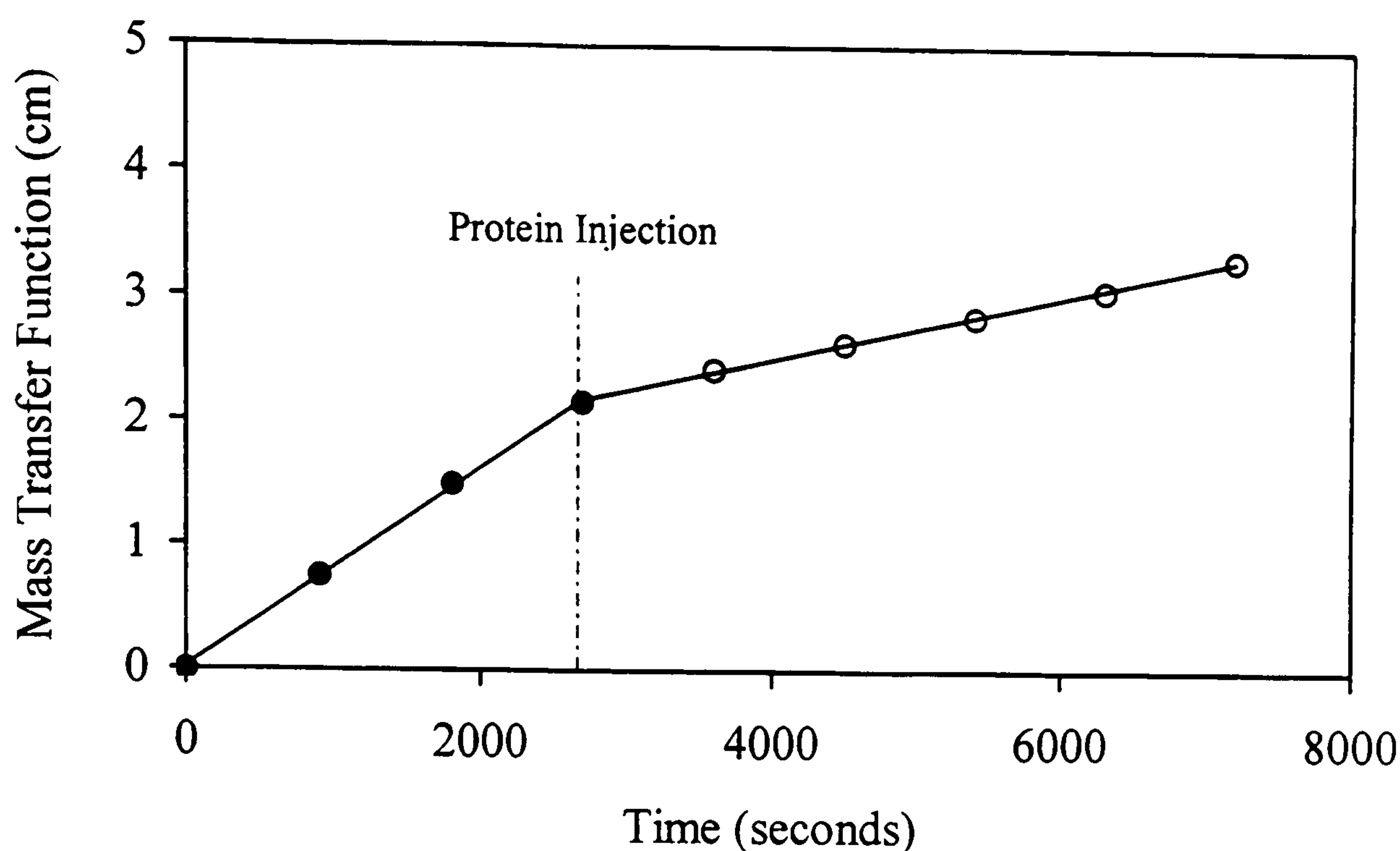


Figure 7-15 The effect of adding BSA during extraction of chloramphenicol from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR=1.

In the rest of this section results are presented for the effect of biosurfactant concentration on the interfacial tension and overall mass transfer coefficient of the liquid-liquid extraction system. In Figures 7-16 to 7-19 the effect of biosurfactant concentration on the overall mass transfer coefficient is presented. It can be seen that in all cases increasing the concentration of the biosurfactant results in a decrease in the overall mass transfer coefficient. This is consistent with the hypothesis that these compounds would affect the mass transfer process. The most notable effects were caused by β-casein and BSA, where the overall mass transfer coefficient was reduced to 2.51×10^{-4} and 2.65×10^{-4} respectively at a concentration of 0.001g/l. For both of these proteins no further reduction in the overall mass transfer coefficient was observed with increasing concentration. For the other two biosurfactants, cytochrome c and PC, similar reductions in the overall mass transfer coefficient were achieved, but at higher concentrations. The reductions observed for all these biosurfactants are similar to the magnitude of the effect caused by the filtered fermentation broth on the overall mass transfer coefficient.

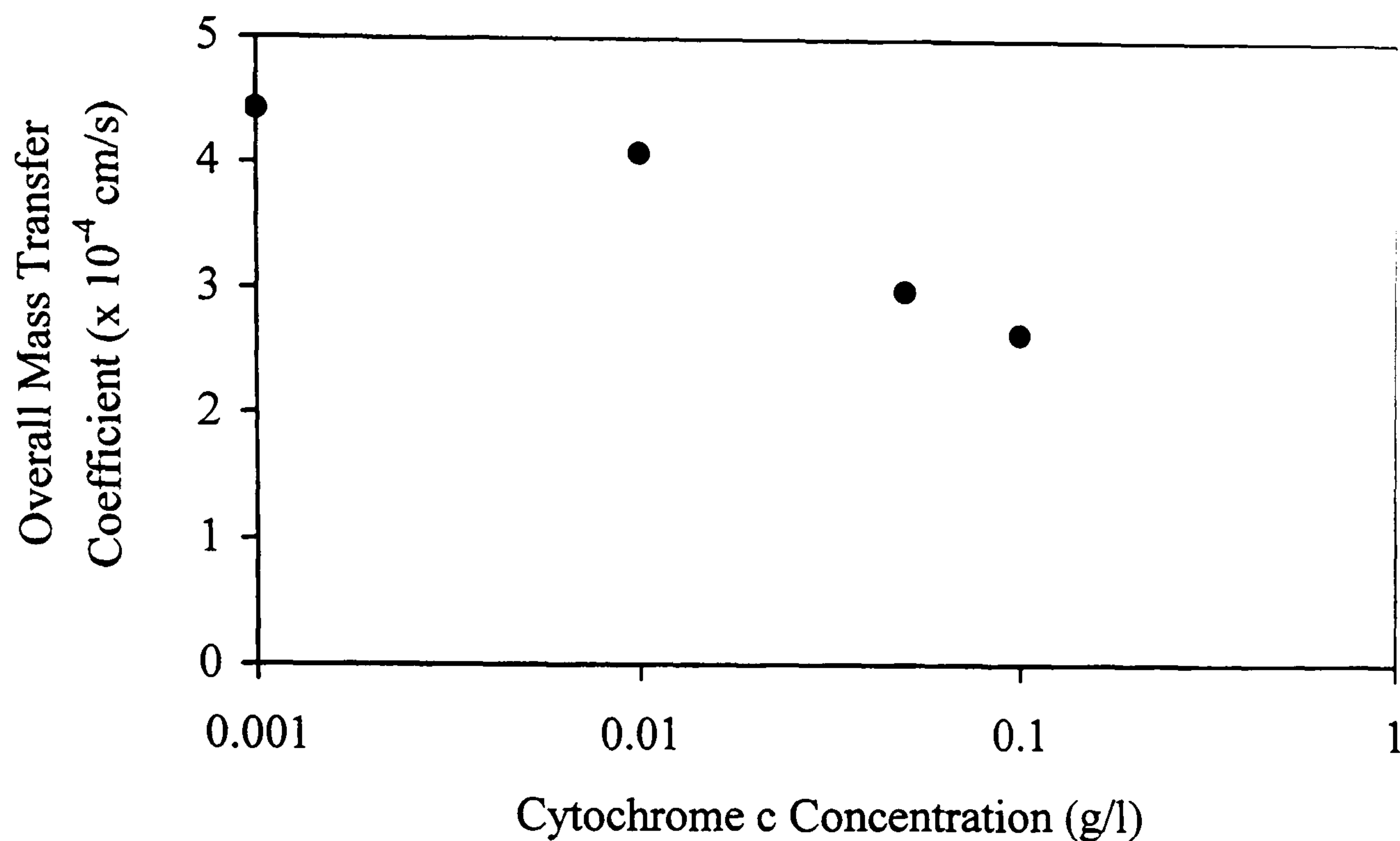


Figure 7-16 The effect of cytochrome c concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

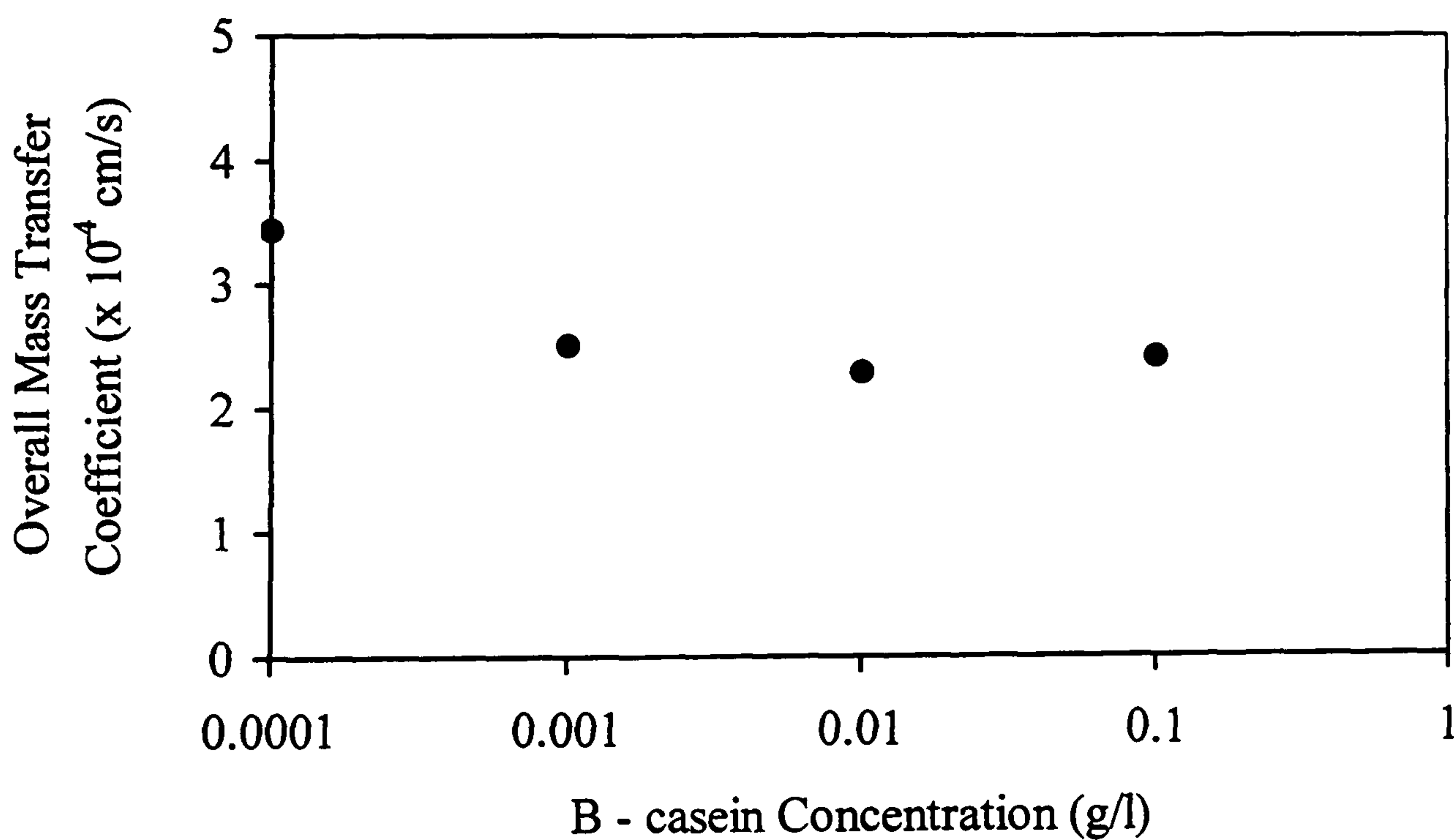


Figure 7-17 The effect of β – casein concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

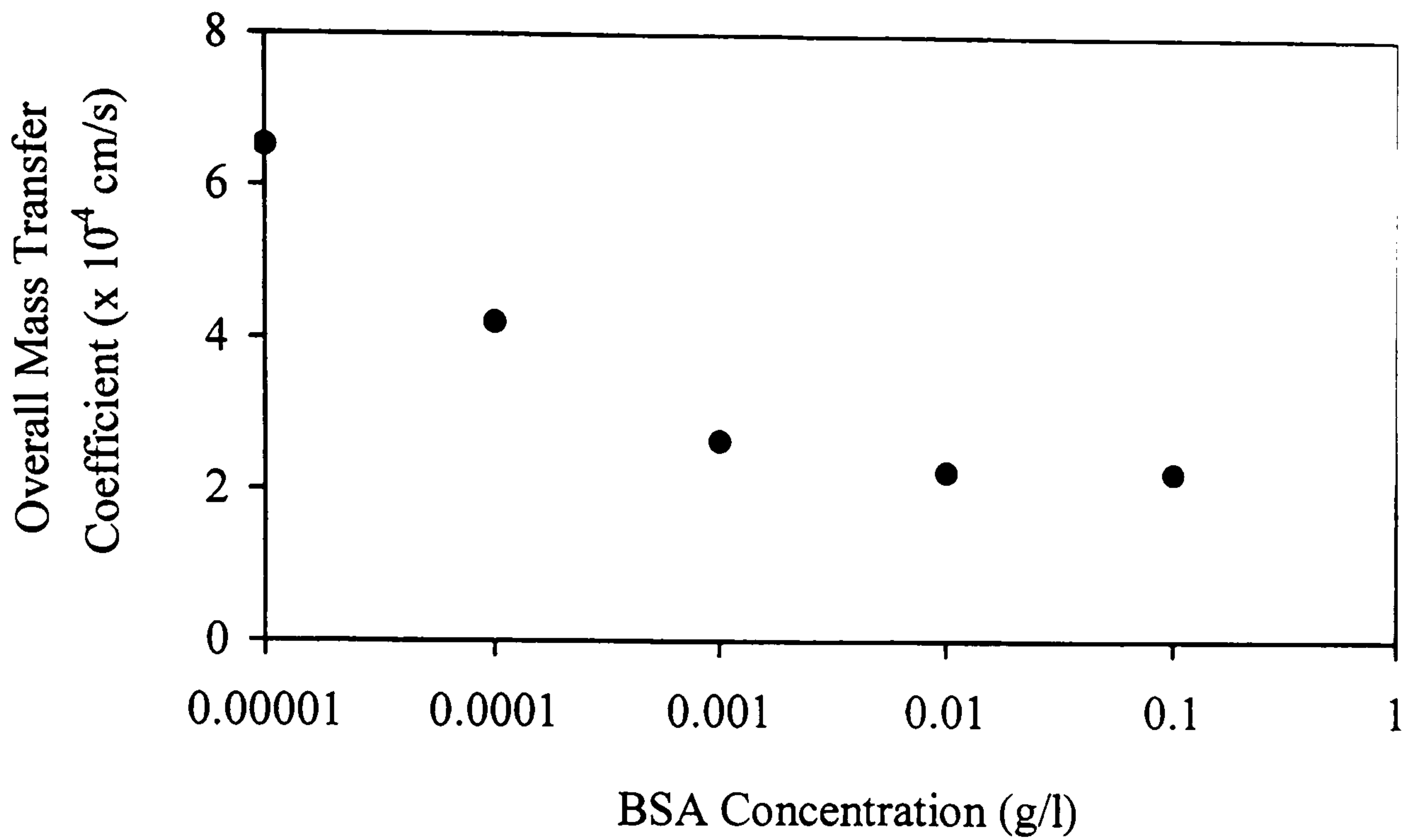


Figure 7-18 The effect of BSA concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

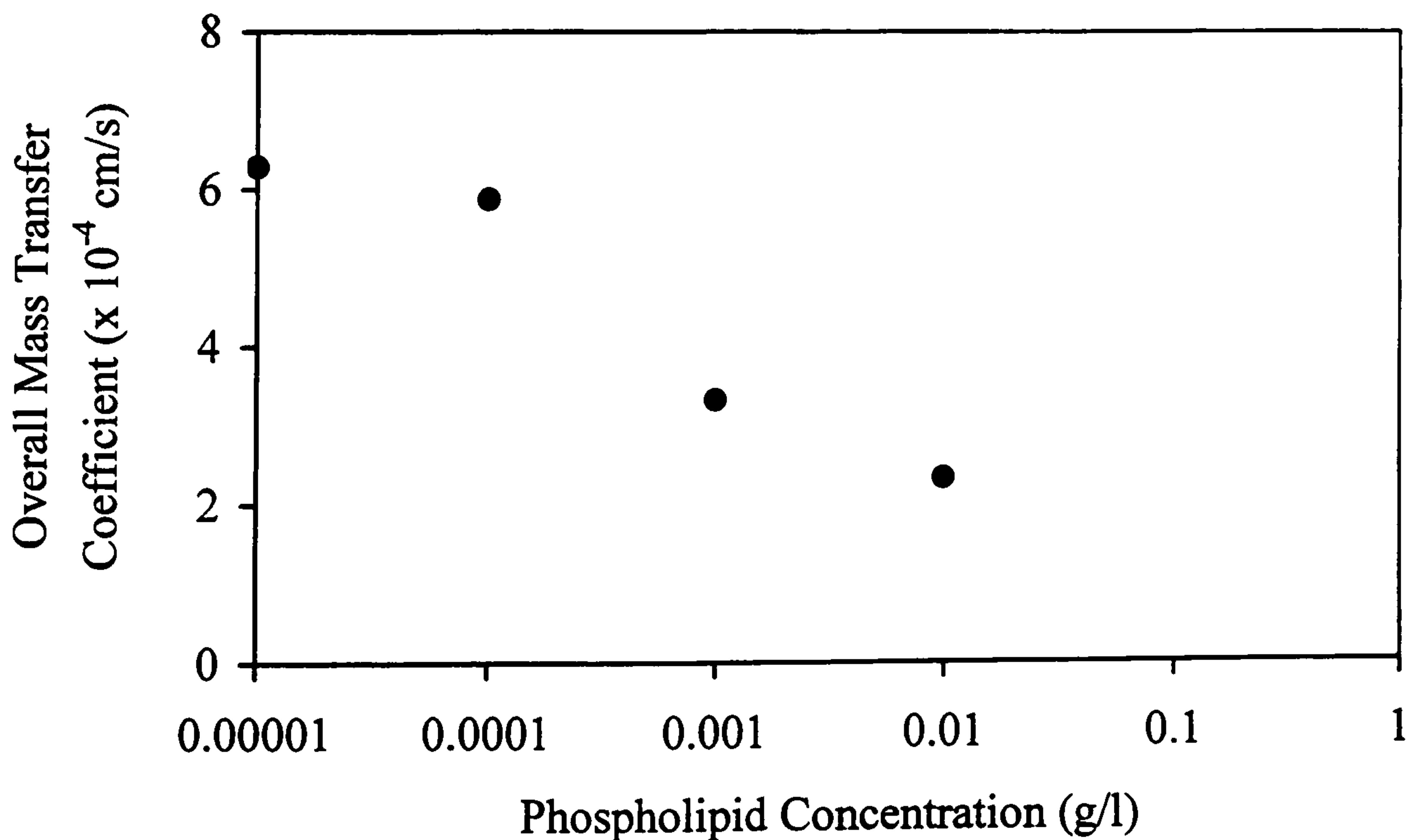


Figure 7-19 The effect of phospholipid concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

The effect of the biosurfactant concentration on interfacial tension was also examined for the three proteins, bovine serum albumin (BSA), β-casein and cytochrome C, and the results are presented in Figure 7-20. The value of the interfacial tension for the chloramphenicol solution with 1-octanol in the absence of any surface-active compounds was found to be 8.84 mN/m. It is seen in Figure 7-20 that both β-casein and BSA reduced the interfacial tension with increasing concentration. However, cytochrome c appeared to have little effect on the interface with only a small reduction in the interfacial tension at the highest concentration examined.

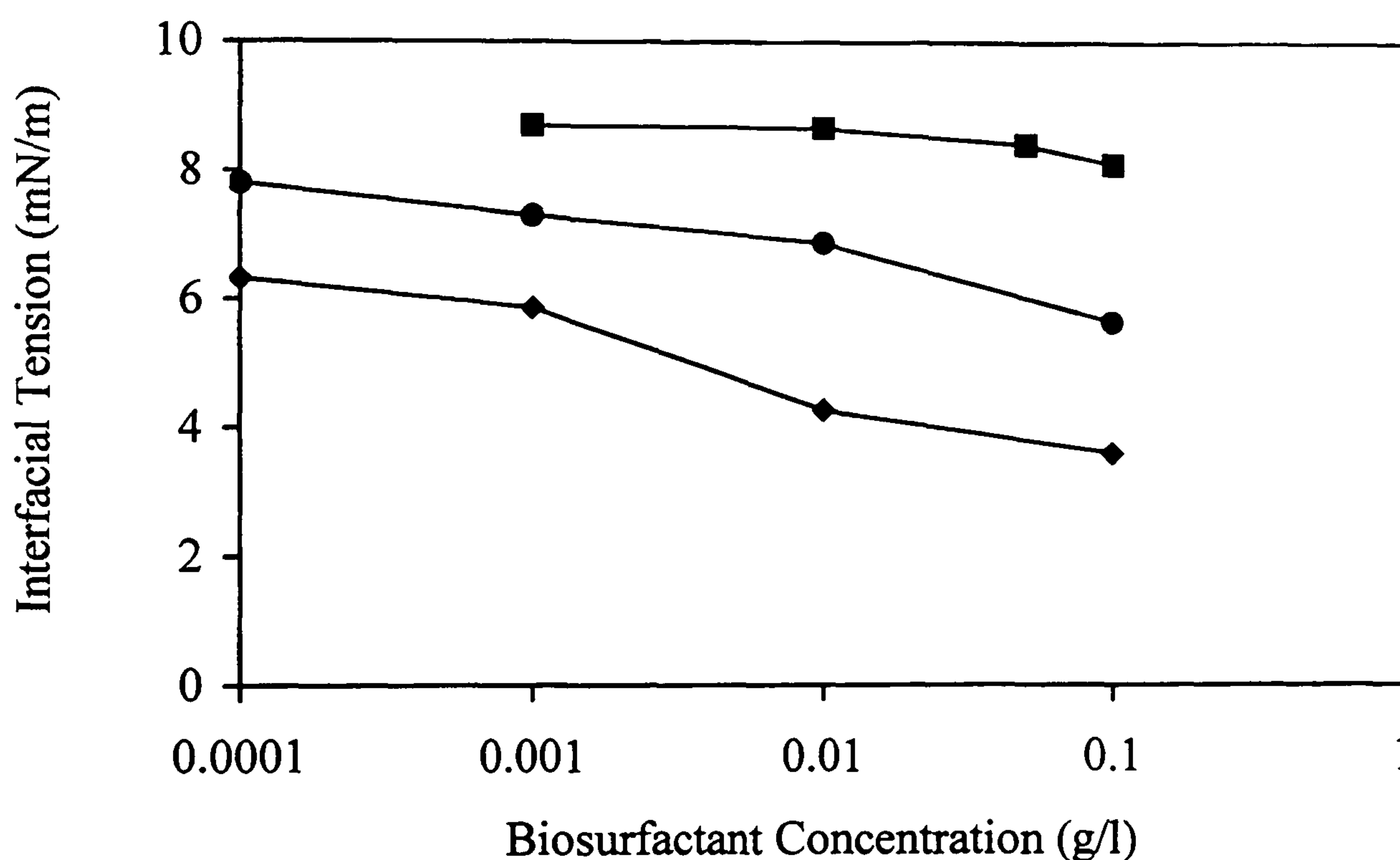


Figure 7-20 The effect of biosurfactant concentration on the interfacial tension between PBS and 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 7, temperature = 25°C, and a PVR = 1. Biosurfactants used were; cytochrome c (■), β-casein (◆), and BSA (●).

These results correlate with the results of mass transfer experiments where both β-casein and BSA were found to have the greatest effect on the overall mass transfer coefficient. The mass transfer results presented earlier showed that both β-casein and BSA reached a minimum at a concentration of 0.001 g/l. However in Figure 7-20 it is seen that beyond this concentration there is still a reduction in the interfacial tension with increasing concentration. It is postulated that this effect can be explained by reconfiguration of the proteins at the interface (Fainerman *et al.*, 1998). At low concentrations the interface may become saturated with protein molecules and

to the system, the protein molecules that are already present at the interface may reorientate themselves to allow further protein molecules to adsorb (Fainerman *et al.*, 2000). This process may not result in any further resistance to mass transfer but will cause the interfacial tension to be reduced as more surface-active moieties will be present there.

In this section it has been show that known biosurfactant compounds can dramatically affect the mass transfer during liquid-liquid extraction. More importantly, it has been shown that very low concentration levels are required to cause this effect. The compounds examined here are typical of compounds that may be produced by bacteria, or are contained in the cell structure of the bacteria that may be released into the fermentation medium during growth or downstream processing.

7.9 The Effect of Antifoam Agents on Solute Extraction

Foaming is a major problem in many fermentation processes, and there are many techniques available to reduce or retard foam using either a mechanical device or an antifoaming agent (Hall *et al.*, 1973). Chemical anti foaming agents act by displacing surface-active compounds at the gas-liquid interface with alternative surface active compounds (the anti foaming agent) which will either destabilise the foam or prevent its formation. Many antifoaming agents are developed for particular processes and the molecular structures are not usually published in the public domain. However, they are generally based on either silicon or polyglycol compounds.

In this section the effect of polypropylene glycol (PPG), on mass transfer was investigated using two different molecular weight polymers, 400 and 2000 g/mol. These compounds are slightly soluble in aqueous solutions and the effect of the solubilised PPG was investigated. PPG is a simple polymeric antifoaming agent, and the two molecular weights relate to different polymer chain lengths. Results are also presented for the effect of adding PPG 2000 to solutions of the filtered fermentation broth, the biomass, and the protein BSA.

In Figure 7-21 the effect of adding PPG 400 on the extraction of chloramphenicol from water is shown. It is seen that the addition of PPG 400 has no apparent effect on the mass transfer process, except for the lowest concentration examined where there was an increase in the value of the overall mass transfer coefficient. Surface tension measurements (not shown) indicate that PPG 400 was adsorbing to the interface, however, its presence had no influence on the extraction of chloramphenicol.

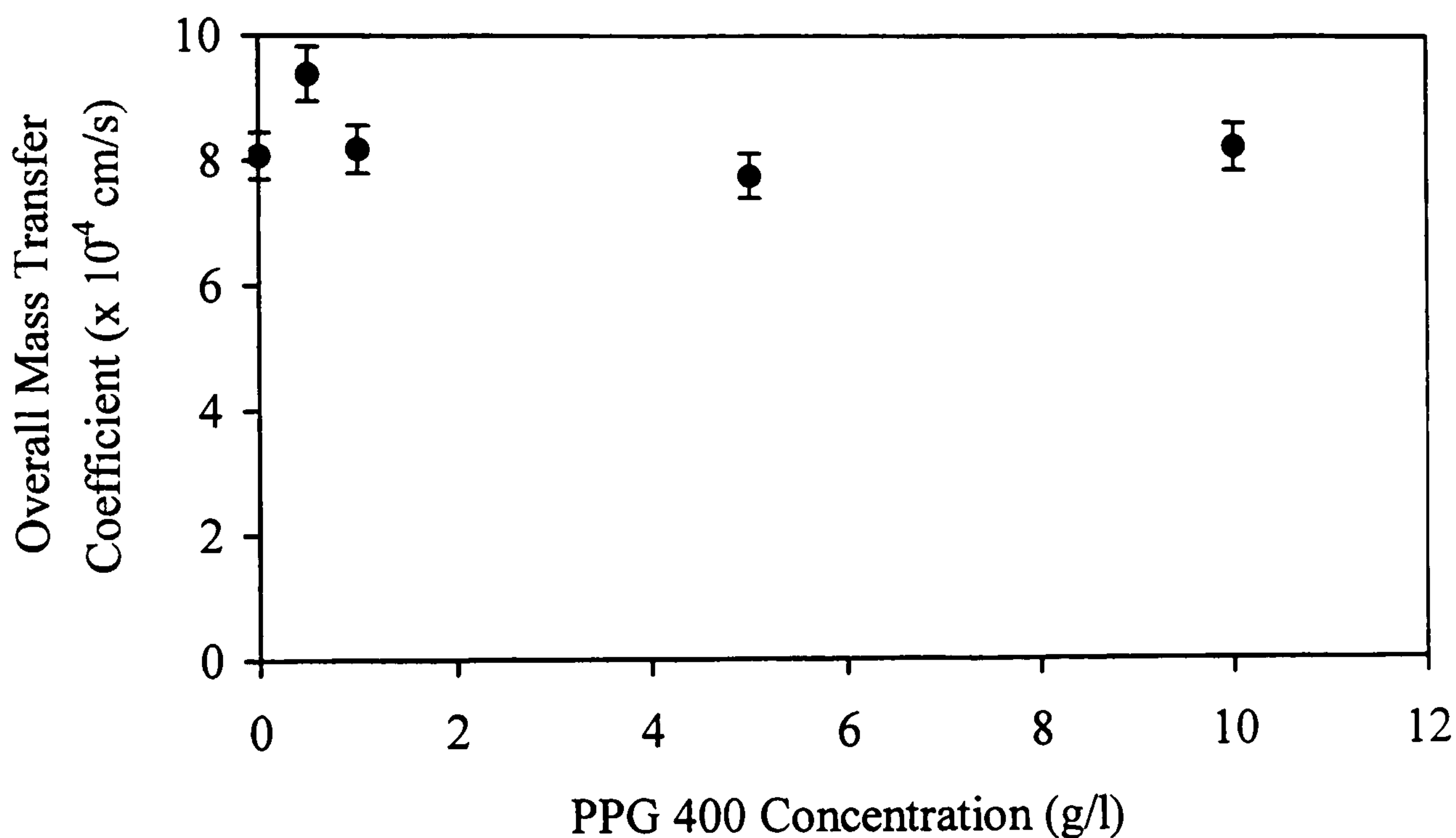


Figure 7-21 The effect of added PPG 400 concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised into 1-octanol. Initial chloramphenicol concentration of 250µm, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

The increased non-polar hydrocarbon chain length of PPG 2000 resulted in it having a reduced aqueous solubility compared to PPG 400. Therefore, the concentration range studied was considerably lower than for PPG 400. The effect of the adding PPG 2000 on extraction of chloramphenicol from water is shown in Figure 7-22. At low concentrations there was a slight increase in the overall mass transfer coefficient, however, this diminishes at the highest concentration studied. Surface tension measurements showed a reduction to 40.4 mN/m for a 20 mg/l aqueous solution of PPG 2000. For concentrations beyond 20 mg/l, the surface tension measurements did not

2000. For concentrations beyond 20 mg/l, the surface tension measurements did not vary. The surface tension measurements indicated that PPG 2000 was adsorbing to the interface, and at low concentrations this adsorption had a slight positive effect on the mass transfer process. During Lewis cell experiments using the higher concentrations of PPG 2000 (200 and 500 mg/l) the aqueous phase went milky white indicating the emulsification of the solvent phase, which remained stable for at least two months.

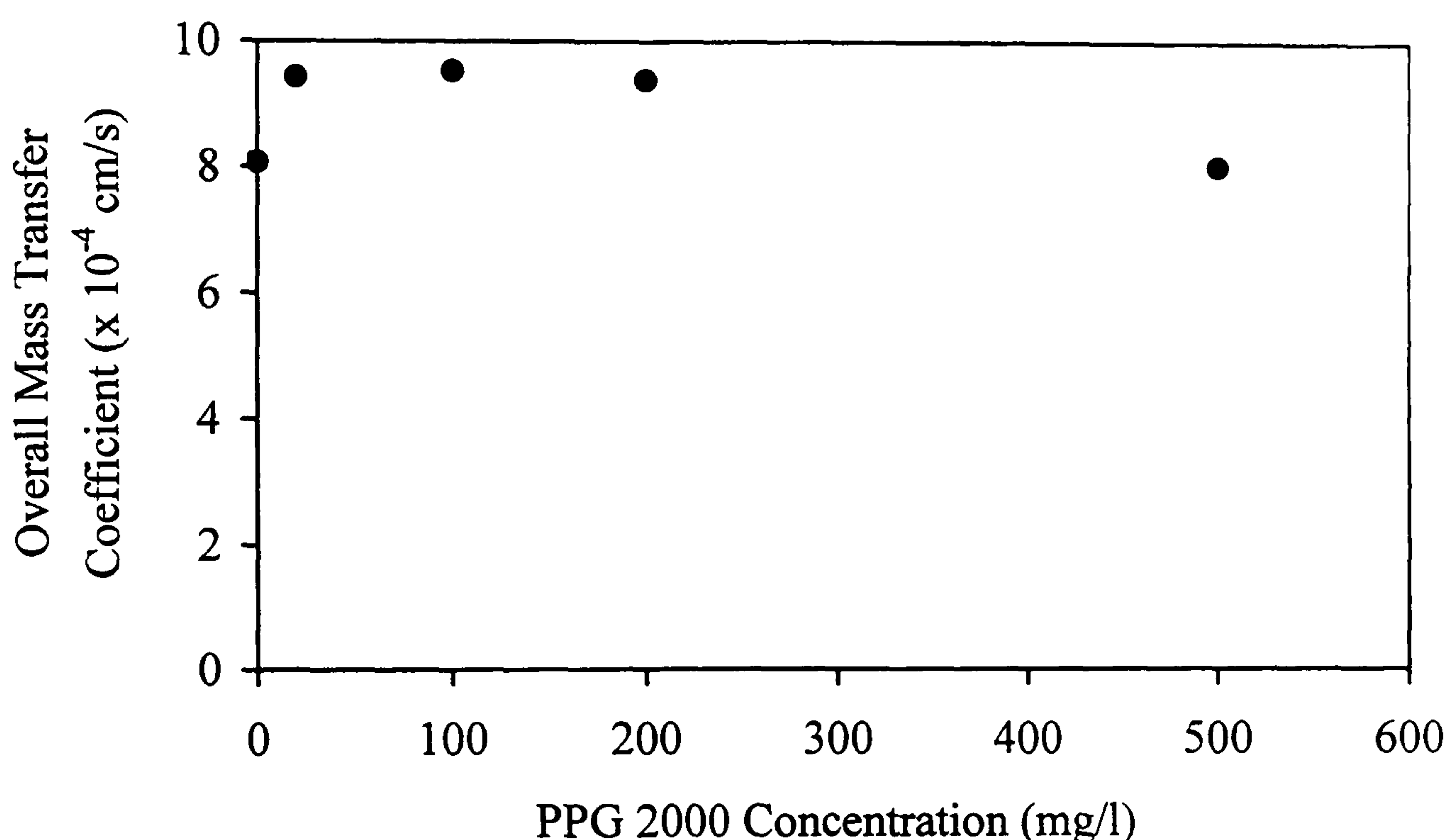


Figure 7-22 The effect of added PPG 2000 concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 250µm, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

The reason for the increase in the overall mass transfer coefficient observed for PPG 2000, and that observed at the lowest concentration of PPG 400 is unknown. In studies of gas-liquid mass transfer systems (Kawase and Moo-Young, 1987, and Morão *et al.*, 1999), the addition of anti-foaming compounds has been associated with both enhanced and reduced K_La measurements. However, from these studies the nature of the enhancement is often attributed to the effects on bubble size and coalescence, and no indication of an effect on liquid film mass transfer was given.

The effect of anti-foam on fermentation broth and protein solutions was also investigated by adding PPG 2000 to filtered fermentation broth, washed biomass and a protein solution, in all cases the concentration of PPG 2000 was 100mg/l.

For extraction from filtered fermentation broth the addition of PPG 2000 increased the overall mass transfer coefficient from 3.42 x 10^{-4} cm/s to 5.37 x 10^{-4} cm/s, an increase of 57%. The addition of PPG 2000 was found to reduce the surface tension from 47.31 mN/m to 36.79 mN/m. Given this reduction in the surface tension, it would appear that the anti-foam preferentially adsorbed to the interface where it reduced the resistance to mass transfer. However, the resistance is still greater than that for extraction from the clean system, suggesting that PPG 2000 only partially displaces the broth components.

When extraction was performed from a washed biomass solution the overall mass transfer coefficient decreased from 5.18 x 10^{-4} cm/s to 4.4 x 10^{-4} cm/s, a reduction of 15%. The surface tension of the biomass solution was 63.01 mN/m, with the added PPG 2000 the surface tension was reduced to 38.33 mN/m. During these experiments the biomass was seen to flocculate and large aggregates were observed at the two-phase interface. The addition of PPG 2000 seems to have caused an increase in flocculation and adsorption of biomass to the interface leading to either an increase in the resistance to mass transfer or a reduction in the interfacial area available for mass transfer.

PPG 2000 was also added to a 0.01 g/l solution of the protein BSA. In the absence of PPG 2000 the addition of BSA substantially reduced the overall mass transfer coefficient from 8.03 x 10^{-4} cm/s, for extraction from water, to 2.24 x 10^{-4} cm/s when the protein was present. When PPG 2000 was added to the system no effect was observed with a value of 2.28 x 10^{-4} cm/s recorded for the overall mass transfer coefficient. The addition of PPG 2000 also had no effect on the surface tension of the solution. It would seem that the protein preferentially adsorbed to the interface preventing PPG 2000 from influencing the system in any way.

The effect of the anti-foaming agents examined here seems to be inconclusive, with both enhancing and reducing effects being observed, therefore, further investigation would seem appropriate. Furthermore, the effect that the antifoaming agent may have on the bacteria was not examined here. Many anti-foams are insoluble in water, and if

they are carbon based they may be utilised by the micro-organism, and it has been reported (Desai, 1987) that bacteria will produce further surface-active compounds to aid in the dispersal and utilisation of insoluble carbon sources.

7.10 The Effect of Surfactants on Solute Extraction

Apart from anti foaming agents, other surface-active compounds may be added to the culture medium during the course of fermentation. Novel extraction methods, such as colloidal liquid aphrons, emulsion liquid membranes and reverse micelles, rely on the surface-active properties of added compounds to enhance or enable the extraction process. During these processes the surface-active compounds may be released into the bulk medium from where they may interfere with interfacial processes.

This section looks at the effect of adding an anionic or a cationic surfactant to the extraction system. The anionic surfactant was sodium dodecyl sulphate (SDS), and the cationic surfactant was dodecyl-trimethyl-ammonium bromide (DTAB), both of these compounds have also found use as a two-phase stabiliser in colloidal liquid aphrons (Matsushita *et al.*, 1992)

7.10.1 SDS

SDS is a common anionic surfactant that is widely used in many industrial and laboratory process. The molecular structure of SDS was previously presented in Figure 2-1. As with the previous sections, an investigation of the effect of the added compound on the equilibrium partition coefficient was undertaken. However, it was not possible to obtain a value for the partition coefficient due to the formation of an emulsion that was resistant to centrifugation. Even when unshaken a faint milky appearance, which was sufficient to interfere with UV absorbence measurements, moved into each phase, and was resistant to centrifugation. Therefore, the partition coefficients used to determine the value of the overall mass transfer coefficient was taken to be that for extraction from water. In Figure 7-23 the effect of the addition of SDS on the surface tension is presented. The surface-active nature of SDS caused the surface tension to reduce with increasing concentration, which indicates that SDS had

surface tension to reduce with increasing concentration, which indicates that SDS had adsorbed to the interface. The surface tension decreased up to a SDS concentration close to 2 g/l after which it remained constant. This trend is similar to other investigations (Agble and Mendes-Tatsis, 2000) which reported that SDS has a critical micelle concentration (CMC) of 2.36 g/l.

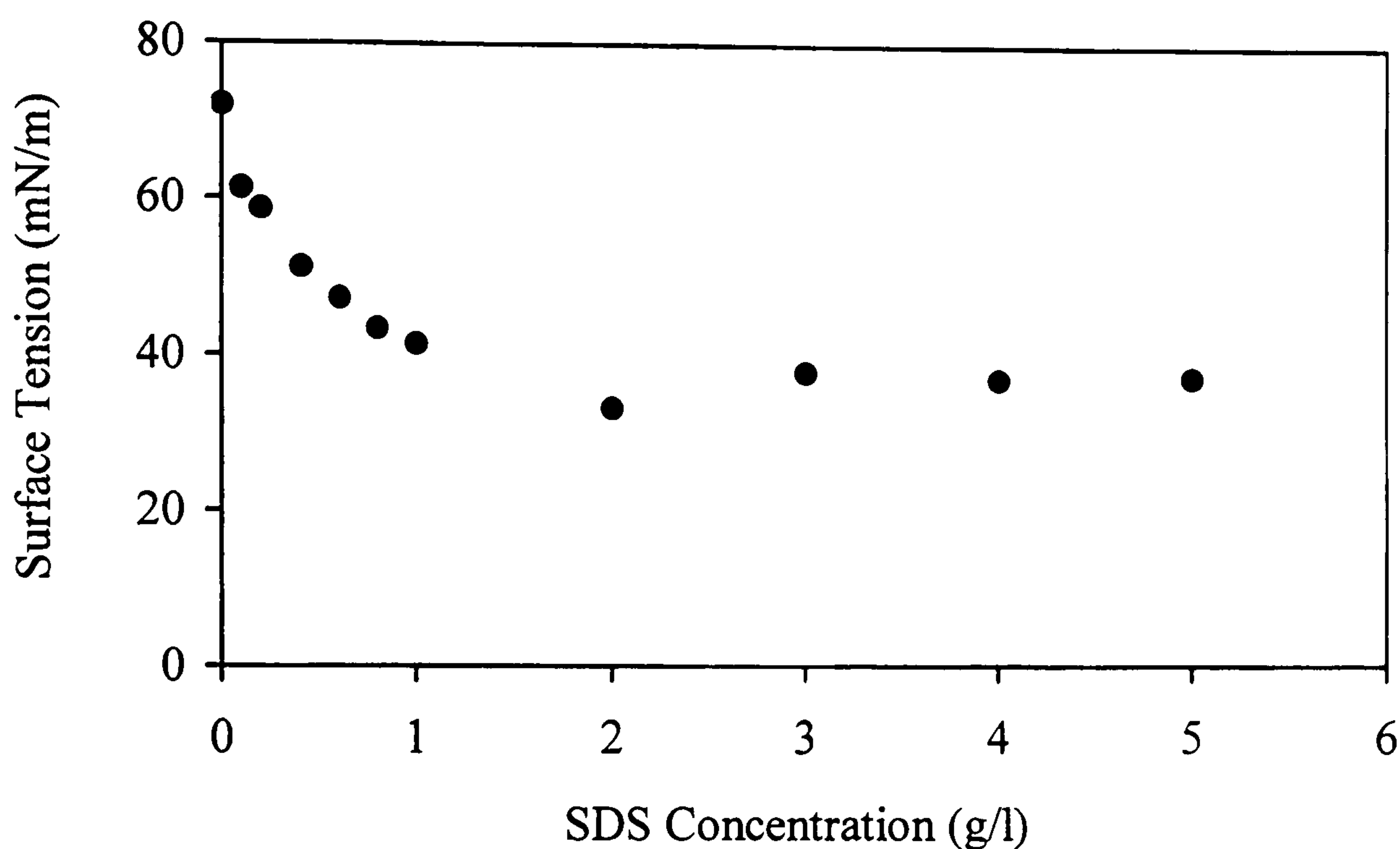


Figure 7-23 The effect of added SDS concentration on the surface tension of chloramphenicol solutions. Initial chloramphenicol concentration of 250μm, pH = 7, temperature = 25°C.

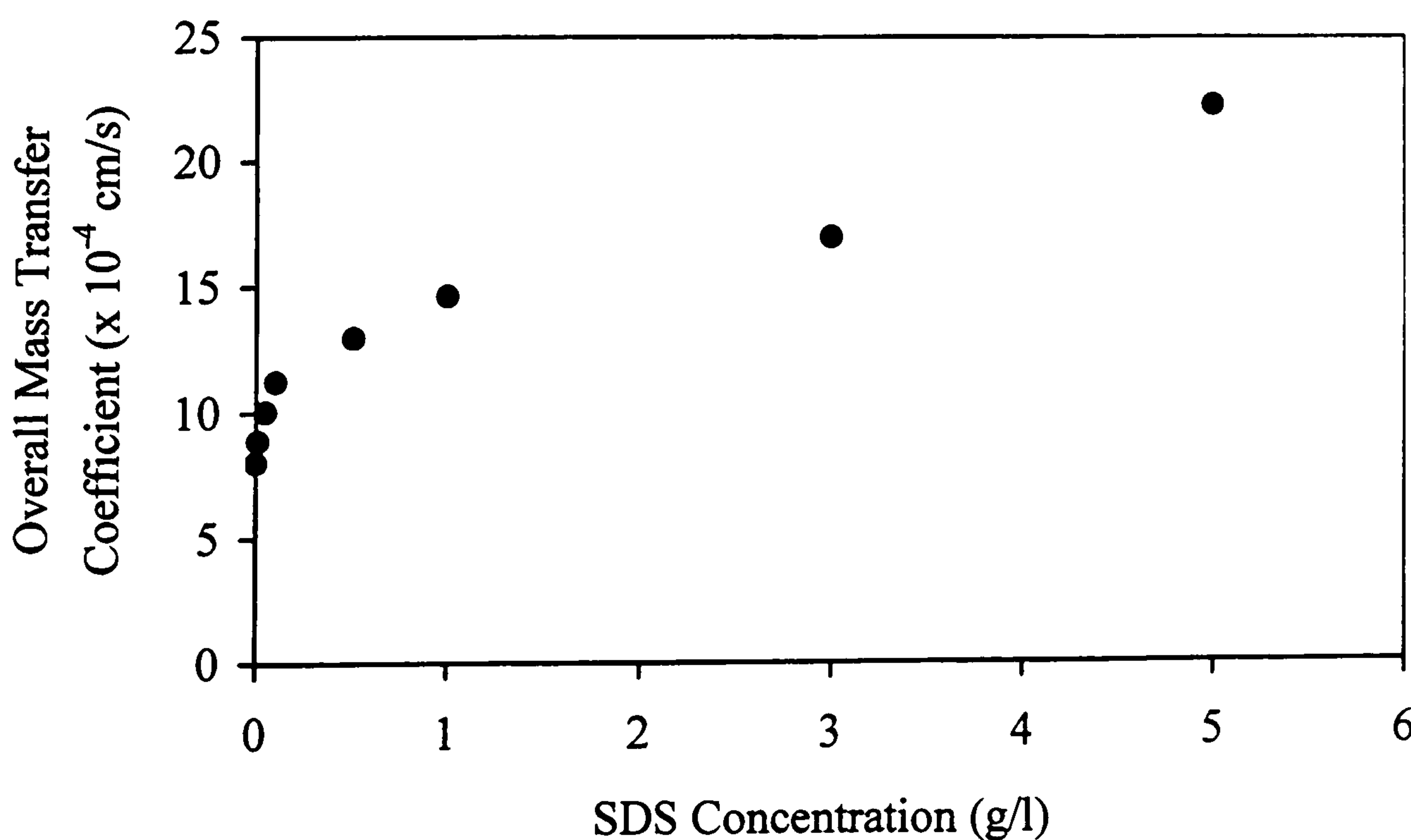
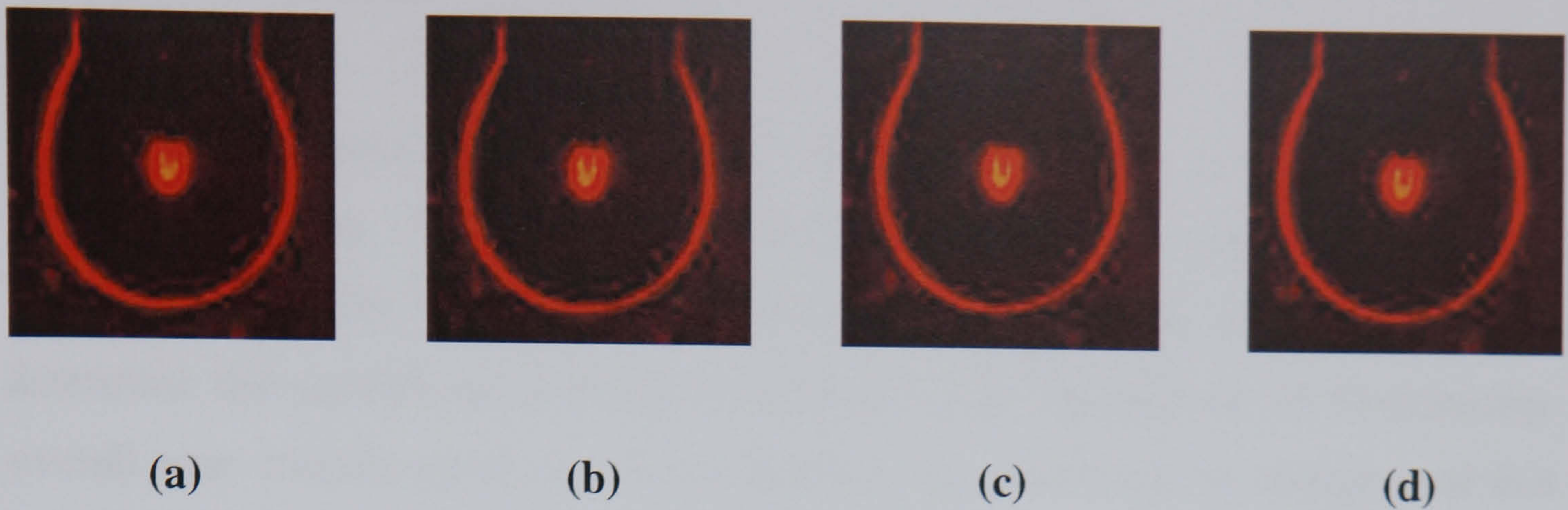


Figure 7-24 The effect of added SDS concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq}

In Figure 7-24 the effect of SDS concentration on the overall mass transfer coefficient is shown. It can be seen that adding SDS to the extraction system causes substantial increases in the value of the overall mass transfer coefficient, even at concentrations as low as 0.01 g/l. Initially the increase in the overall mass transfer coefficient with SDS concentration is quite steep, after 0.1 g/l the rate of increase reduces. At the highest concentration of SDS examined (5 g/l) the value of the overall mass transfer coefficient was 22.23 x 10^{-4} cm/s, an increase of 174% over extraction from water.

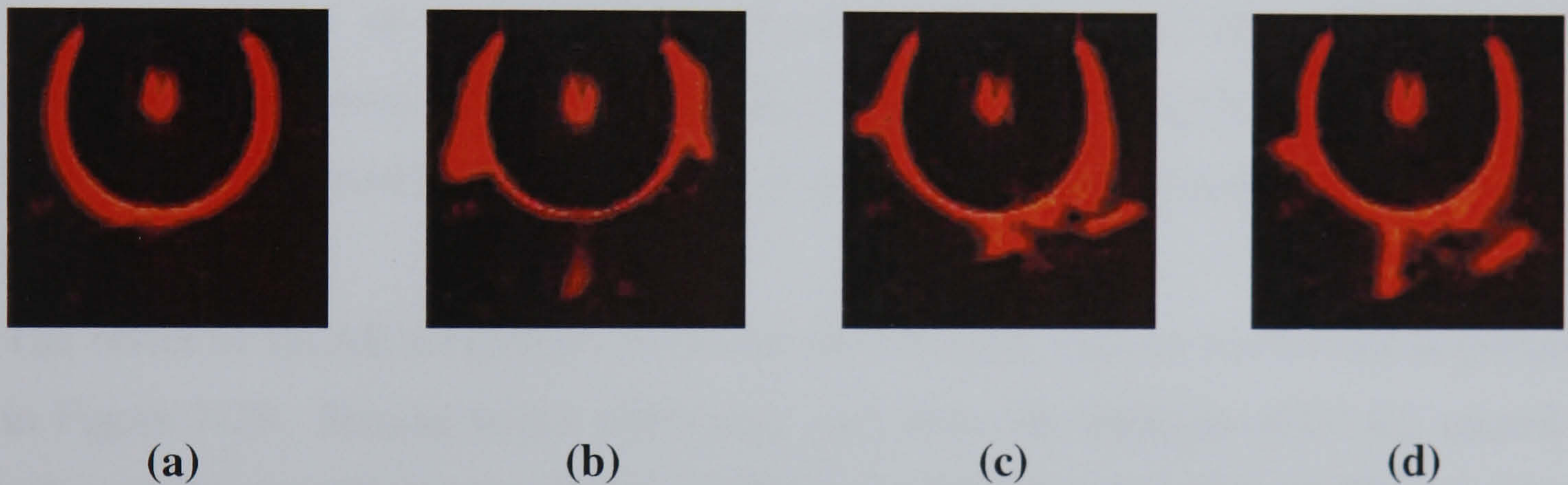
In previous studies of liquid-liquid extraction the addition of SDS was found to increase overall mass transfer coefficients (Pursell *et al.*, 1999). In that study the enhancement was attributed to interfacial turbulence (Section 3.5), and thus, a Schlieren investigation of the two-phase system was conducted. Pictures of the two phase interface of a drop are shown in Figures 7-25 to 7-27 for situations where either SDS was not present or the SDS concentration was 0.1 g/l and 5 g/l, respectively. For the case where no SDS was present (Figure 7-25) the two-phase interface can be clearly seen, and no movement or eruptions from the interface are observed. Where SDS has been added (Figures 7-26 and 7-27) movement at the drop surface is observed. This movement is due to localised changes in the interfacial tension of the drop and will result in rapid surface renewal and movement on both sides of the interface, and thus will enhance mass transfer. By comparison of Figures 7-26 and 7-27 it is possible to say that the effect is more apparent at the higher SDS concentration.

When SDS was added to the extraction system increased overall mass transfer coefficients were observed, and this increase was caused by the presence of interfacial turbulence. Interfacial turbulence will reduce the resistance to mass transfer by increasing fluid motion close to the interface, in effect giving rise to a surface renewal mass transfer mechanism, similar to that described in Section 3.3.4.2. The cause of interfacial turbulence may be due to partitioning of the surfactant into the solvent. Thus, the surfactant will cross the interface, and in doing so may cause the reduction in the interfacial tension required to initiate interfacial turbulence (Section 3.5).



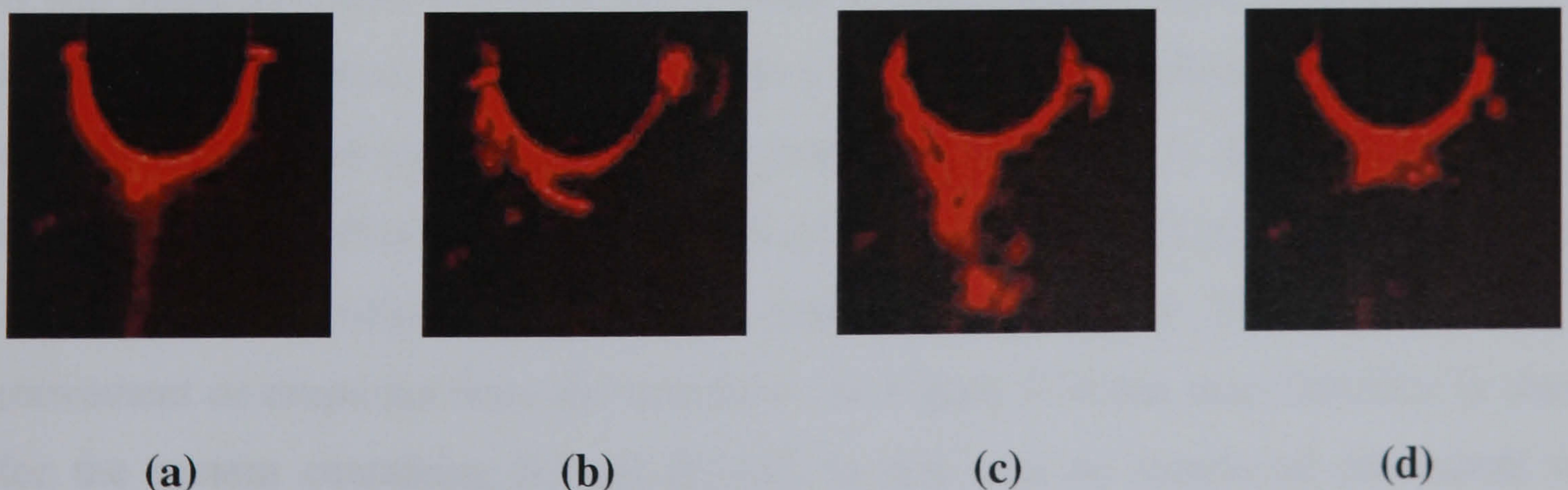
(a) (b) (c) (d)

Figure 7-25 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water into 1-octanol, at (a) 0 seconds, (b) 30 seconds, (c) 60 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C.



(a) (b) (c) (d)

Figure 7-26 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with 0.1 g/l SDS added, into 1-octanol, at (a) 0 seconds, (b) 10 seconds, (c) 30 seconds, and (d) 40 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C.



(a) (b) (c) (d)

Figure 7-27 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with 5 g/l SDS added, into 1-octanol, at (a) 0 seconds, (b) 5 seconds, (c) 20 seconds, and (d) 30 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C.

7.10.2 DTAB

DTAB is a common cationic surfactant that is widely used in many industrial and laboratory process. The molecular structure of DTAB was presented previously in Figure 2-1. Similar to the investigation of the effect of SDS, it was not possible to determine the equilibrium partition coefficient. For the purpose of determining the overall mass transfer coefficient it was assumed that there was no change, and that the value for extraction from water was used.

The effect of the addition of DTAB on the surface tension is presented in Figure 7-28. The surface-active nature of DTAB causes the surface tension to reduce with increasing concentration; this indicates that DTAB has adsorbed to the interface. The surface tension decreases up to a DTAB concentration close to 4 g/l after which it remains constant. This trend was similar to other investigations (Agble and Mendes-Tatsis, 2000) which reported that DTAB has a critical micelle concentration (CMC) of 4.93 g/l.

The effect of DTAB concentration on the overall mass transfer coefficient is presented in Figure 7-29. Similar to the effect seen with SDS, the addition of DTAB caused the value of the overall mass transfer coefficient to increase steadily, reaching a value of 23.78 x 10^{-4} cm/s at a DTAB concentration of 7.5 g/l. However, in contrast to SDS, low concentrations of DTAB only caused a minor increase, with significant increases occurring at concentrations above 2 g/l.

It was postulated that, like SDS, the increase in mass transfer was due to the presence of interfacial turbulence, and to confirm this a Schlieren investigation of the liquid-liquid system was carried out. Pictures of the two-phase interface are shown in Figure 7-30 and 7-31 for DTAB concentrations of 0.5 g/l and 5 g/l, respectively. The case for when no surfactant is added was presented previously in Figure 7-25, and showed no movement or eruptions from the interface. In Figure 7-30 the drop interface is shown for the system containing 0.5 g/l DTAB; in this case no interfacial movement was observed. In the corresponding experiment conducted in the Lewis cell only a slight increase in the overall mass transfer coefficient was recorded. In Figure 7-31, the effect of adding 5 g/l DTAB was examined; in this series of pictures considerable movement

of adding 5 g/l DTAB was examined; in this series of pictures considerable movement of the drop was observed, and this corresponded to the situation where a greatly enhanced overall mass transfer coefficient was recorded.

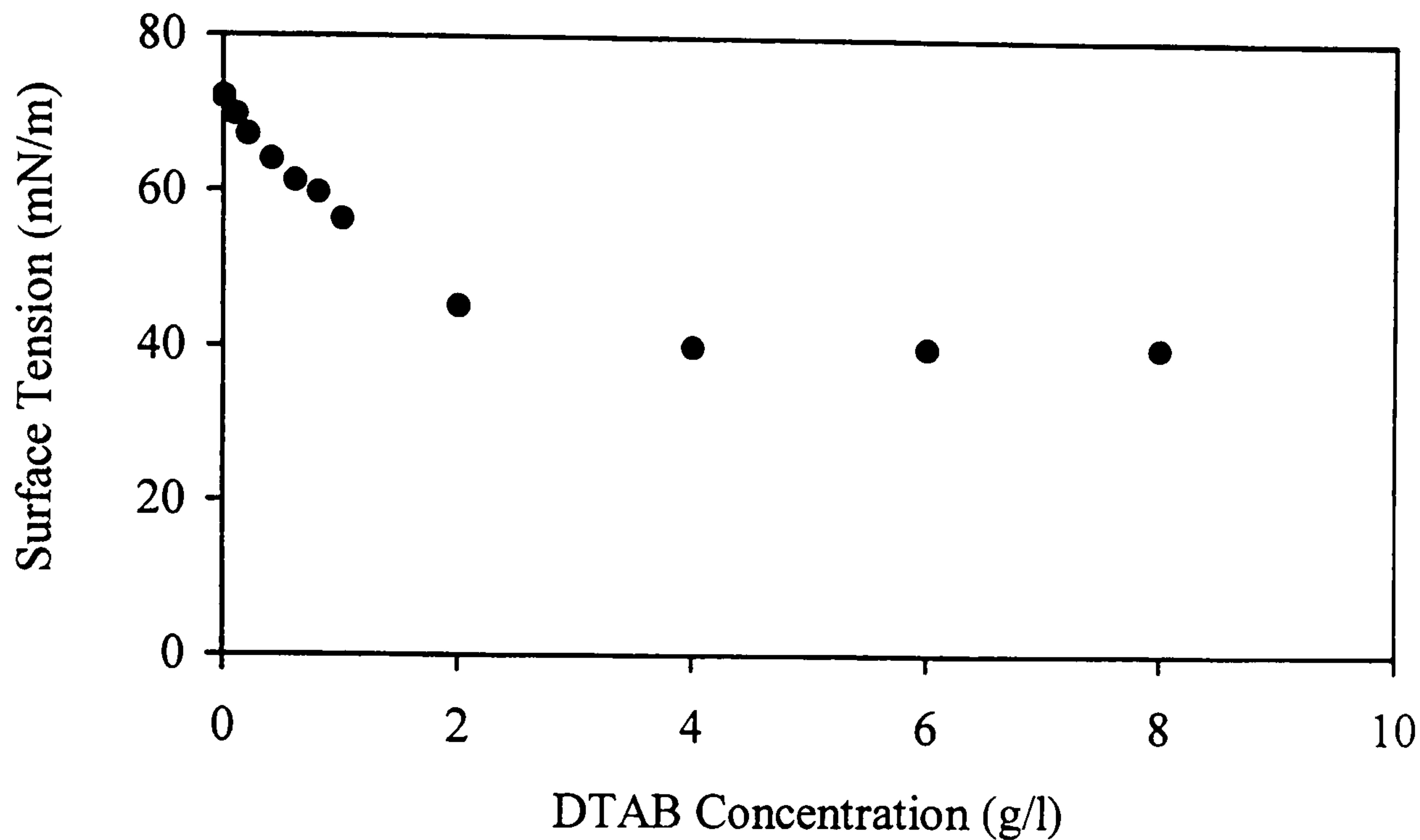


Figure 7-28 The effect of added DTAB concentration on the surface tension of chloramphenicol solutions. Initial chloramphenicol concentration of 250μm, pH = 7, temperature = 25°C.

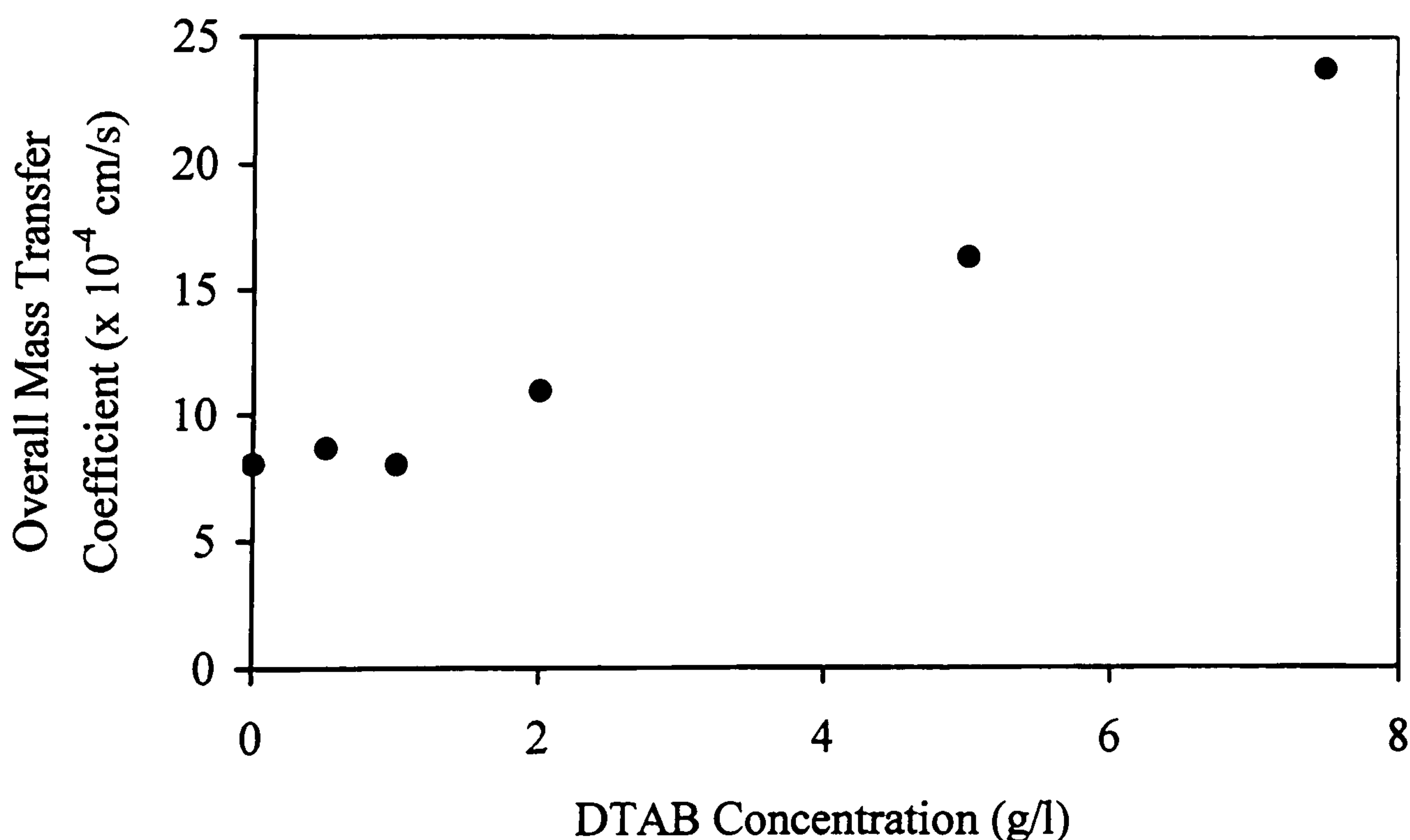
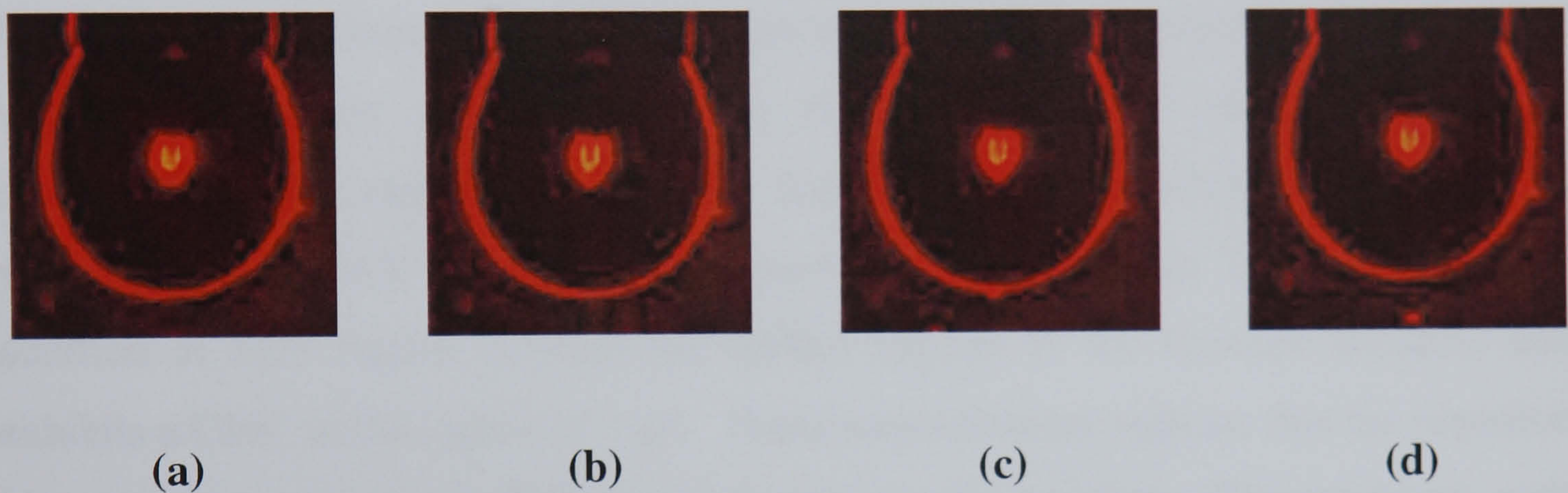


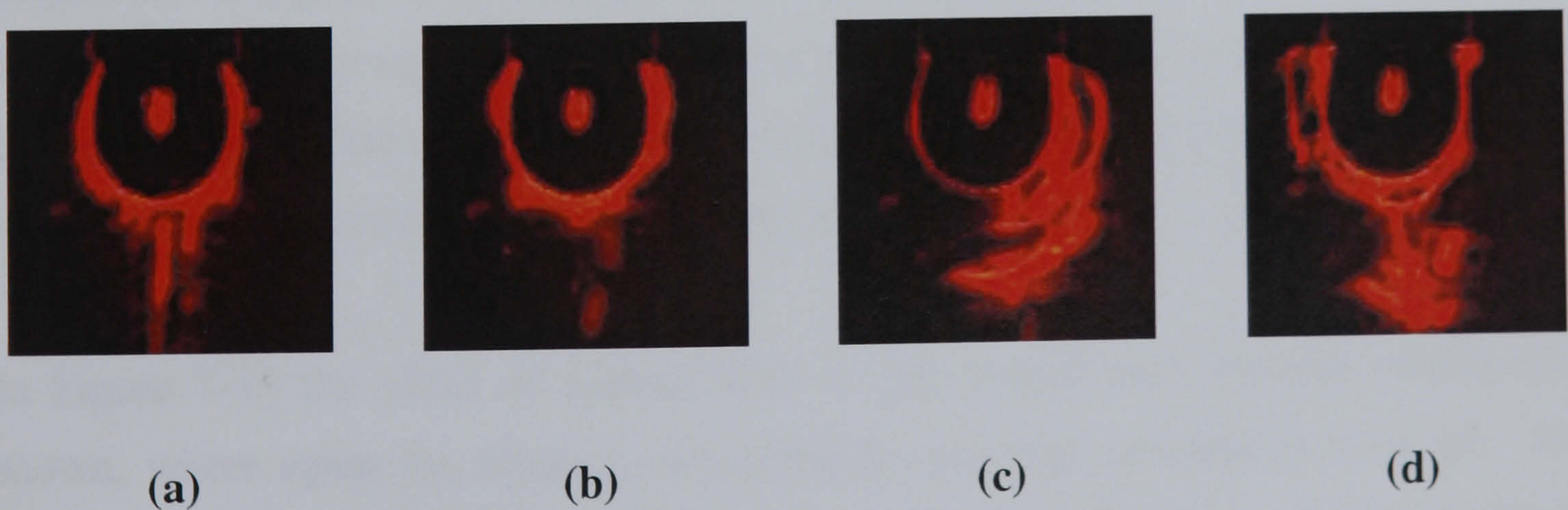
Figure 7-29 The effect of added DTAB concentration on the overall mass transfer coefficient for chloramphenicol extraction from de-ionised water into 1-octanol. Initial chloramphenicol concentration of 250μm, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

Similar to SDS, the cationic surfactant DTAB was found to greatly enhance mass transfer by causing interfacial turbulence, and this may be attributed to the surfactant crossing the interface and causing a localised reduction in interfacial tension. However, the effect only became apparent at concentrations above 2 g/l, below this concentration slight increases in overall mass transfer coefficients were recorded, and it is possible that interfacial turbulence was responsible, but this was not observed from the Schlieren investigation.



(a) (b) (c) (d)

Figure 7-30 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with 0.5 g/l DTAB added, into 1-octanol, at (a) 0 seconds, (b) 10 seconds, (c) 30 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C.



(a) (b) (c) (d)

Figure 7-31 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with 5 g/l DTAB added, into 1-octanol, at (a) 5 seconds, (b) 10 seconds, (c) 30 seconds, and (d) 60 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, pH = 5.5, temperature = 23°C.

7.10.3 Surfactant and Biomass

The surfaces of bacterial cells generally have a negative charge due to the properties of the cell wall components. This section details results obtained when ionic surfactants where added to biomass solutions. It was postulated that the adsorption of a charged species would repel the charged cells, and in the light of the previous results where SDS and DTAB caused interfacial turbulence, that biomass would damp interfacial movement.

In Figure 7-32 surface tension measurements for the biomass-SDS system are shown, where the biomass concentration was kept constant at 0.14 g/l and the SDS concentration was varied. The biomass concentration corresponded to a quantity that was found previously to result in a saturated interface (Section 7.7.3). However, the addition of SDS further reduced the surface tension of the biomass solution, and it exhibits a CMC in the region of 1 g/l. These measurements indicate that the presence of biomass does not prevent SDS from adsorbing to the interface. The minimum surface tension recorded was similar to the value when biomass was not present (Figure 7-23), although the CMC concentration is lower which may be the result of a mixed monolayer at the interface.

This suggests that SDS prevents biomass adsorption, or masks the effect that biomass has on the interface. Both the bacterial cells and the surface-active portion of SDS are negatively charged, and therefore they will repel one another and compete for adsorption sites. Compared to the biomass, SDS is smaller and more surface active. It is likely that it would adsorb more quickly to the interface, and due to similar charge it would prevent adsorption of biomass to the interface.

In Figure 7-33 the effect of adding SDS on the overall mass transfer coefficient is shown, where again the biomass concentration was kept constant at 0.14 g/l. With increasing SDS concentration the value of the overall mass transfer coefficient increases. Initially the rate of increase is quite steep, but after 1 g/l the rate reduces reaching a value of 17.82 x 10^{-4} cm/s at a SDS concentration of 5 g/l. This result is similar to the case when the biomass was not present (Figure 7-24). However, the extent of mass transfer enhancement is lower than when the biomass was absent.

During the Lewis cell experiments large aggregates (2mm^3 or larger) were seen in the bulk aqueous phase, however, due to the density of the biomass it was not possible to observe the interface. If these aggregates did adsorb to the interfacial region then it is possible that they would damp interfacial movement, or restrict the interfacial area, thus, reducing the overall mass transfer coefficient compared to the zero biomass case.

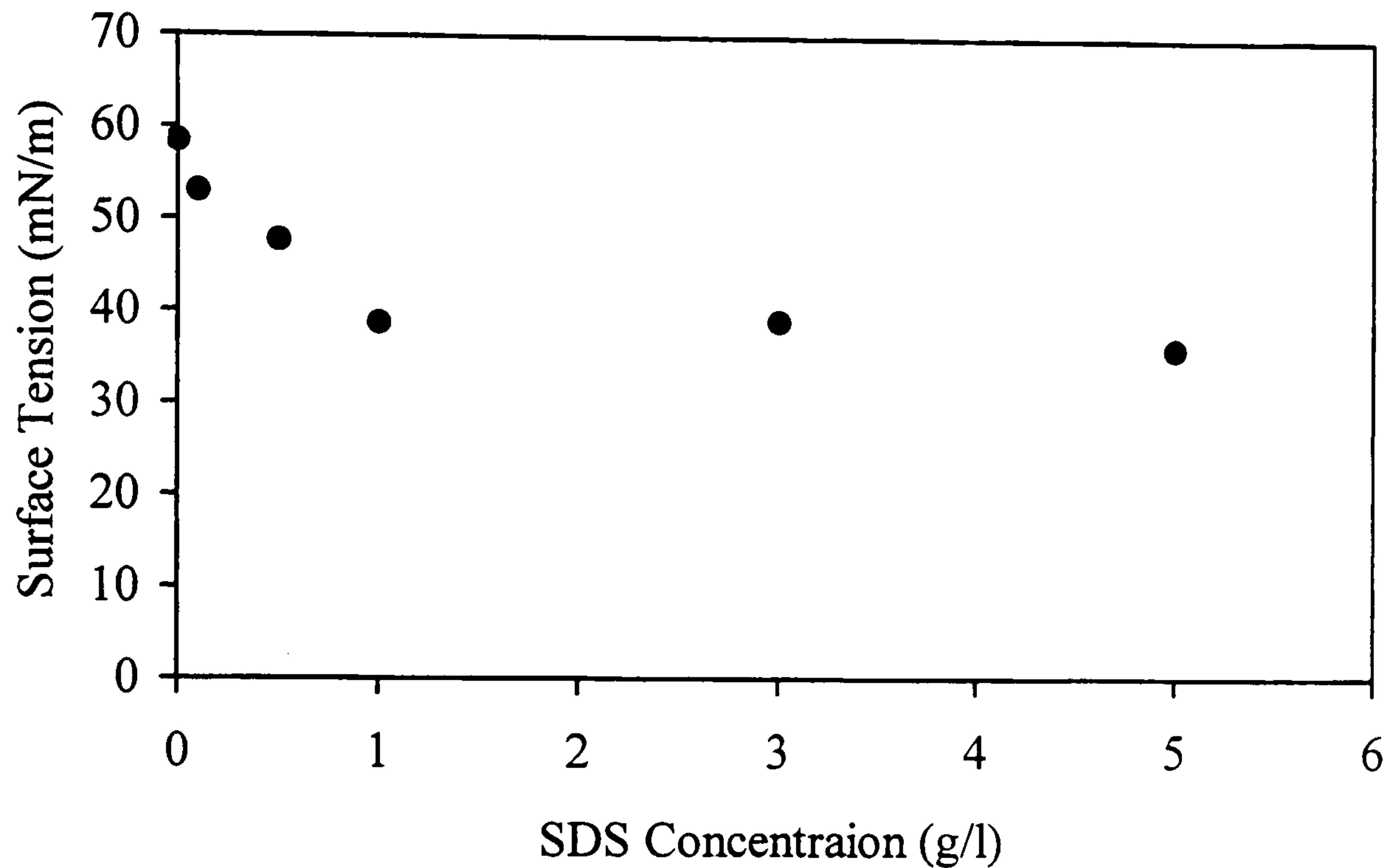


Figure 7-32 The effect of added SDS concentration on the surface tension of biomass solutions. Initial chloramphenicol concentration of 250µm, a biomass concentration of 0.14 g/l, pH = 5.5, temperature = 25°C.

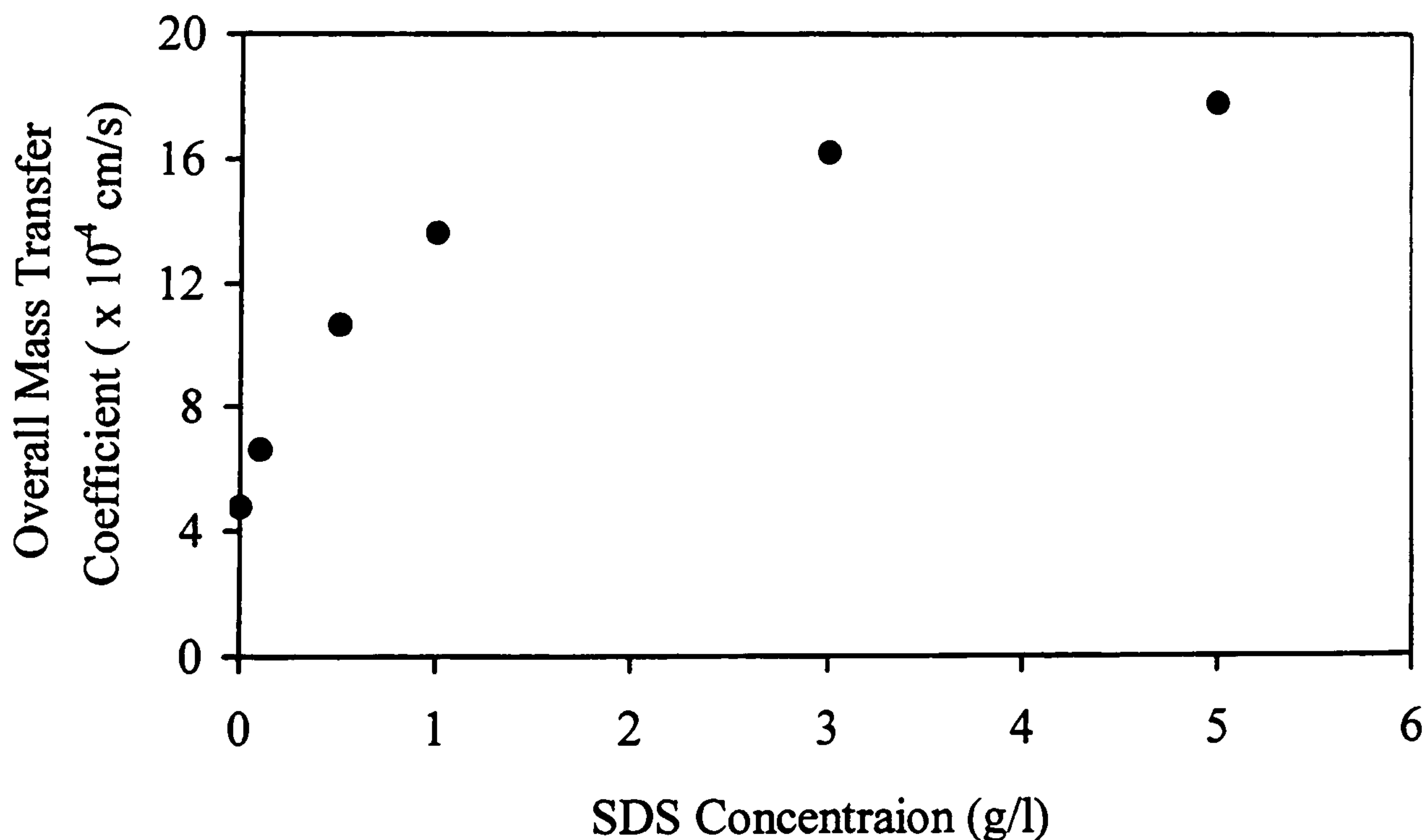


Figure 7-33 The effect of added SDS concentration on the overall mass transfer coefficient for chloramphenicol extraction from a biomass solution into 1-octanol. Initial chloramphenicol concentration of 250µm, a biomass concentration of 0.14 g/l, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR=1.

To investigate the presence of interfacial turbulence a Schlieren investigation of this system was conducted. In Figure 7-36 images are shown for extraction where biomass is present but no surfactants have been added. In these pictures no interfacial movement is observed, however, when SDS was added to the system, shown in Figure 7-37, interfacial movement, which will enhance mass transfer, was observed. In comparison to Figure 7-27 where a similar concentration of SDS is present, but no biomass, it is seen that the interfacial movements are as prominent with biomass present. From this it is concluded that the biomass does not prevent or damp interfacial turbulence from occurring when repelled by a like charge at the interface.

The effect of the cationic surfactant DTAB on the biomass extraction system was also investigated where the biomass concentration remained constant at 0.14 g/l, and the concentration of DTAB was varied. In Figure 7-34 surface tension measurements for the DTAB-biomass system are shown; it is seen that up to a DTAB concentration of 1 g/l the surface tension decreased, there after it remained constant. This indicated that the biomass does not prevent DTAB from adsorbing to the interface where it exerts its surface-active properties. The minimum value of the surface tension was similar to that when the biomass was absent (Figure 7-28), however, as with the SDS-biomass system the CMC was reduced to around 1 g/l. This lower CMC value may indicate that the biomass does partially adsorb to the interface, but the greater surface activity of DTAB caused a reduction in the surface tension.

In contrast to the SDS-biomass system, the addition of DTAB to the biomass extraction system did not show a similar effect on mass transfer to that observed when biomass was not present. In Figure 7-35 the effect of adding DTAB on the overall mass transfer coefficient is shown, where again the biomass concentration was kept constant at 0.14 g/l. The addition of DTAB would appear to have no effect, and the value of the overall mass transfer coefficient remained constant at the value when no DTAB was added. This could be due to the surface-active portion of DTAB, which is positively charged, attracting the negatively charged bacteria. Thus, DTAB could be adsorbed on to the biomass and prevented from crossing the interface where it may initiate interfacial turbulence. The absence of interfacial turbulence is confirmed by the pictures of the interface shown in Figure 7-38, where no movement of the interface was observed.

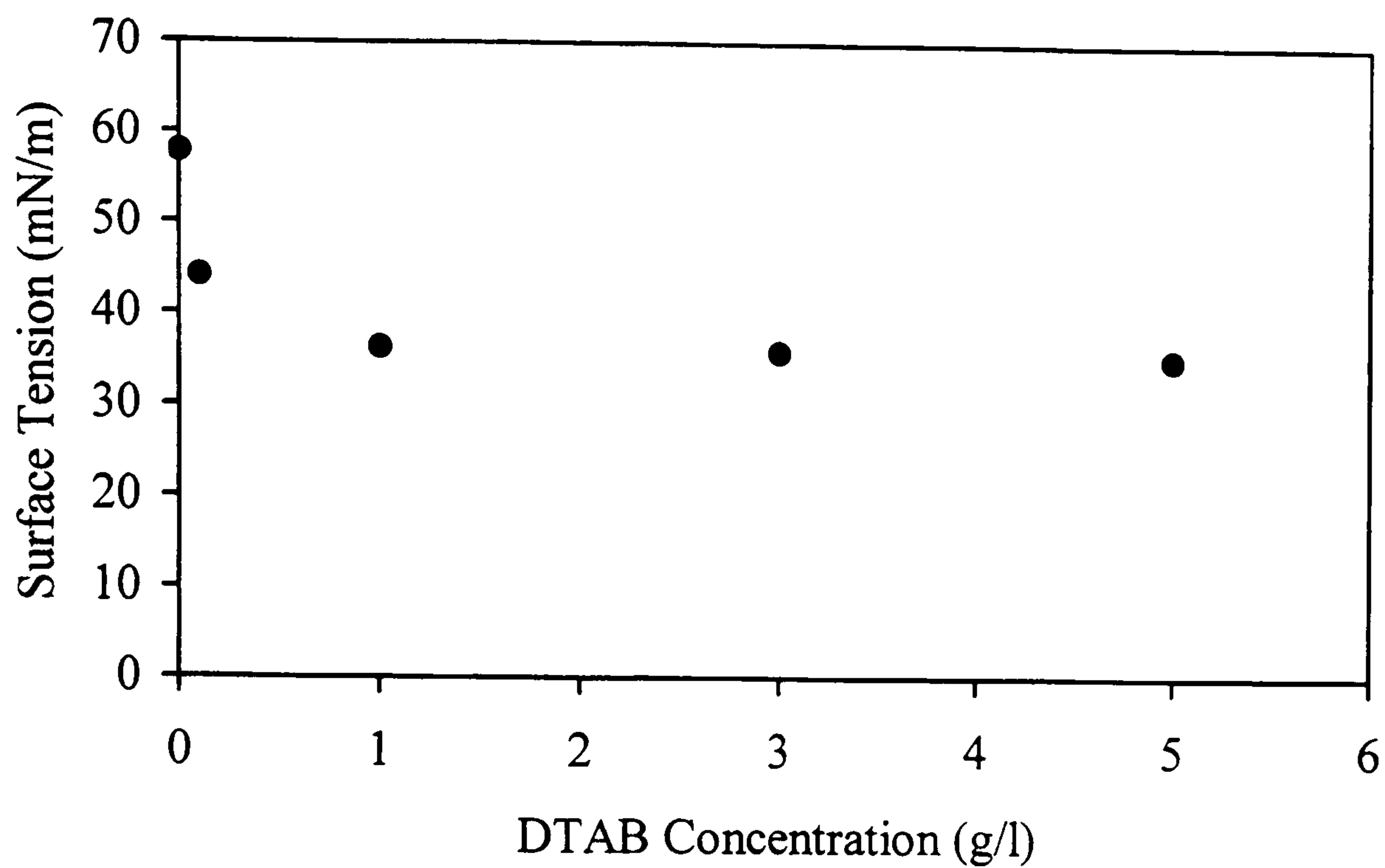


Figure 7-34 The effect of added DTAB concentration on the surface tension of biomass solutions. Initial chloramphenicol concentration of 250μm, a biomass concentration of 0.14 g/l, pH = 5.5, temperature = 25°C.

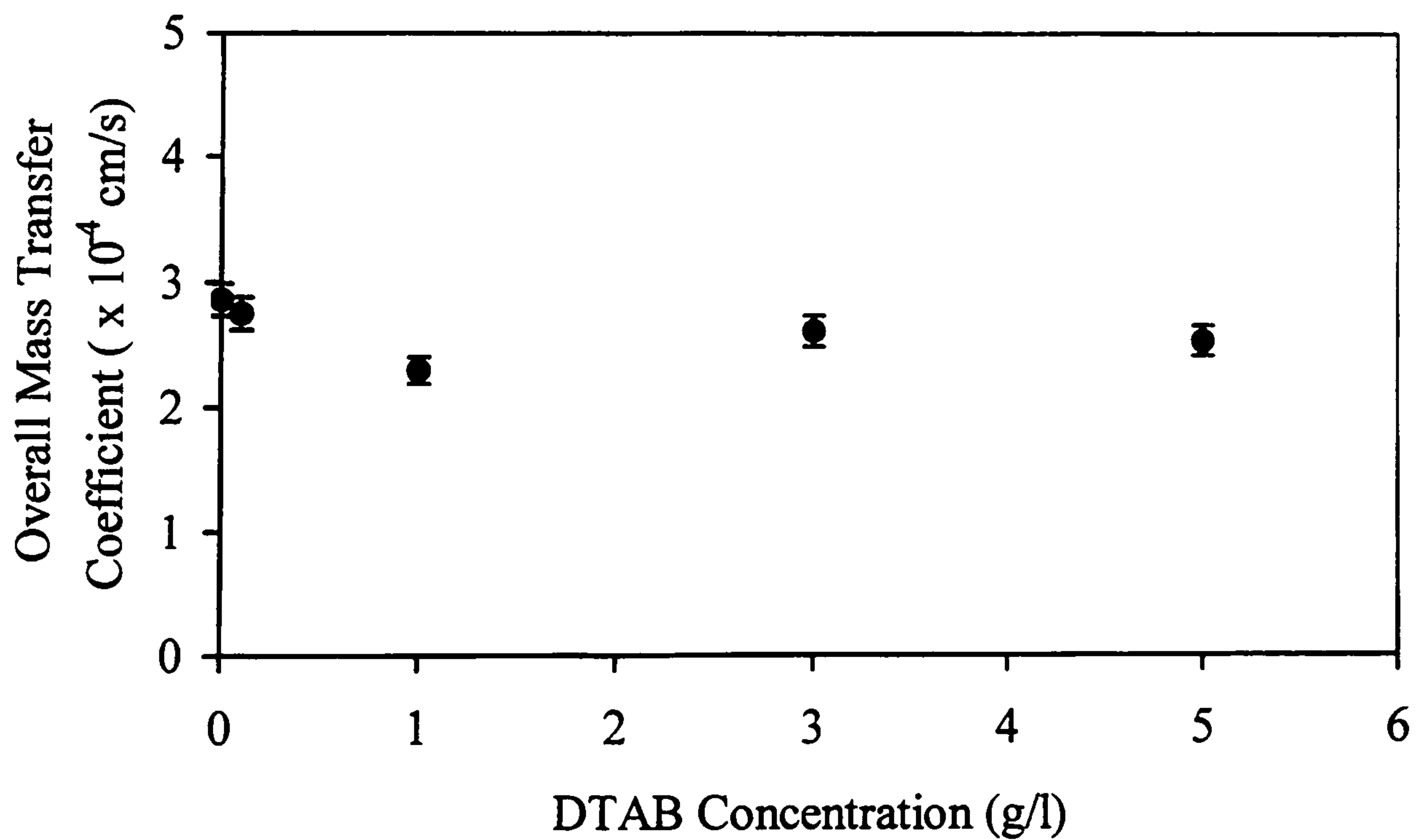
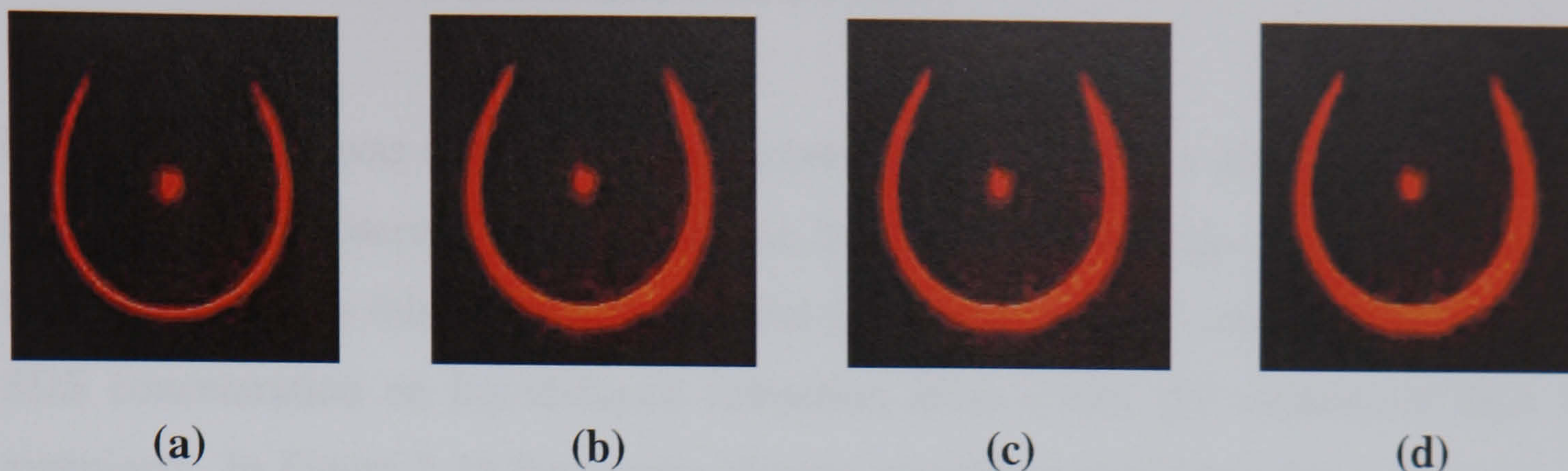
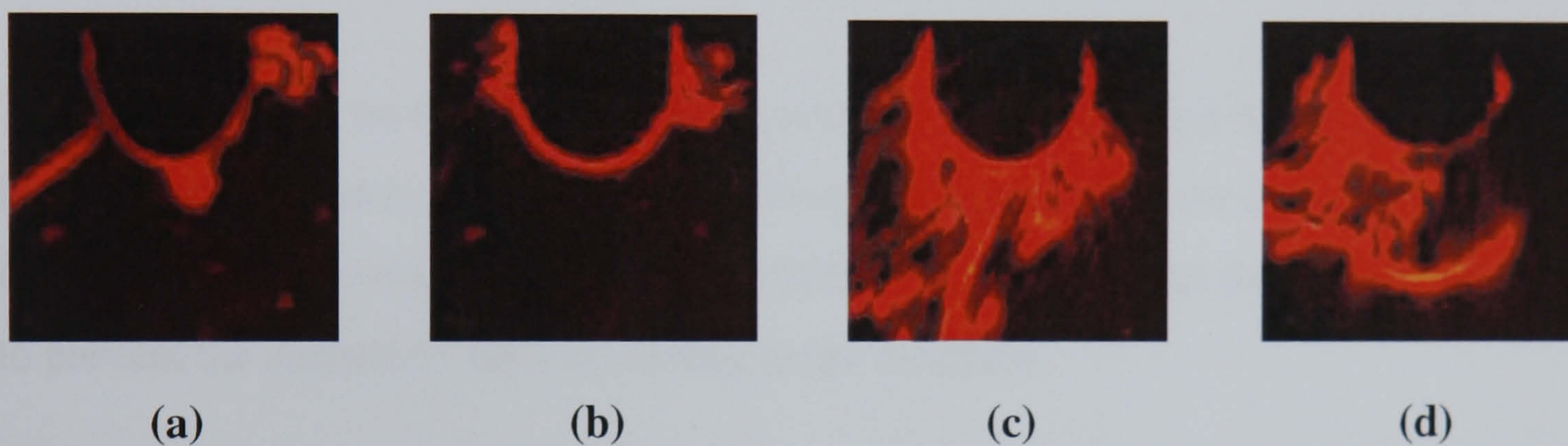


Figure 7-35 The effect of added DTAB concentration on the overall mass transfer coefficient for chloramphenicol extraction from a biomass solution into 1-octanol. Initial chloramphenicol concentration of 250μm, a biomass concentration of 0.14 g/l, pH = 5.5, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR=1.



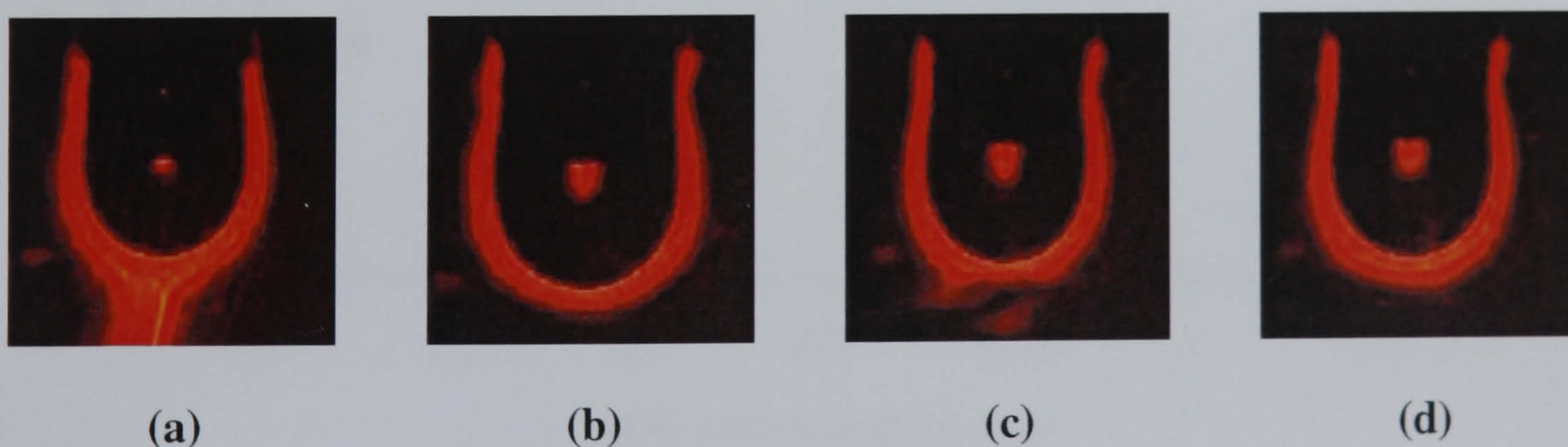
(a) (b) (c) (d)

Figure 7-36 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with biomass added, into 1-octanol, at (a) 0 seconds, (b) 30 seconds, (c) 60 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, a biomass concentration of 0.14 g/l, pH = 5.5, temperature = 23°C.



(a) (b) (c) (d)

Figure 7-37 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with biomass and SDS added, into 1-octanol, at (a) 5 seconds, (b) 10 seconds, (c) 30 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, a biomass concentration of 0.14 g/l, and a SDS concentration of 5 g/l, pH = 5.5, temperature = 23°C.



(a) (b) (c) (d)

Figure 7-38 Images of the two-phase interface during extraction of chloramphenicol from de-ionised water with biomass and DTAB added, into 1-octanol, at (a) 0 seconds, (b) 30 seconds, (c) 60 seconds, and (d) 90 seconds after drop formation. Initial chloramphenicol concentration of 250 μm, a biomass concentration of 0.14 g/l, and a DTAB concentration of 5 g/l, pH = 5.5, temperature = 23°C.

7.10.4 Surfactant and Protein

In Section 7.8 it was seen that biosurfactant compounds had a similar affect on mass transfer as was observed for fermentation broth. Therefore, one of these compounds, BSA, was used in this section as a model fermentation broth solution. The effect of SDS concentration on liquid-liquid extraction from a 0.01 g/l solution of BSA was examined. In Figure 7-39 the results of mass transfer experiments show an increase in the overall mass transfer coefficient with increasing SDS concentration, these results are similar to those seen previously when SDS was added to water and biomass extraction systems. It is likely that the increase is due to interfacial turbulence, however, on this occasion a Schlieren investigation was not performed.

The effect of SDS on the surface tension was also examined, and it was found that with increasing SDS concentration the surface tension decreased to the CMC value observed in Figure 7-23 at a concentration of 1 g/l SDS. This implies that the protein was unable to prevent the surfactant from absorbing to the interface.

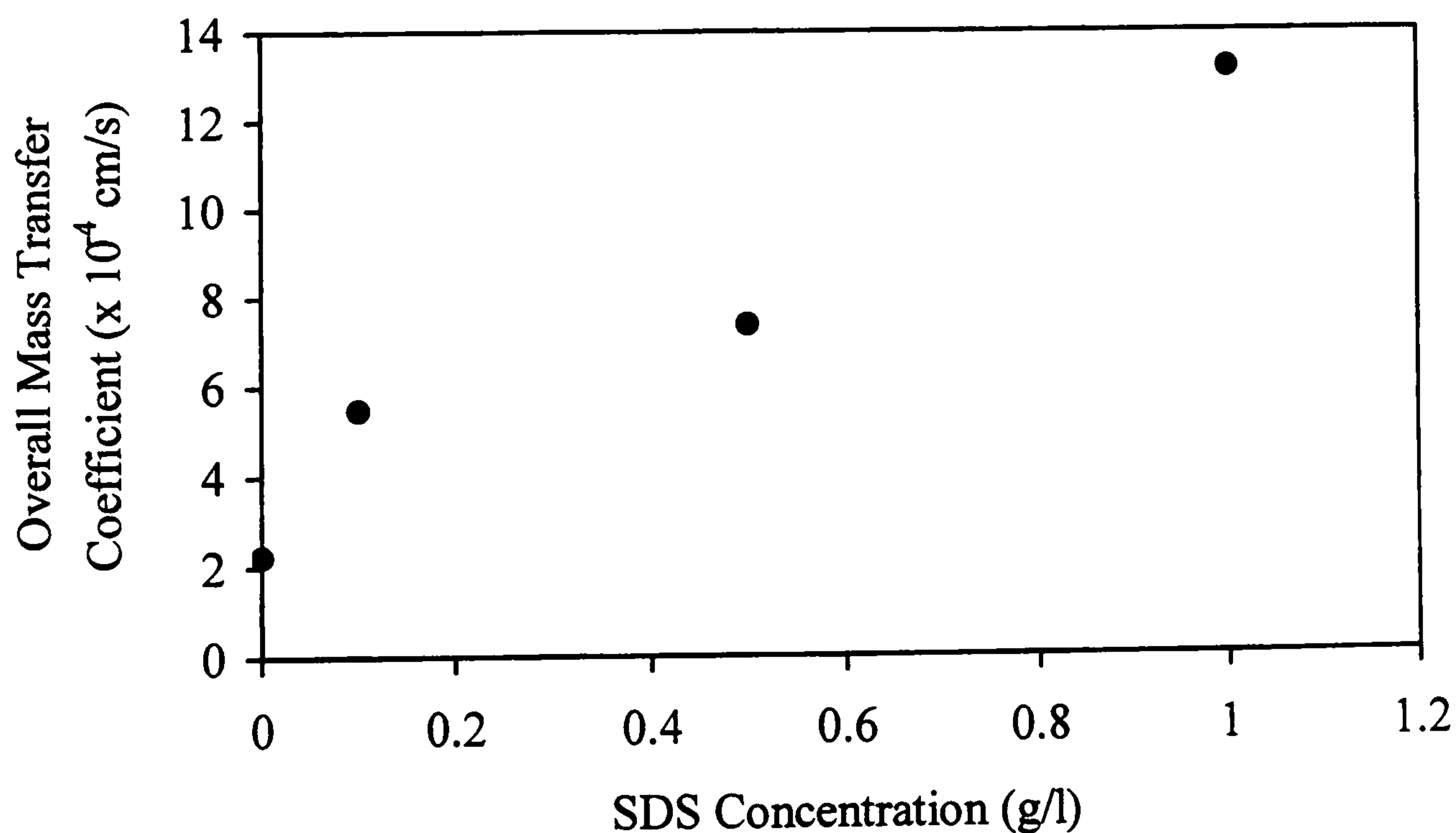


Figure 7-39 The effect of added SDS concentration on the overall mass transfer coefficient for chloramphenicol extraction from PBS with BSA, into 1-octanol. Initial chloramphenicol concentration of 250μm, a BSA concentration of 0.01 g/l, pH = 7, N_{Aq} = 90, N_{Sol} = 250, temperature = 25°C, and PVR = 1.

7.11 Summary of Results and Discussion

An experimental study has been conducted using the antibiotic chloramphenicol as the model solute to allow an investigation of solute extraction from fermentation broth using a physical liquid-liquid extraction process.

A Lewis cell has been used to study effects on the mass transfer process. A summary of the main results and conclusions drawn from that work are presented in the following sections. The effects that fermentation broth components had on extraction are discussed in Section 7.11.1. In order to compare these effects with known compounds a number of biosurfactants were added to the systems, this work is summarised in Section 7.11.2. Finally, in Section 7.11.3, a summary of the effects of non-ionic and ionic surfactants on extraction is presented.

7.11.1 Effects of Fermentation Broth Components On Mass Transfer

Solute extractions were performed from whole and filtered fermentation broth where a similar reduction in the overall mass transfer coefficient was observed. However, when extraction was performed with only the biomass present there was less of a reduction. From this it is postulated that the soluble components in the fermentation broth have a stronger influence on the interface and dominate effects on the mass transfer process when present.

The effect of filtered fermentation broth on solute extraction was investigated further, initially by diluting the broth to reduce the concentration of compounds present. It was found that the overall mass transfer coefficient decreased as the concentration of the broth increased, with a 70% reduction recorded for extraction from the complete filtered broth. The reduction in mass transfer coincided with a reduction in the surface tension; however, a minimum value was not observed suggesting that the interface was not saturated. In order to ascertain the types of compounds causing the effect on mass transfer the filtered fermentation broth was split into eight weight fractions using ultrafiltration membranes. The fraction corresponding to a weight range of 10000 to

30000 Daltons had the greatest impact upon mass transfer and surface tension. although the reductions seen were not a large as that caused by the complete filtered broth. This suggests that components of the filtered broth act synergistically to affect interfacial processes.

The effect of biomass was investigated and it was found that the overall mass transfer coefficient decreased with increasing amounts of biomass. However, no minimum in the trend was observed. This correlated with surface tension measurements where a reduction in tension was observed. However a minimum was observed at a concentrations of 0.125 g/l. The surface tension measurements suggest that the interface became saturated with microbial cells and no further interfacial adsorption occurred. However, it is possible that multi-layers of biomass formed at the interface which provided a further resistance to mass transfer. It is proposed that biomass affects extraction by reducing the interfacial area available for mass transfer, and multi-layers of biomass provide a resistance to solute transfer up to the interface.

7.11.2 Effect of Biosurfactants on Mass Transfer

The fraction of the ultrafiltered fermentation broth retained in the weight range of 10000 to 30000 Daltons was found to have the greatest effect on mass transfer and surface tension. This weight range corresponds to macromolecular compounds such as proteins and phospholipids that are produced in biological systems, and are known to be surface active. The effect of these types of compounds on an otherwise "clean" extraction system was investigated were the proteins; bovine serum albumin, cytochrome c and β-casein; and the phospholipid phosphatidylcholine were individually added to the system. When added at a concentration of 10 mg/l all four compounds were found to reduce the overall mass transfer coefficient to the level found for filtered fermentation broth, and noticeable effects on mass transfer were observed for concentrations as low as 0.01 mg/l. These results indicate that only small quantities of macromolecular compounds need to be produced during the fermentation process for there to be a significant effect on mass transfer and thus performance of the separation process.

7.11.3 Effect of Surfactants on Mass Transfer

The effect on extraction of the anti-foaming agent polypropylene glycol (PPG) was investigated. Surface tension measurements indicated that PPG did adsorb to the interface, however, no discernible effect on mass transfer was observed. In conjunction with the biological systems studied above PPG had mixed effects. When added to filtered fermentation broth the overall mass transfer coefficient was seen to increase. In conjunction with the protein BSA, PPG had no effect, but when PPG was added to a biomass system a further reduction in mass transfer was observed. Thus, the anti-foaming agent gave rise to differing effects, and it is recommended that further investigation is conducted in order to ascertain the different effects occurring in these systems.

The addition of the ionic surfactants SDS and DTAB to the extraction system was investigated. When extraction was performed from an otherwise clean system the addition of either surfactant was found to greatly enhance mass transfer. Using the Schlieren technique to observe the interfacial region it was found that the surfactants caused interfacial turbulence, and the increase in mass transfer is attributed to movement at the interface caused by this phenomenon. The cause of the interfacial turbulence may be due to the transfer of the surfactant across the interface. During chloramphenicol extraction, SDS or DTAB will also be extracted into the solvent phase. As it passes through the interface the surfactant may cause the localised reduction in the interfacial tension required to initiate interfacial turbulence.

The effect of SDS and DTAB on extraction from a biomass solution was also studied. The maximum reduction in surface tension caused by either SDS or DTAB was found to be unaffected by the presence of biomass, however, for both surfactants the value of the CMC was reduced. This indicates that the ionic surfactant dominates at the interface, although some interaction with the biomass does occur. When SDS was added to an extraction system containing biomass the enhanced mass transfer rates where still observed, and through a Schlieren investigation this was again found to be due to interfacial turbulence. However, when DTAB was added to the extraction system containing biomass no enhancement of mass transfer was found, and interfacial

turbulence was not observed. DTAB may have been prevented from transferring across the interface because it was adsorbed onto the surface of the cell wall through electrostatic interaction, and thus, it was unable to create the conditions at the interface required to initiate interfacial turbulence. SDS was also added to an extraction system containing the protein BSA, where enhanced mass transfer rates were observed, and again, this is attributed to interfacial turbulence.

Chapter 8

Reactive Extraction: Results and Discussion

8.1	Introduction	165
8.2	Extraction Equilibrium	165
	8.2.1 Co-Extraction	166
	8.2.2 The Equilibrium Constant	167
8.3	Modelling Extraction Equilibrium	170
8.4	The Effect of System Conditions on Extraction Equilibrium	174
	8.4.1 The Effect of Feed Composition on Solute Extraction	174
	8.4.2 The Effect of the Value of the Equilibrium Constant	178
	8.4.3 The Effect of a Second Competing Anion	179
	8.4.4 Extraction of Multi-Valent Anions	182
	8.4.5 The Effect of Phase Volume Ratio on Extraction Equilibrium	182
8.5	Co-Extraction During Mass Transfer	183
8.6	Mass Transfer Modelling	186
	8.6.1 Interfacial Flux Balance	187
	8.6.2 Mass Transfer Mechanism	188
	8.6.3 Determining Film Mass Transfer Coefficients	190
8.7	The Effect of Co-Extraction on Mass Transfer	192
8.8	Extraction from Fermentation Broth	195
	8.8.1 The Effect of Filtered Fermentation Broth	196
	8.8.2 The Effect of Biomass	198
8.9	The Effect of Surfactants on Extraction	199
8.10	Summary of Results and Discussion	201
	8.10.1 Equilibrium Extraction	201
	8.10.2 Mechanism of Mass Transfer	202
	8.10.3 Effects of Added Compounds	203

8.1 Introduction

In this chapter a reactive liquid-liquid extraction process was used to investigate the effects of fermentation broth components on solute extraction. The model system used was the extraction of the amino acid phenylalanine using the ion exchange reagent Aliquat 336 dissolved in 1-octanol. Fermentation broth will contain many anionic compounds, therefore, the results presented in this chapter will focus on how co-extraction of other anions affects solute recovery. Other anions present in the fermentation medium are expected to affect solute recovery mainly by affecting the equilibrium of the extraction reaction. In addition high levels of co-extraction will be in contrast to the process aim which is to concentrate the solute and also to separate it from similar compounds.

In Section 8.2 the occurrence of co-extraction is demonstrated, and the equilibrium constants that describe the extraction and co-extraction reactions are determined. These equilibrium constants are then used in Section 8.3 as the basis for modelling the equilibrium extraction process. In Section 8.4 the effect of system conditions on extraction equilibrium is investigated using the model of extraction and experimental extraction data. In Section 8.5 the occurrence of co-extraction during mass transfer is demonstrated, and in Section 8.6 a new description of the mass transfer process is presented which takes into account co-extraction during mass transfer. The effect that co-extraction has on mass transfer is examined in Section 8.7. In Section 8.8 extraction from fermentation broth, and the effect of the biomass, is investigated. Finally, the effects of two surfactants on mass transfer are explored in Section 8.9. A summary of the main results from this Chapter are given in Section 8.10.

8.2 Extraction Equilibrium

The aim of the liquid-liquid extraction process is to increase the concentration of the solute and to separate it from other compounds present in the fermentation broth. Using a reactive extraction mechanism it should be possible to separate the solute from non-polar compounds that do not partition into the solvent and which will not be extracted by the ion exchange reagent. The ion exchange reagent will not extract cationic

compounds, but other anionic compounds are liable to extraction. For the system under study it has been reported that co-extraction of other anionic compounds does occur (Behr and Lehn, 1973; Hano *et al.*, 1991; Scarpello and Stuckey, 2000). In Section 8.2.1 the occurrence of co-extraction during reactive extraction is demonstrated, and in Section 8.2.2 the equilibrium constants for the competing reactions are determined.

8.2.1 Co-Extraction

In Figure 8-1 the occurrence of co-extraction is highlighted for the simplest possible system where phenylalanine was extracted using Aliquat 336, and sodium hydroxide was added to the aqueous phase to allow the phenylalanine to exist in the anionic form. Any excess hydroxide ions not used in the neutralisation of phenylalanine are available for extraction. Details of the extraction reaction mechanisms were given previously in Section 5.5. Figure 8-1 presents the equilibrium concentrations of the products of the phenylalanine extraction reaction, which are solvent phase Aliquat-phenylalanine and aqueous phase chloride (Equation 5-1). It can be seen that the concentration of chloride is greater than Aliquat-phenylalanine for all the given initial phenylalanine concentrations. If co-extraction were not occurring then these two concentrations would be equal, but they are not equal. The extent of co-extraction is given by the difference between the two product concentrations, and as no other anions are present this must be due to hydroxide extraction. Also shown in Figure 8-1 is the line $y = x$, which represents complete extraction of phenylalanine. It can be seen that complete extraction does not occur, but at low initial phenylalanine concentrations extraction is almost complete. However, this near complete extraction also gives rise to a high level of co-extraction, with over four times the amount of phenylalanine being co-extracted. As the concentration of phenylalanine increases the level of co-extraction decreases, however, the fraction of phenylalanine extracted also decreases. Co-extraction occurs, and varies, because each extraction mechanism is related through the thermodynamics of the system. In Section 8.3 a model of the extraction system is given that will allow prediction of the extent of solute extraction and any co-extraction. However, before this can be done the equilibrium constant for the extraction reactions must be determined.

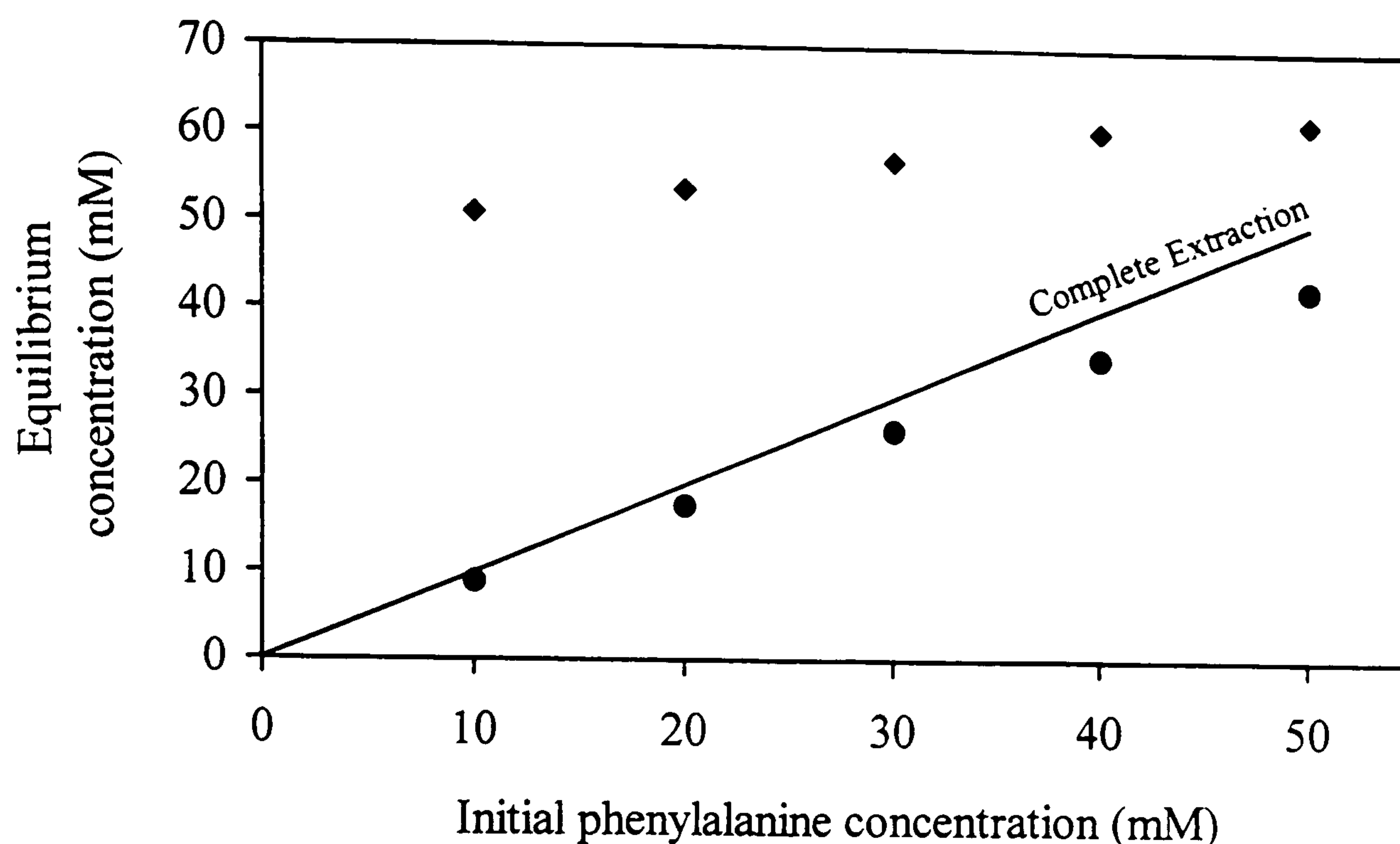


Figure 8-1 Variation in the equilibrium concentrations of the reaction products (Aliquat-phenylalanine, •, and chloride, ♦) from the phenylalanine extraction reaction where the initial phenylalanine concentration was varied from 10mM to 50mM. Initially, 100 mM sodium hydroxide was added to the aqueous phase, and 200 mM Aliquat 336 added to the solvent phase, temperature = 25°C, PVR = 1.

8.2.2 The Equilibrium Constant

Co-extraction occurs because the ion exchange reagent is not specific to phenylalanine and the extent of extraction and co-extraction is governed by the thermodynamics of the extraction reactions. This thermodynamic description is usually given in the form of an equilibrium constant for each reaction, and where multiple reactions occur the equilibrium conditions for each reaction are related. This relationship will affect the extent of product formation from each reaction.

The equilibrium constant for a reaction is described as the product of each species' activity raised to the power of its stoichiometric number. For liquid phase reactions the activity of each species can be described by the species mole fraction, which for low concentrations can be given as the concentration (Smith and Van Ness, 1987). For the cases of phenylalanine and hydroxide extraction the equilibrium constants, which were

defined previously in Section 5-5, are given as,

$$K^{*}_{Phe} = \frac{C_{QPhe,e}C_{Cl,e}}{C_{Phe,e}C_{QCl,e}} \tag{8-1}$$

$$K^{*}_{OH} = \frac{C_{QOH,e}C_{Cl,e}}{C_{OH,e}C_{QCl,e}} \tag{8-2}$$

The value of the equilibrium constant may be determined using experimental concentration measurements plotted according to Equations 8-1 and 8-2, and shown in Figures 8-2 and 8-3 for phenylalanine and hydroxide respectively. The same data was used in both Figures 8-2 and 8-3; gathered from a series of 25 equilibrium extraction experiments where the initial concentration of both phenylalanine and sodium hydroxide was varied.

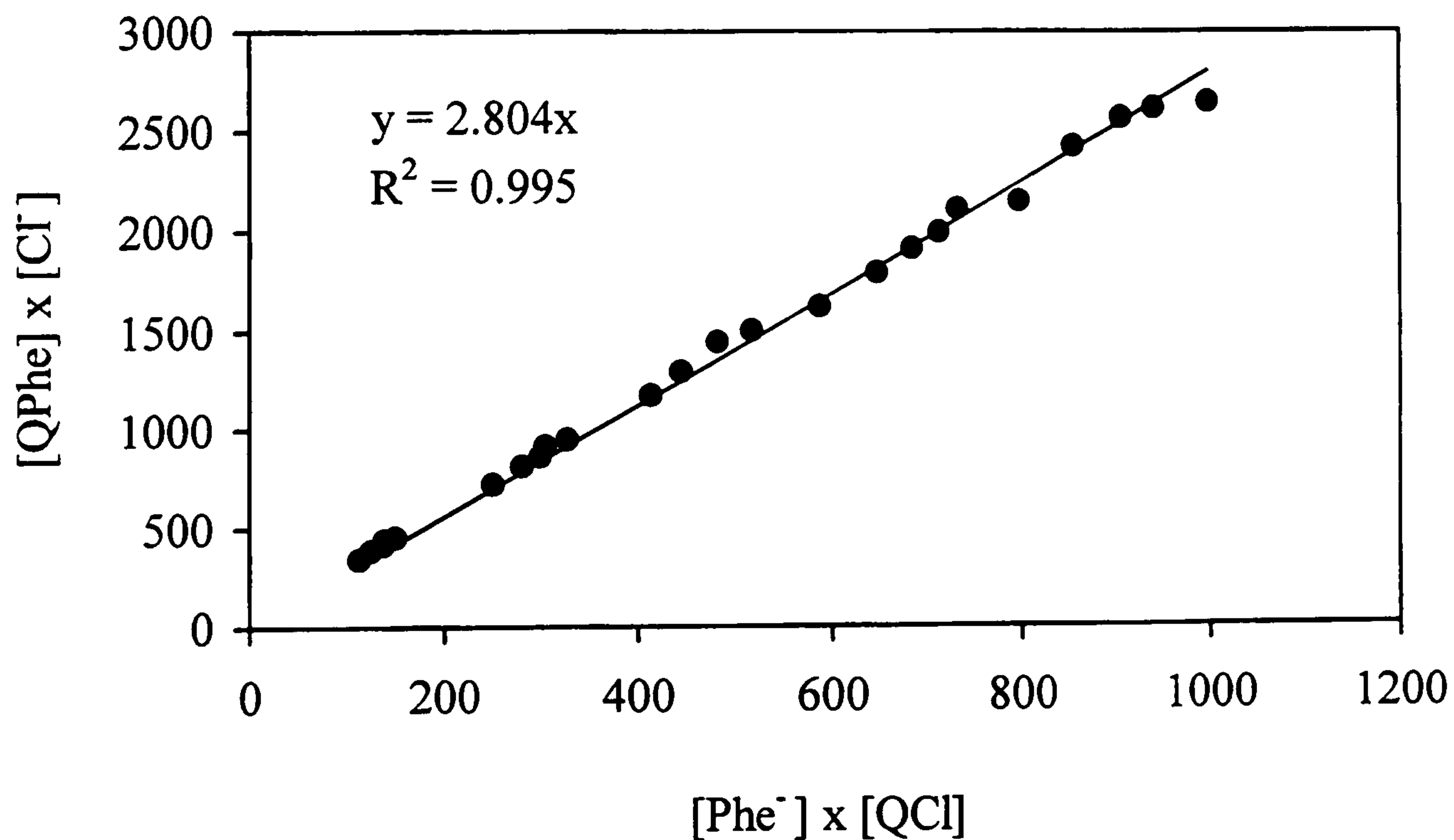


Figure 8-2 Plot to determine the equilibrium constant for the phenylalanine extraction reaction, K^{*}_{Phe}. Initially; 10-50 mM phenylalanine and 50-100mM sodium hydroxide added to the aqueous phase, 200mM Aliquat 336 added to the solvent phase, temperature = 25°C, and PVR = 1.

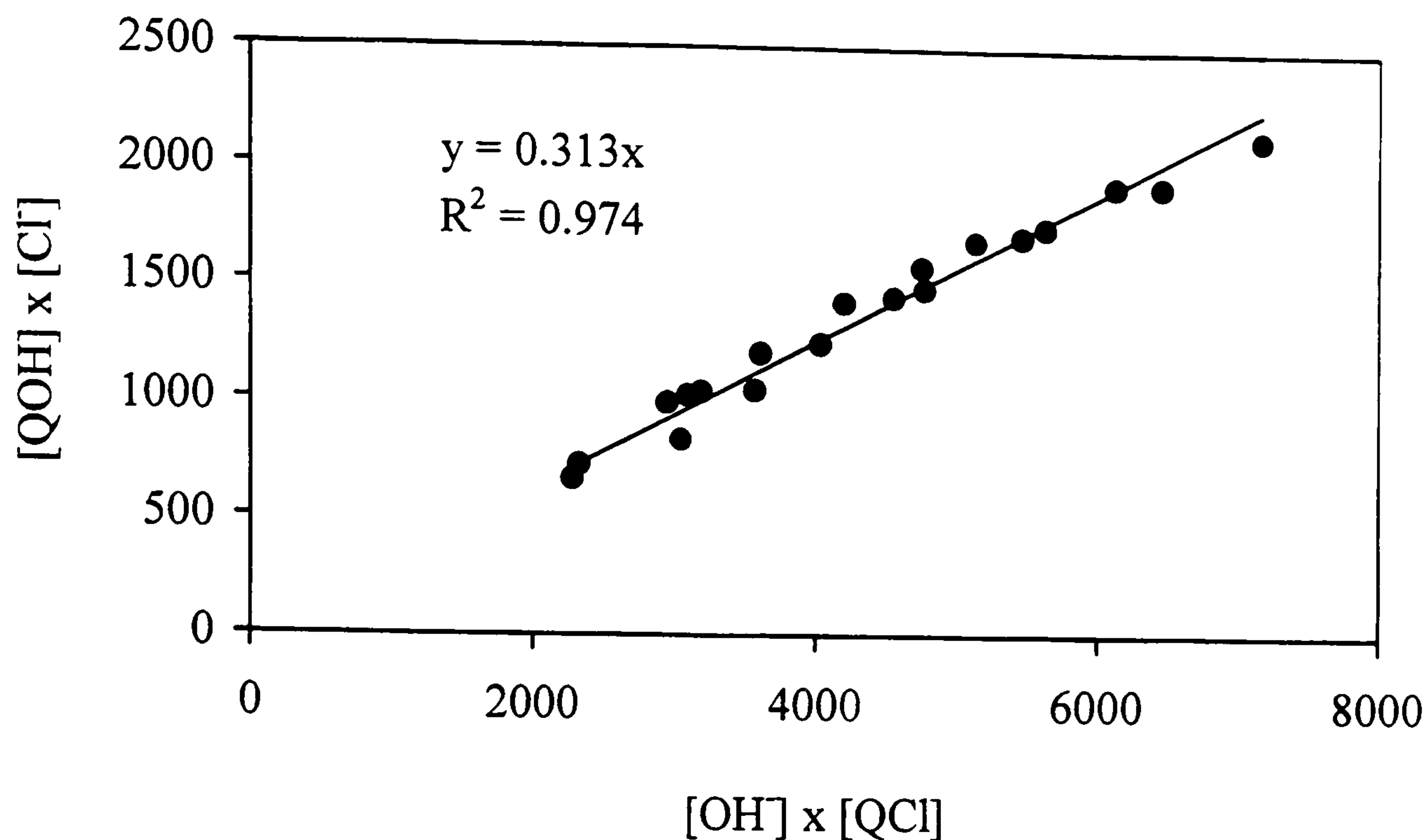


Figure 8-3 Plot to determine the equilibrium constant for the hydroxide extraction reaction, K^*_{OH}. Initially; 10-50 mM phenylalanine and 50-100mM sodium hydroxide added to the aqueous phase, 200mM Aliquat 336 added to the solvent phase, temperature = 25°C, and PVR = 1.

In Figure 8-2 and 8-3 it can be seen that good linear fits to the data are obtained, and the value of the equilibrium constant is given by the slope of the fitted line. The equations describing the fitted lines are also given in the figures; therefore, the values of the equilibrium constants are 2.804 and 0.313 for the phenylalanine and hydroxide extraction reactions, respectively.

Scarpello (1998) suggested that the hydroxide equilibrium constant was a function of the initial phenylalanine concentration, and varied as a result of varying levels of co-extraction. However, the thermodynamic description of an equilibrium constant defines it as only a function of temperature, and not composition. In Figure 8-3 it is seen that for this work a constant value of the equilibrium constant is obtained for situations where both initial phenylalanine and hydroxide concentrations have been varied. When determining the initial and equilibrium concentrations of hydroxide and Aliquat-hydroxide species, the effect of the acid neutralisation reaction must be taken into account. Hydroxide is added to the system to dissociate phenylalanine; in order for it to do this a hydroxide ion reacts with the hydrogen ion associated with phenylalanine, so as to maintain the ionic product of water. Thus, some of the initial hydroxide ions added to the system are converted to water and take no part in the equilibrium

description or the extraction process. The amount of hydroxide removed in this way will be stoichiometrically equivalent to the number of dissociated moles of phenylalanine present. When the removal of hydroxide ions is not taken in to account in mass balances, the value of the equilibrium constant determined is seen to vary with concentration, which may explain the result reported by Scarpello (1998). In the remainder of this chapter all quoted concentrations of hydroxide have been corrected for the neutralisation reaction.

8.3 Modelling Extraction Equilibrium.

In this section the equilibrium conditions of the extraction process are modelled using the description of equilibrium given by the equilibrium constants for the two extraction reactions. This model, which will be verified using experimental data, will then be used to explore the effect of system conditions on extraction and co-extraction, and where possible the predictions will be supported with experimental results. In some cases experimental evidence may not be readily obtainable and the model will be used alone to describe the extraction conditions that may arise; this is done with the confidence of the supporting experimental data for other conditions.

The model uses the equilibrium constant of each reaction to describe the relative concentrations of each species at equilibrium, and mass balances described in terms of initial concentrations and the extent of reaction will allow the composition of each species to be predicted. The equilibrium constant is correctly defined using mole fractions. In the case examined here the total moles remains constant. The volumes of the phases are equal, and thus concentrations may be used to describe the equilibrium constant. The definitions for each species are,

$$C_{phe,e} = C_{phe,0} - E_{Phe} \tag{8-3}$$

$$C_{OH,e} = C_{OH,0} - E_{OH} \tag{8-4}$$

$$C_{QCl,e} = C_{QCl,0} - E_{Phe} - E_{OH} \tag{8-5}$$

$$C_{Qphe,e} = E_{Phe} \tag{8-6}$$

$$C_{QOH,e} = E_{OH} \tag{8-7}$$

$$C_{Cl,e} = E_{Phe} + E_{OH} \tag{8-8}$$

Where, E_{Phe} and E_{OH} are the extents of the two extraction reactions. E_{Phe} and E_{OH} are equal to the moles of phenylalanine and hydroxide that react, and for a phase volume ratio of 1, are equal to the number of moles of each Aliquat-anion complex formed. Substitution of the above expressions into the definitions of the equilibrium constants gives,

$$K^{*}_{Phe} = \frac{E_{Phe}\left(E_{Phe} + E_{OH}\right)}{\left(C_{Phe,0} - E_{Phe}\right)\left(C_{QCl,0} - E_{Phe} - E_{OH}\right)} \qquad (8\text{-}9)$$

$$K^{*}_{OH} = \frac{E_{OH}\left(E_{Phe} + E_{OH}\right)}{\left(C_{OH,0} - E_{OH}\right)\left(C_{QCl,0} - E_{Phe} - E_{OH}\right)} \qquad (8\text{-}10)$$

Using Equations 8-1 and 8-2 a third expression may be defined as the ratio of the two equilibrium constants,

$$\frac{K^{*}_{Phe}}{K^{*}_{OH}} = K^{*}_{R} = \frac{E_{Phe}\left(C_{OH,0} - E_{OH}\right)}{E_{OH}\left(C_{Phe,0} - E_{Phe}\right)} \qquad (8\text{-}11)$$

This third expression is defined to allow the solution of the resulting expressions to be less complicated. For a given extraction system the initial concentrations will be defined, and equilibrium constants for the two reactions are already known. Therefore, in Equations 8-9 to 8-11 there are 2 unknowns. Any two of the three above Equations are independent and may be solved simultaneously to obtain a value of the extent of the two reactions.

An expression for E_{OH} in terms of E_{Phe} is obtained by re-writing Equation 8-11,

$$E_{OH} = \frac{E_{Phe}\, C_{OH,0}}{E_{Phe} + K^{*}_{R}\, C_{Phe,0} - K^{*}_{R}\, E_{Phe}} \qquad (8\text{-}12)$$

Equation 8-12 may now be substituted into Equation 8-9 to eliminate E_{OH^-} and then rearranged to give a cubic polynomial

$$E_{Phe}^3 + b\,E_{Phe}^2 + c\,E_{Phe} + d = 0 \qquad (8\text{-}13)$$

where,

$$b = \frac{K_{Phe}^{*}\left(C_{Phe,0} + C_{QCl,0} - C_{OH,0} - K_R^{*}\left(2C_{Phe,0} + C_{QCl,0}\right)\right) + K_R^{*}\,C_{Phe,0} + C_{OH,0}}{K_R^{*}\left(K_{Phe}^{*} - 1\right) - K_{Phe}^{*} + 1} \qquad (8\text{-}14)$$

$$c = \frac{K_{Phe}^{*}\,C_{Phe,0}\left(K_R^{*}\left(C_{Phe,0} + 2\,C_{QCl,0}\right) - C_{QCl,0} - C_{OH,0}\right)}{K_R^{*}\left(K_{Phe}^{*} - 1\right) - K_{Phe}^{*} + 1} \qquad (8\text{-}15)$$

$$d = \frac{K_{Phe}^{*}\,K_R^{*}\,C_{Phe,0}^2\,C_{QCl,0}}{K_R^{*}\left(K_{Phe}^{*} - 1\right) - K_{Phe}^{*} + 1} \qquad (8\text{-}16)$$

Exact solutions of cubic polynomials are available, and the form of the solution that corresponds to the situation encountered here is given below,

$$E_{Phe} = \pm 2\sqrt{\frac{-p}{3}}\,\cos\left[\left(\frac{\phi}{3}\right) + 120\,k\right] \qquad (8\text{-}17)$$

Where,

$$\phi = \cos^{-1}\left(\sqrt{\frac{27\,q^2}{-4\,p^3}}\right) \qquad (8\text{-}18)$$

$$p = \frac{3c - b^2}{3} \qquad (8\text{-}19)$$

$$q = \frac{27d - 9bc + 2b^3}{27} \tag{8-20}$$

The sign at the start of the right hand side of Equation 8-17 is taken to be opposite to the sign of q, and the constant k has the value 0, 1 or 2 depending on which root is required. The root is chosen subject to the physical constraints of the extraction system such that $0 \leq E_{Phe} \leq C_{phe,0}$. Following evaluation of E_{Phe} using Equation 8-17, the extent of the co-extraction reaction, E_{OH}, may be determined using Equation 8-12.

In the following section this model will be used to predict the effect of initial conditions on solute extraction and co-extraction. First, however, the validity of this model will be demonstrated by comparison of predicted values of E_{Phe} and E_{OH} to those determined from extraction experiments. In Figure 8-4 data obtained from equilibrium experiments are presented where the initial hydroxide anion and phenylalanine anion concentrations have been varied. The data used in Figure 8-4 to validate the model is different to that used in Figures 8-2 and 8-3 to determine the equilibrium constant. Use of this data would be inappropriate because the model is based on the equilibrium description given by this data, and therefore should correlate with the predicted values.

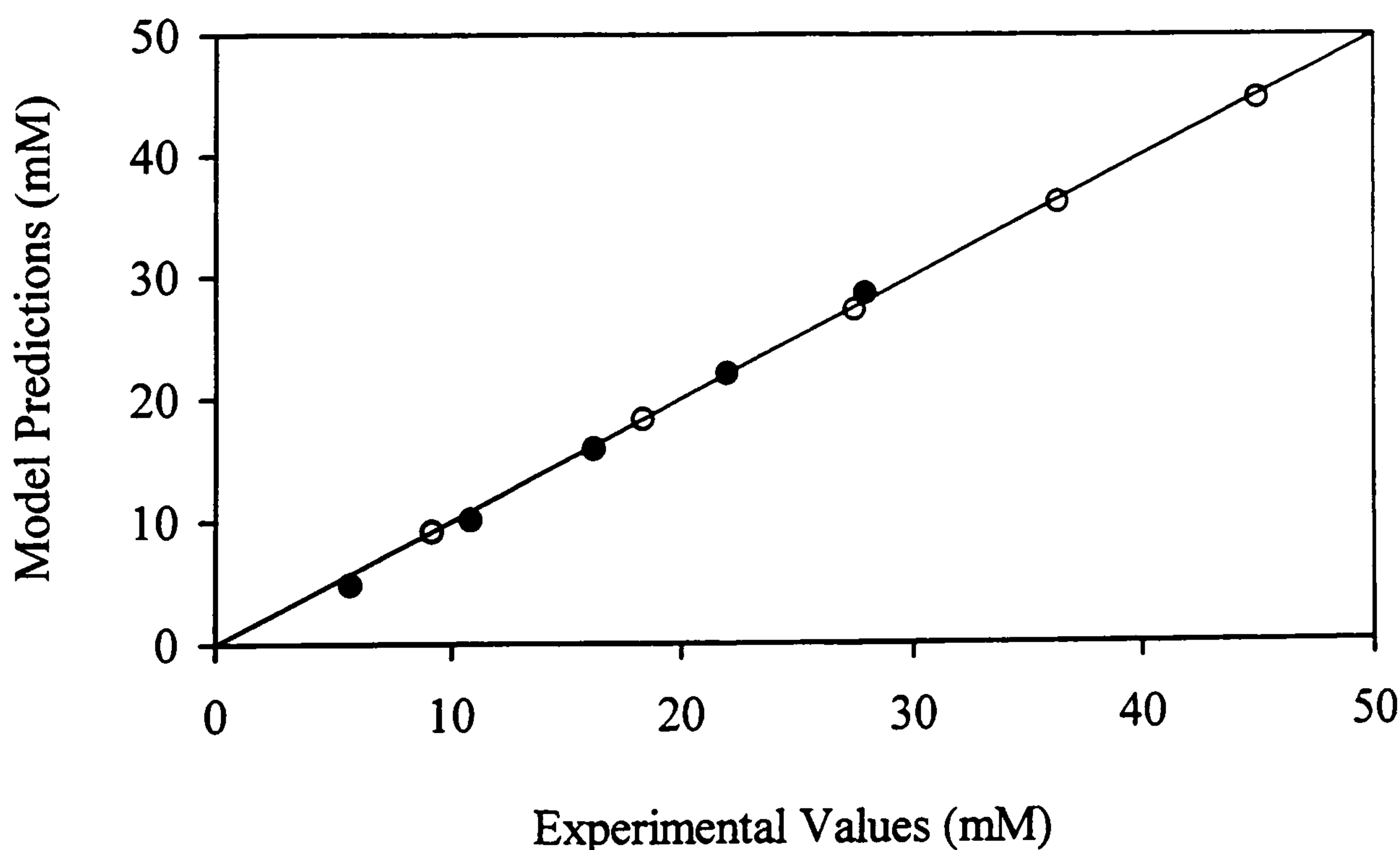


Figure 8-4 Comparison of experimental results to model predictions for the extent of reaction; E_{Phe}, phenylalanine extraction reaction, o, and, E_{OH}, hydroxide extraction reaction, •. Initial phenylalanine anion concentration, 10-50mM, Initial hydroxide anion concentration, 10-60mM, initial Aliquat 336 concentration = 200mM, temperature = 25°C, and PVR = 1.

In Figure 8-4 it can be seen that the ratio of the predicted values to the experimentally determined values lies on or very close to the line y = x. This indicates that the model can be used to predict the equilibrium conditions for the extraction of phenylalanine using Aliquat 336 where a competing anion is present.

8.4 The Effect of Co-Extraction on Extraction Equilibrium

In this section the effect of system conditions on extraction equilibrium was investigated using the model developed in the previous section, and extraction data obtained from experiments. In Section 8.4.1 the effect of the initial reactant concentrations are examined, while the effect of varying the equilibrium constant is theoretically considered in Section 8.4.2. In Section 8.4.3 the effect of a second competing anion is examined. The ability of Aliquat 336 to extraction di-valent compounds is briefly examined in Section 8.4.4, and finally the effect of the phase volume ratio on extraction is presented in Section 8.4.5.

8.4.1 The Effect of Feed Composition on Solute Extraction

The composition of the media received from a fermentation reactor will change over time due to variable fermenter performance, and changes in the initial feedstock composition. It was shown in Figure 8-1 that the level of extraction and co-extraction was dependent upon the composition of the initial solution. Therefore, in this section the effect of feed composition was examined further by looking at the effect of varying solute and competing anion concentrations on the extent of the two reactions, and also the effect of extractant concentration is considered.

In Figure 8-5 the effect of increasing the initial phenylalanine anion concentration on the extent of the phenylalanine extraction reaction for different initial hydroxide anion concentrations is presented. E_{Phe} is equal to the reduction in the aqueous phase concentration of phenylalanine, and is also equal to the concentration of phenylalanine-Aliquat in the solvent phase, when the PVR is 1. It can be seen from Figure 8-5 that the extent of the phenylalanine extraction reaction decreases slightly with an increase in the initial hydroxide anion concentration, with the effect being more pronounced at higher

initial phenylalanine concentrations. As the initial phenylalanine concentration is increased the extent of reaction increases, however, the extraction yield of phenylalanine decreases.

An increase in the extent of reaction with phenylalanine concentration is to be expected according to the thermodynamic description of the reaction at equilibrium. Given an increase in the concentration of a reactant the system will respond by increasing the product concentrations so as to maintain the distribution described by the equilibrium constant. The yield decreases with increasing phenylalanine concentration because there is only an increase in one of the reactants, to maintain the extraction yield the concentration of the ion exchange reagent should also be increased. As the concentration of hydroxide is increased there will be an increase in hydroxide extraction (see below) resulting in an increase in chloride, which is a common product for both reactions. This will cause the species concentration to be redistributed in favour of the reactants of the phenylalanine extraction reaction.

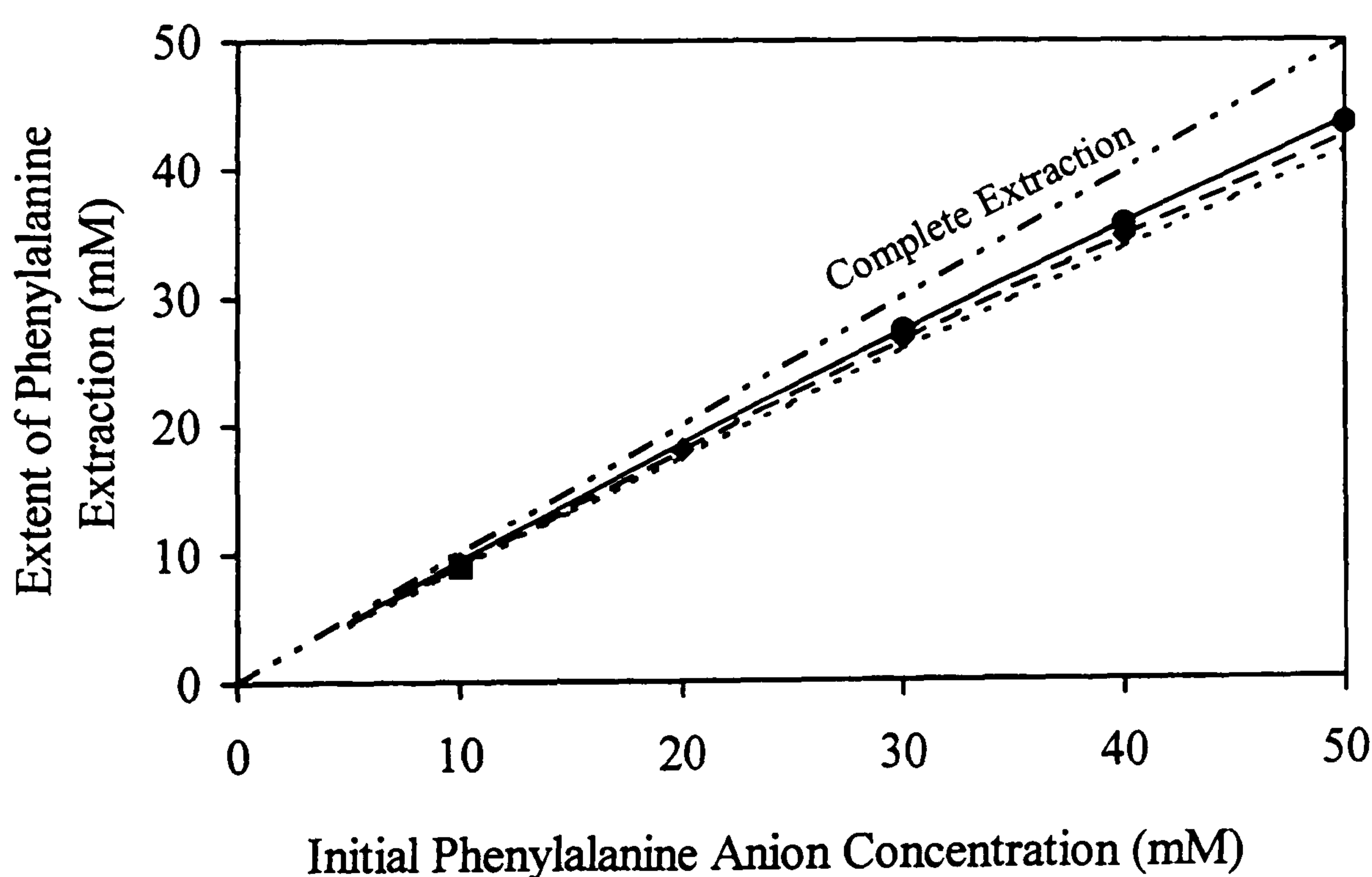


Figure 8-5 The change in E_{Phe}, the extent of the phenylalanine extraction reaction, with increasing initial phenylalanine anion concentration for initial hydroxide concentrations of 30mM (• and —), 60mM (♦ and —), and 90mM (■ and ---). Initial Aliquat 336 concentration = 200mM, and PVR = 1. Markers denote experimental data and the lines are the model predictions.

In Figure 8-6 the effect of increasing the initial hydroxide anion concentration on the extent of the hydroxide extraction reaction for different initial phenylalanine anion concentrations is presented. E_{OH} is equal to the reduction in the aqueous phase concentration of hydroxide, and is also equal to the concentration of hydroxide-Aliquat in the solvent phase when the PVR is 1. Figure 8-6 shows trends similar to those seen in Figure 8-5, however, the effect of both the phenylalanine and the hydroxide concentrations are more pronounced on the extent of the hydroxide extraction reaction.

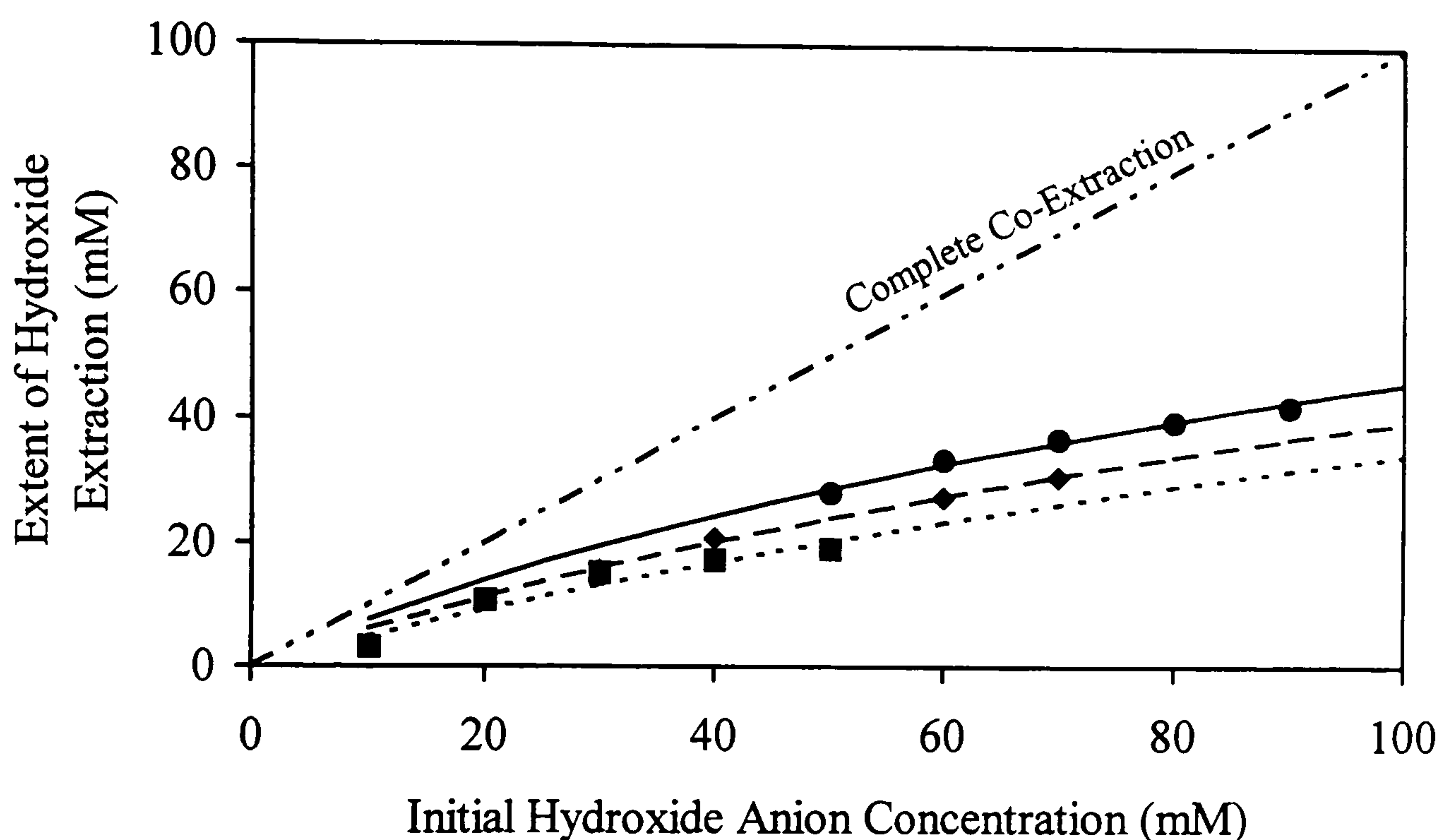


Figure 8-6 The change in E_{OH}, the extent of the hydroxide extraction reaction, with increasing initial hydroxide anion concentration for initial phenylalanine concentration of 10mM (• and —), 30mM (♦ and —), and 50mM (■ and ---). Initial Aliquat 336 concentration = 200mM, temperature = 25°C, and PVR = 1. Markers denote experimental data and the lines are the model predictions.

These two concentrations have a greater effect on the extraction yield of hydroxide because the value of the equilibrium constant for hydroxide extraction is an order of magnitude lower than for phenylalanine extraction, and is below unity indicating that the reverse reaction is thermodynamically favoured. When a reactant concentration is increased only a fraction will be converted to the products, and similarly as a product concentration is increased (as caused by increased phenylalanine extraction yielding more chloride) the equilibrium will shift to a greater extent towards the reactants than was observed for phenylalanine extraction.

In Figure 8-7 the effect of the concentration of Aliquat 336 on the extent of both extraction reactions is shown. When there is excess ion exchange reagent, increasing its concentration will increase the extent of both extraction reactions. It is seen that more phenylalanine is extracted than hydroxide, due to the higher value of the equilibrium constant for the phenylalanine reaction. Also shown is the fraction of extracted material that is phenylalanine. This parameter decreases as the concentration of Aliquat 336 increases. At higher Aliquat 336 concentrations the extraction fraction of phenylalanine will tend towards the mole fraction of the initial aqueous phase (which in this case was 0.5), as both reactions will effectively go to completion.

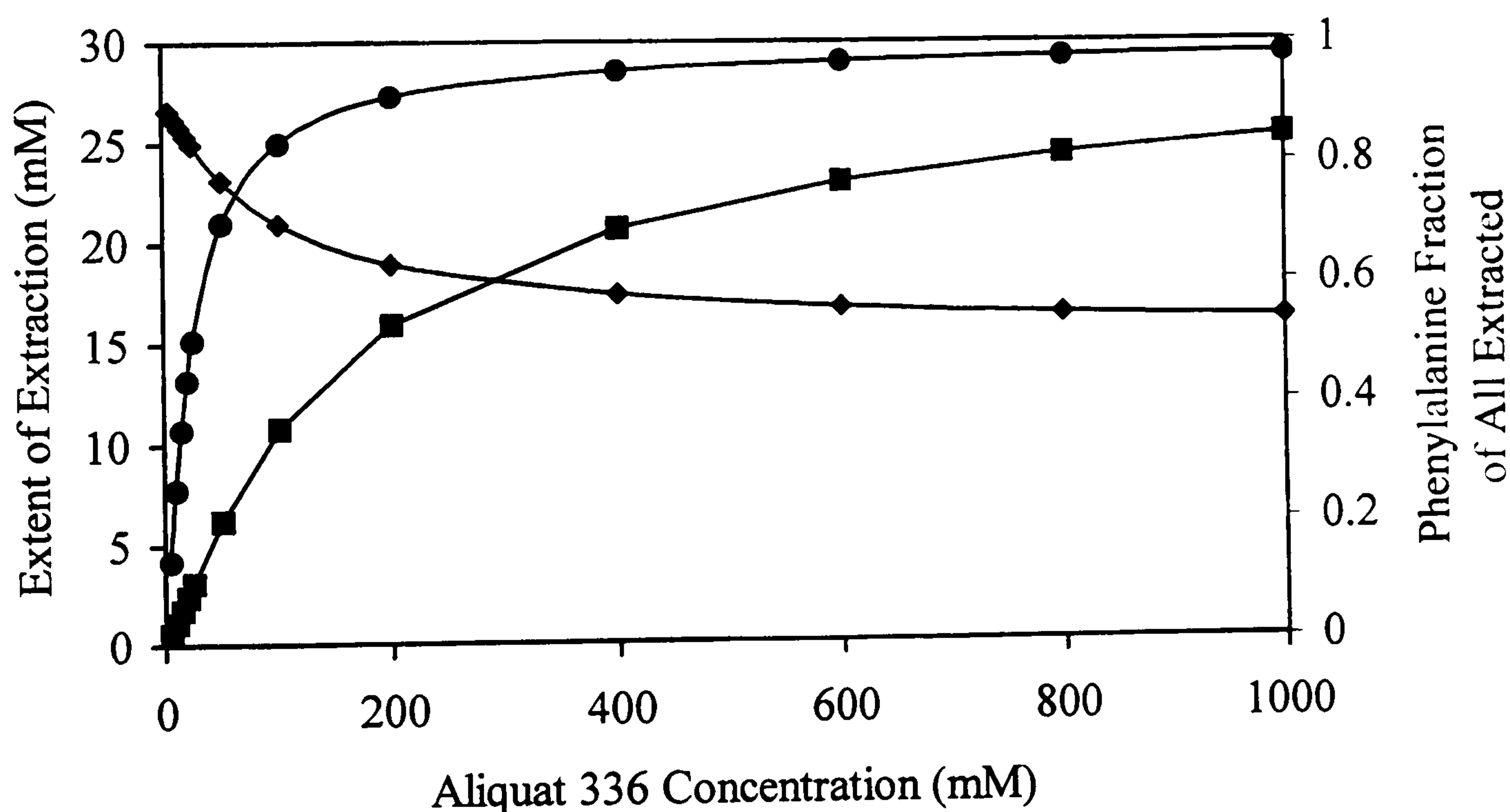


Figure 8-7 The effect of the Aliquat 336 concentration on the extent of the extraction, •, and the co-extraction, ■, reactions. Plotted on the second y-axis is the phenylalanine fraction of all extracted species, ♦. Initially, 30mM phenylalanine and 30mM hydroxide anions in the aqueous phase, and a PVR of 1. Results were based on the model only.

8.4.2 Effect of the Value of the Equilibrium Constant

In Section 8.4.1 the effect of the initial concentration of the reactant species on extraction was examined, where the co-extractable anion was the excess of hydroxide used to dissociate phenylalanine. In that situation the values of the equilibrium constants were fixed at the values determined from experiment, however, the co-extractable species may change, and so will the value of the equilibrium constant for the extraction reaction.

In this section the effect of the value of the equilibrium constant for the co-extraction reaction is investigated. This has been done using only the model of the equilibrium extraction process developed in Section 8.3; experimentally it was not possible to vary the second competing anion, as this had to be hydroxide. The effect of changing the value of the equilibrium constant for the solute extraction reaction was also examined: this situation will be relevant if a similar reaction mechanism occurs but Aliquat is used to extract a different compound, or a different ion exchange reagent is used to extract phenylalanine.

In Figure 8-8 the effects of changing the equilibrium constant for both the extraction, K^*_{Ex}, and co-extraction reactions, $K^*_{Co\text{-}Ex}$, are shown. The initial concentrations of the reactants are constant, at 30mM for both extractable anions and 200 mM for the ion exchange reagent. It can be seen that increasing the value of the equilibrium constant for the co-extraction reaction causes the extent of the phenylalanine extraction reaction to decrease ($K^*_{Ex} = 2.8$). A similar trend is seen for the other values of K^*_{Ex}, and for all cases increasing the value of $K^*_{Co\text{-}Ex}$ beyond 10 gives effectively no further change in the extent of extraction. The extent of the extraction reaction increases with K^*_{Ex}, resulting in an increase in the extraction yield. This is to be expected as an increase in the reaction constant will push the distribution of the species towards product formation, tending toward complete extraction at high K^*_{Ex}.

The effects of the equilibrium constants on the extent of the co-extraction reaction will be analogous to that already described for the extraction reaction. Therefore, to achieve a high level of extraction a high equilibrium constant is required, and to minimise co-

extraction the equilibrium constant for the co-extraction reaction should be considerably lower. When the equilibrium constants are equal it will not be possible to achieve any separation of the solute over the competing anion.

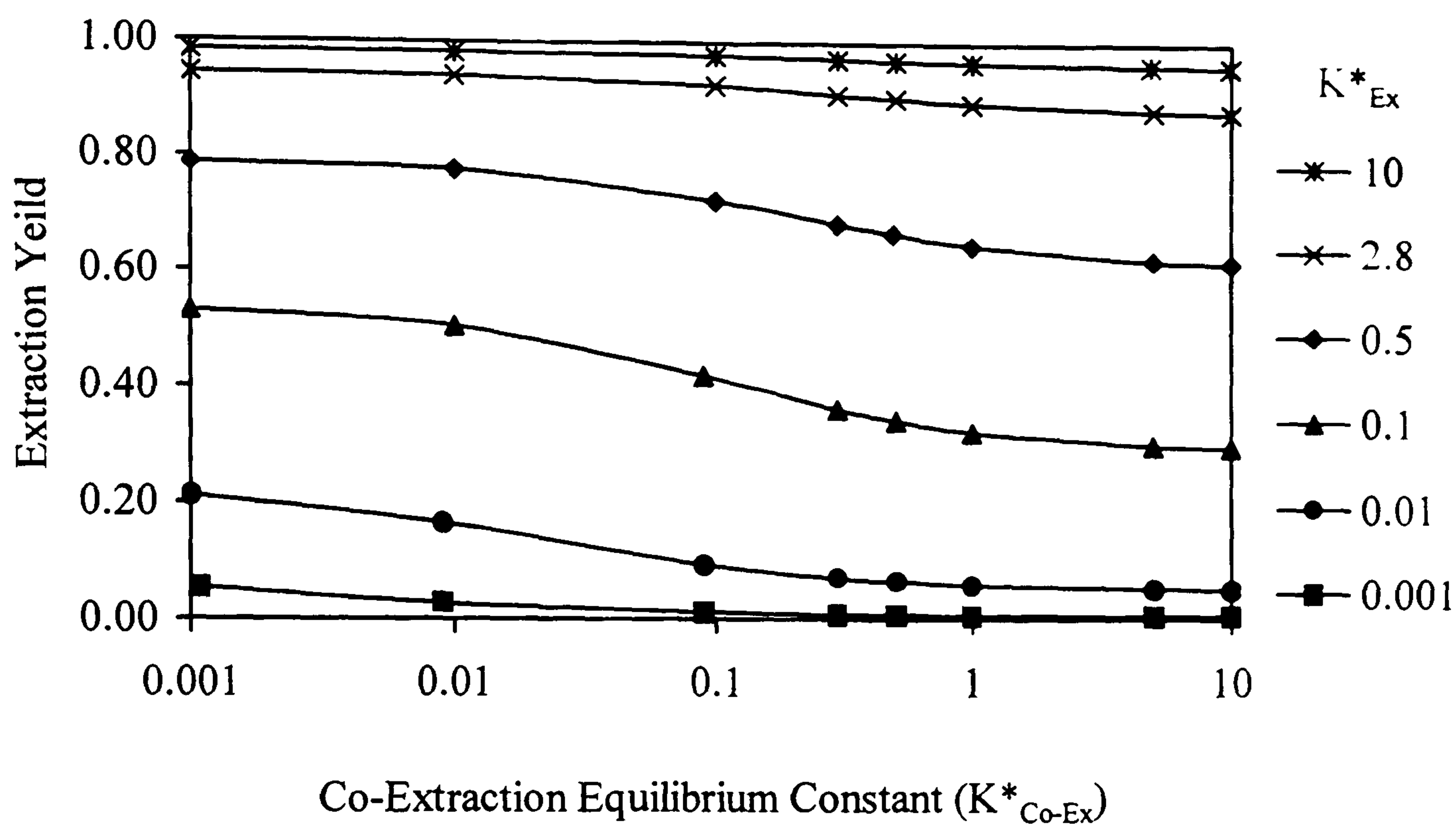


Figure 8-8 The effect of the equilibrium constant for both extraction and co-extraction reactions on the extraction yield of the solute. Initially, 30mM phenylalanine and 30mM hydroxide anions in the aqueous phase, 200mM Aliquat 336 in the solvent phase, and a PVR of 1. Results were based on the model only.

8.4.3 Effect of a Second Competing Anion

So far the model of extraction, and the supporting experimental evidence has been used to investigate situations where only one other anionic species is present in the aqueous phase. However, for extraction from fermentation broth this situation is unrealistic as many ionic compounds are added to fermentation broth to optimise microbial growth and metabolite production, e.g. nutrient salts or buffers. In this investigation the effect of adding bromide ions in the form of the salt potassium bromide was investigated.

In Figure 8-9 the plot used to determine the equilibrium constant for bromide extraction is presented. Like the plots obtained for phenylalanine and hydroxide (Figures 8-2 and 8-3) the data fits a straight line passing through the origin. This confirms the assumed 1:1 stoichiometry for this extraction, and the reaction mechanism may be described similarly to phenylalanine and hydroxide (Equations 5-1 and 5-2). From the gradient of the trendline the equilibrium constant was determined to be 2.66.

This value is similar to the value for phenylalanine extraction, and given the effects seen in Figure 8-8 it is expected that bromide co-extraction will have a greater effect on phenylalanine extraction than hydroxide co-extraction. In Figure 8-10 the effect of initial bromide concentration on the extent of the phenylalanine reaction and co-extraction constant is presented. The co-extraction constant is the ratio of the extent of both extraction reactions to the extent of the phenylalanine extraction reaction. It is seen that with increasing initial bromide concentration the extent of phenylalanine extraction decreases; this occurs in a similar manner to the effect of hydroxide, where an increase in the extraction of bromide causes an increase in the production of chloride. This increase in chloride then pushes the phenylalanine reaction towards the reactants. Thus, extraction of phenylalanine is reduced as a result of increasing bromide extraction, and hence the level of co-extraction increases, as indicated by the increase in the co-extraction constant. These effects are intuitively obvious, and the extraction model presented in Section 8.3 may therefore be extended to account for multiple anion co-extraction. This has not been done in this investigation due to the increased complexity in solving the simultaneous equations that arise.

It is concluded that, in general, co-extracting anions will effect solute recovery and the magnitude of the effect will depend on the value of the equilibrium constant. The nature of the affinity of the ion exchange reagent for a solute was investigated by Hano *et al.* (1991). They correlated the value of the equilibrium constant with the hydrophobicity of the reactant, where increased hydrophobicity resulted in a higher equilibrium constant. Thus, the main opposition to efficient solute extraction will come from hydrophobic anionic compounds present in the extraction medium.

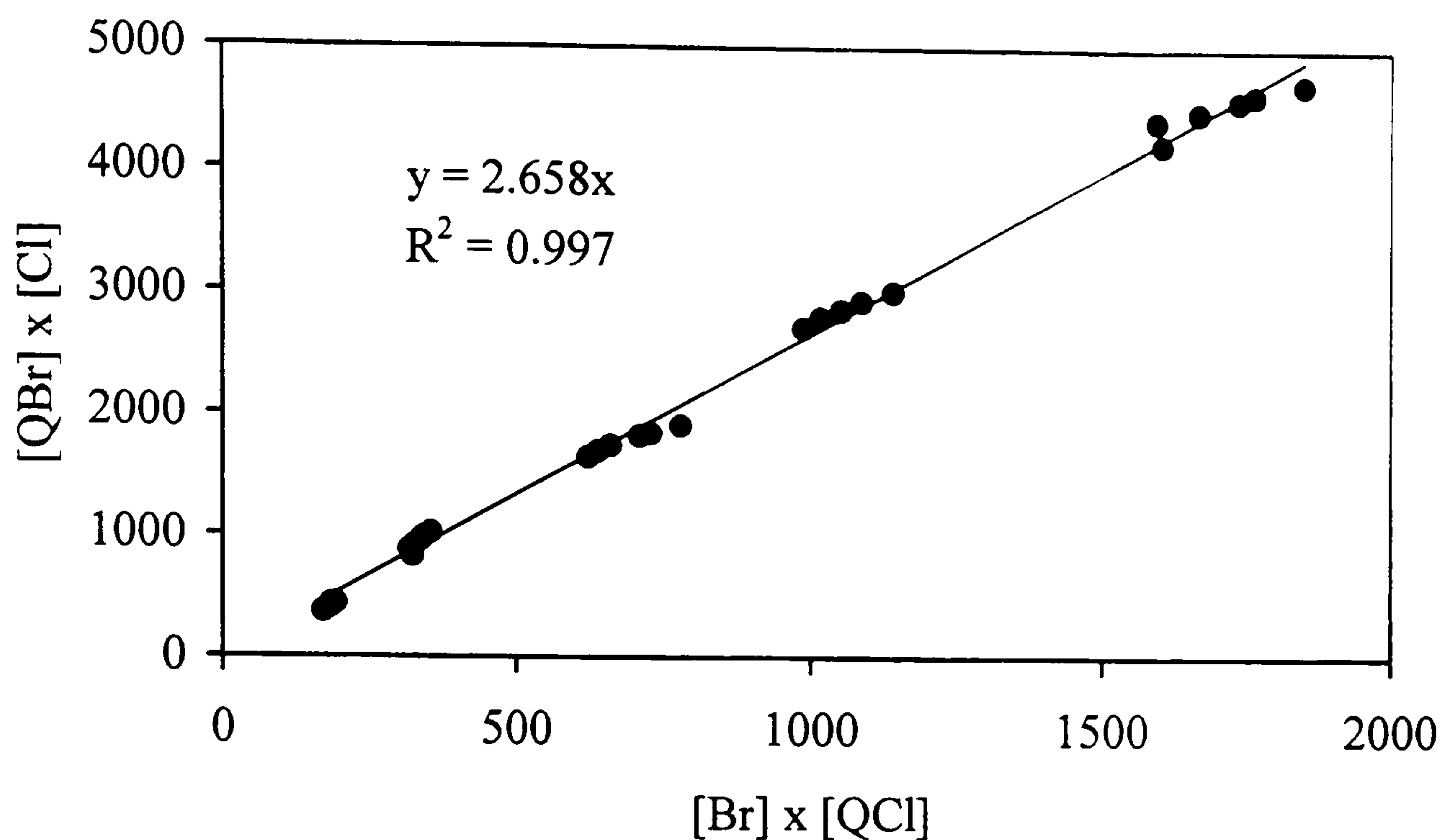


Figure 8-9 Plot to determine the equilibrium constant for the bromide extraction reaction, K^*_{Br}. Initially; 35mM phenylalanine, 25mM hydroxide anions and 10-70mM bromide anions added to the aqueous phase, 200mM Aliquat 336 added to the solvent phase, temperature = 25°C, and PVR = 1.

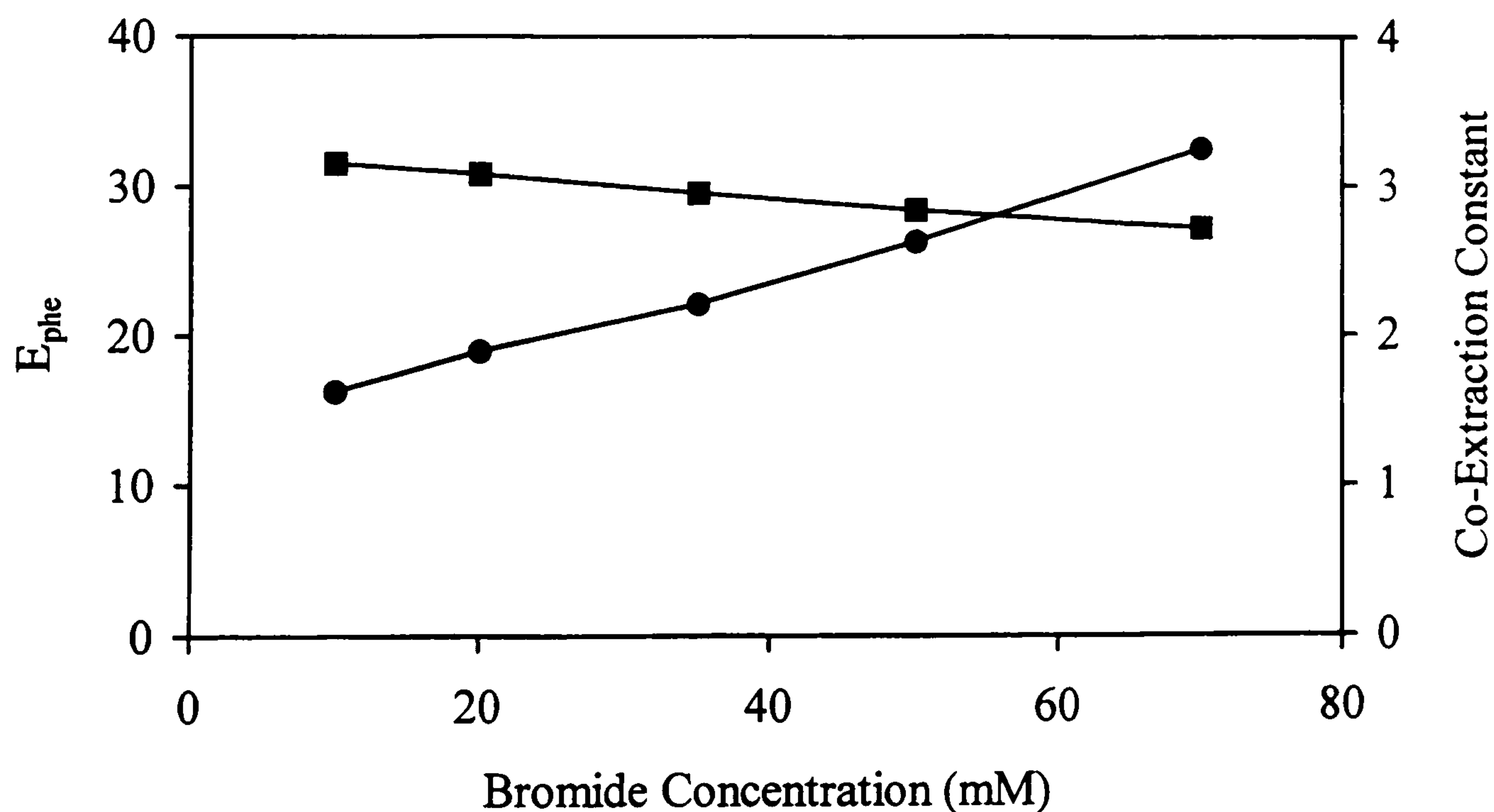


Figure 8-10 The effect of varying the initial bromide concentration from 10 to 70mM. on the extent of the phenylalanine extraction reaction (■, 1st y-axis) and the value of the co-extraction constant (•, 2nd y-axis). Initially: 35mM phenylalanine and 25mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase,, temperature = 25°C, and PVR = 1.

8.4.4 Extraction of Multi-Valent Anions

The extraction and co-extraction reactions examined so far have considered reactions where extraction occurs with a 1:1 stoichometry. However, fermentation broth is likely to contain multi-valent compounds, which could possibly be extracted by Aliquat 336 through a 1:n stoichometry, where n is the valency of the anion. In this study a brief examination of this concept was conducted, and it was found that Aliquat 336 will extract carbonate (CO_3^{2-}), phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}). These compounds are commonly used in fermentation process, and are often added in excess so as not to limit microbial metabolism. These results are in accordance with similar results presented by Hano *et al.* (1991) where equilibrium constants for the extractions where determined. The values of the reported equilibrium constant were low, below the value for hydroxide, and therefore their effect on the extraction process will be less than the effects observed for bromide.

8.4.5 The Effect of PVR on Extraction Equilibrium

During this investigation it became apparent from conflicting results that the phase volume ratio of the two liquid phases had an influence on extraction behaviour. This effect was investigated further, and in Figure 8-11 the effect of the phase volume ratio on the equilibrium constant for the phenylalanine is presented. It can be seen that with increasing relative solvent volume the equilibrium constant for the phenylalanine reaction increases, and a similar effect was observed for the hydroxide reaction constant. This effect would have implications on the operation of an industrial process. Operating with a high PVR would give a high extraction yield as a result of the increased equilibrium constant; however, the equilibrium constant for other species may also be increased resulting in an increase in the level of co-extraction.

The thermodynamic description of the equilibrium constant indicates that it should only be a function of temperature and not composition. Thus, the cause of this effect is unknown, but it may be due to non-idealities of the species. Further investigation of this effect is required.

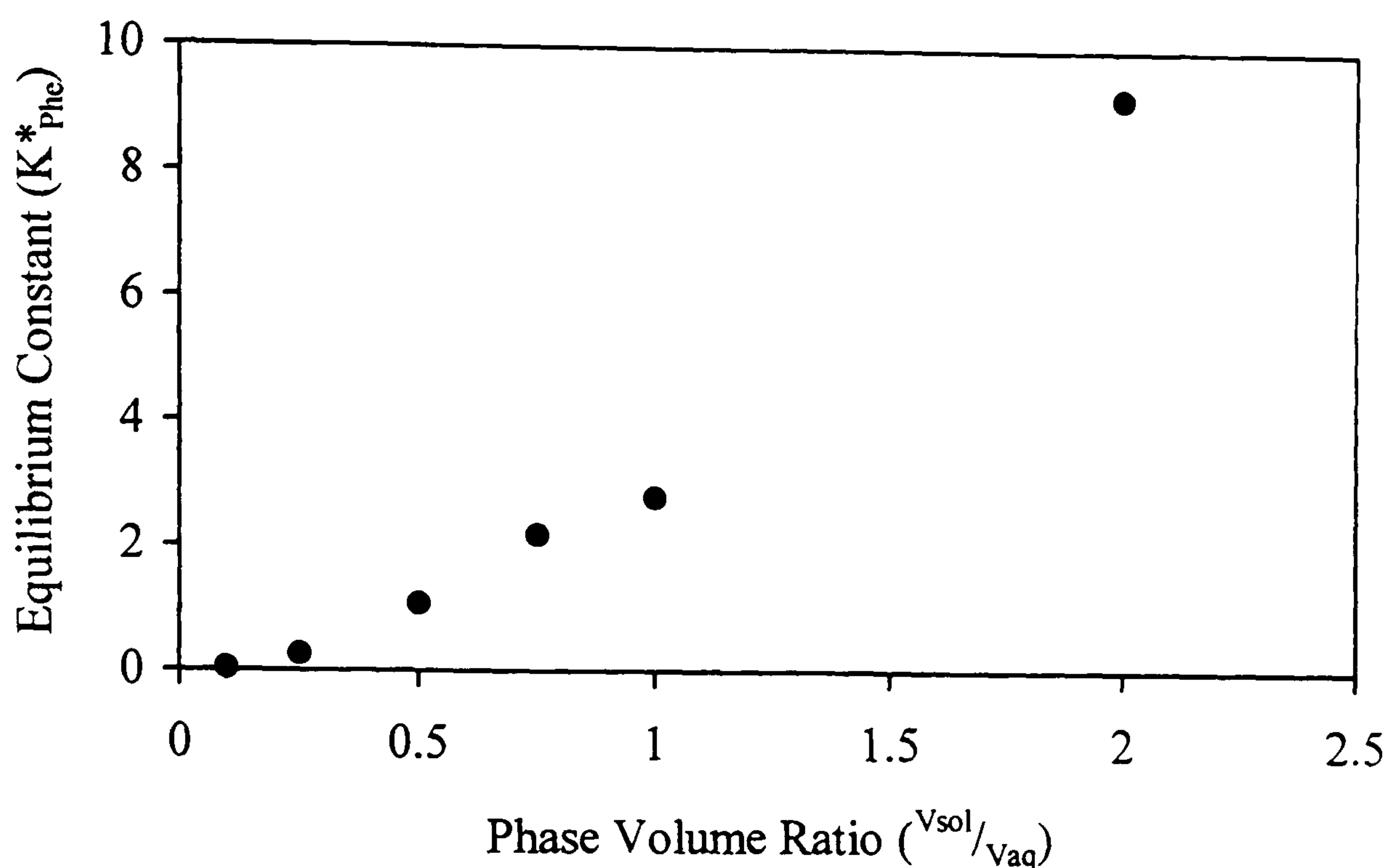


Figure 8-11 The effect of the phase volume ratio on the determination of the equilibrium constant for the phenylalanine reaction. Initially: 10-50mM phenylalanine and 10-50mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, temperature = 25°C.

8.5 Co-Extraction during Mass Transfer

In Section 8.2 it was shown that during the extraction of phenylalanine using Aliquat 336 co-extraction of other anions present in the aqueous phase occurred. In the remaining sections of this chapter the effect of co-extraction and added compounds on mass transfer will be examined. The effect of co-extraction on mass transfer may be highlighted by plotting the amount of phenylalanine extracted against the amount of chloride produced during an extraction. This is shown in Figure 8-12 where there is a linear relationship between the bulk phase concentration of chloride and phenylalanine. This is to be expected as the reaction has a 1:1 stoichometry, and if no co-extraction occurred then the plot would produce a line described by $y = x$ (as shown in Figure 8-12). However, the gradient of the plot is greater than 1 indicating that co-extraction does occur.

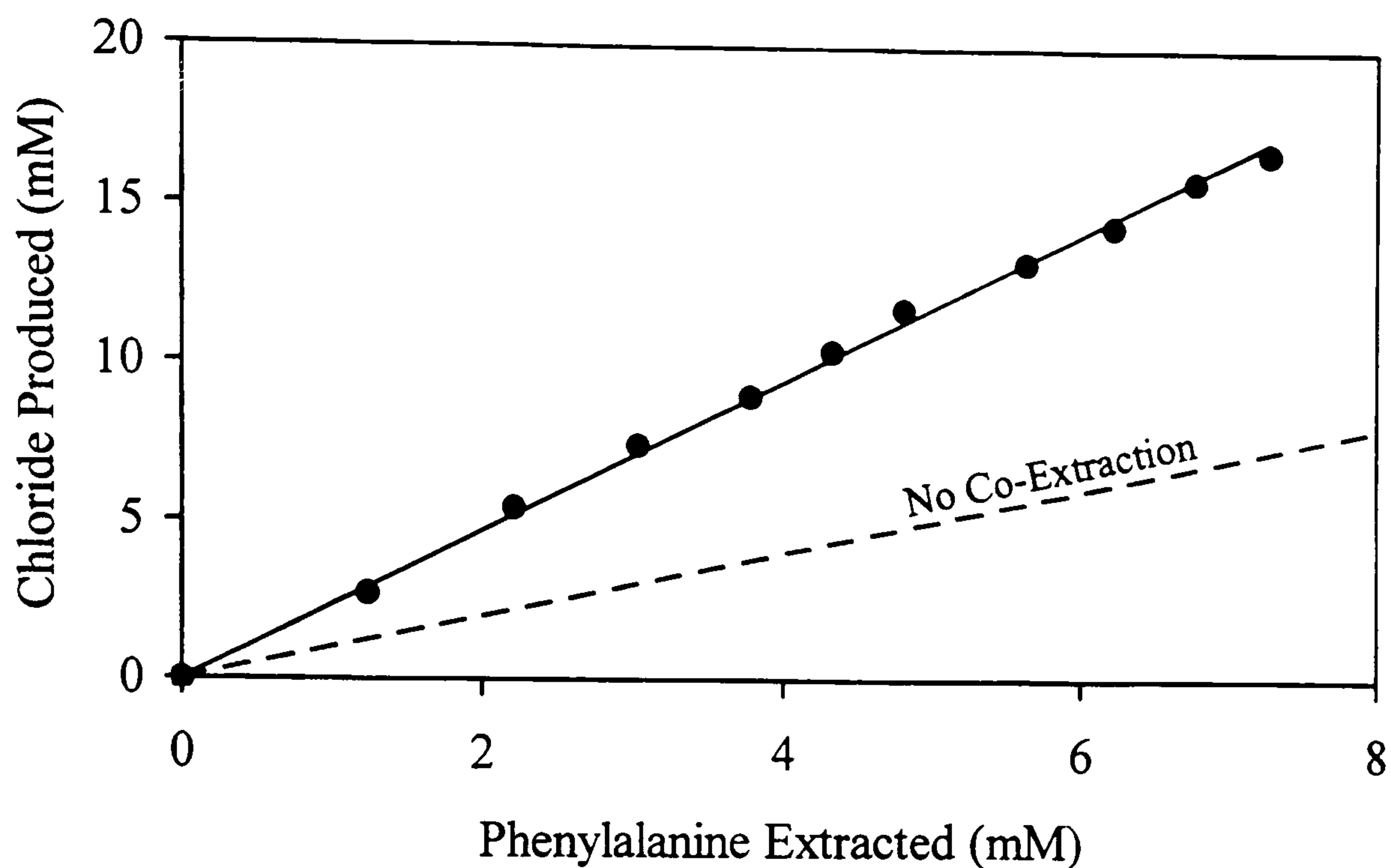


Figure 8-12 Plot of the aqueous phase concentration of chloride against the moles of phenylalanine extracted. Initial conditions: 20mM phenylalanine and 40mM hydroxide anions in the aqueous phase and 200mM Aliquat 336 in the solvent phase, N_{AQ} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1.

From similar results to that shown in Figure 8-12 Scarpello and Stuckey (2000) recognised that the extent of co-extraction could be related to the phenylalanine concentration. They defined the co-extraction constant (α), which they used to construct mass balances for mass transfer modelling. This allowed all species' concentrations to be related to the bulk phenylalanine concentration. The co-extraction constant was defined as,

$$\alpha = \frac{C_{Cl,0} - C_{Cl,t}}{C_{Phe,0} - C_{Phe,t}} \qquad (8\text{-}21)$$

Based on the extraction equilibrium it is possible to define α in terms of the extents of reaction,

$$\alpha = \frac{E_{Phe} + E_{OH}}{E_{Phe}} \tag{8-22}$$

These two definitions for the co-extraction constant (α) are numerically identical except for the sign, where the extent of the two reactions have a positive value (in Equation 8-22) while the change in chloride concentration will be negative (in Equation 8-21). In this study the second version of the co-extraction constant was used as this uses the terms defined when modelling the extraction equilibrium (Section 8.3), thus for a dynamic system the co-extraction constant was determined from the gradient of a plot of $C_{Cl,t}$ vs. $(C_{Phe,0} - C_{Phe,t})$, similar to that shown in Figure 8-12, and α is defined as,

$$\alpha = \frac{C_{Cl,t}}{C_{Phe,0} - C_{Phe,t}} \tag{8-23}$$

In the study of Scarpello and Stuckey (2000) values of the co-extraction constant determined from equilibrium experiments were found to differ from the value determined from Lewis cell experiments for similar conditions. It was reasoned that this difference occurred due to the physical configuration of the mass transfer system. However, in that investigation equilibrium experiments were conducted at a PVR of 0.1, and mass transfer experiments where conducted with a PVR of 1. In Section 8.4.4 it was shown that the PVR had an effect on the values of the equilibrium constants, and therefore on the extents of reaction and the co-extraction constant. The values reported by Scarpello and Stuckey (2000) were similar to values found in this study when a similar PVR was used.

The effect of the initial hydroxide anion concentration on the value of the co-extraction constant determined from either equilibrium experiments, the equilibrium extraction model or Lewis cell experiments are shown in Figure 8-13. It can be seen that for a given set of initial conditions the values determined by the three methods are effectively identical. Therefore, events occurring during mass transfer can be described using the

equilibrium description of mass transfer given in Section 8.3. The implications of this result on mass transfer are discussed further in the next Section where a new mass transfer mechanism is proposed.

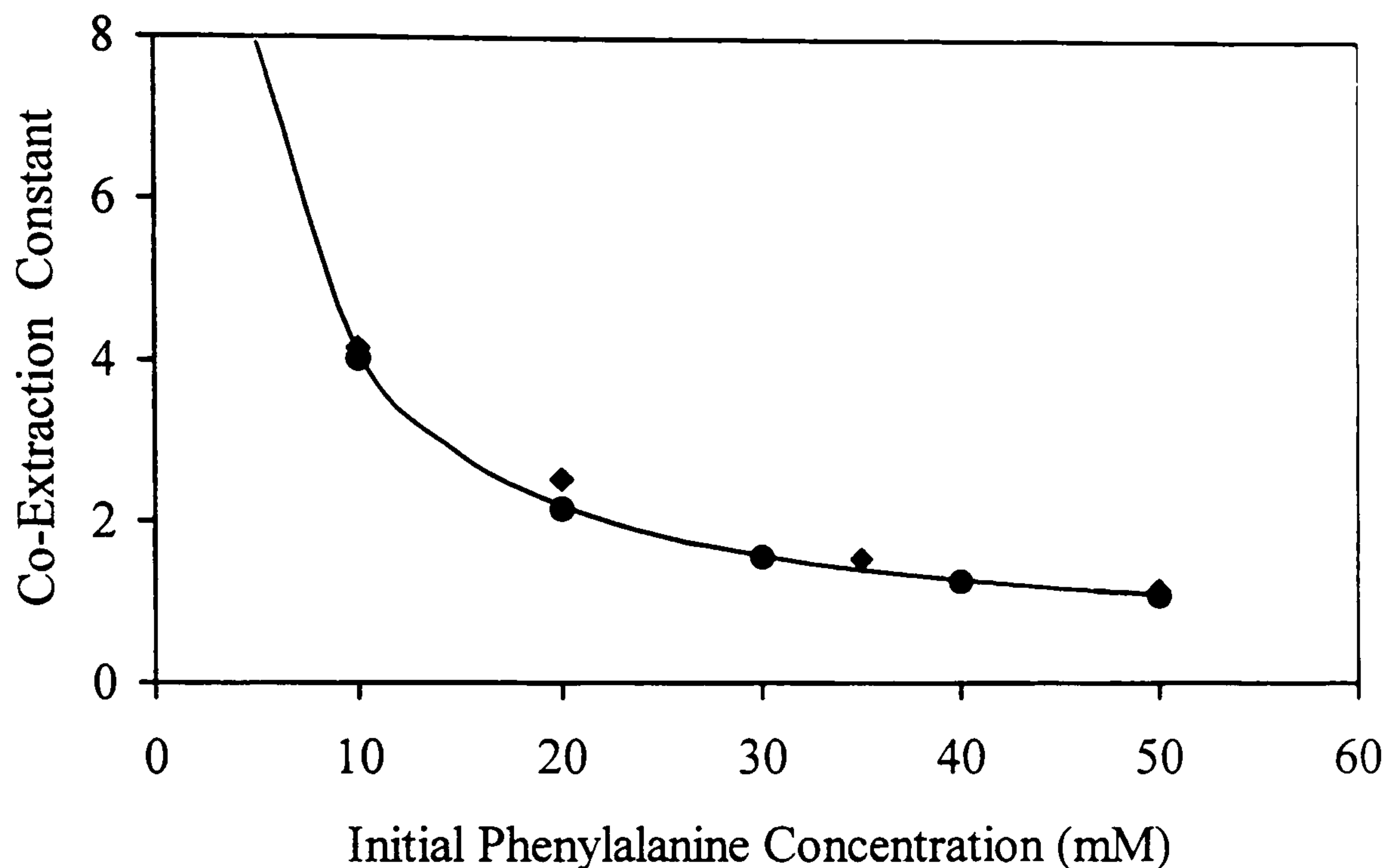


Figure 8-13 The effect of the initial hydroxide anion concentration on the value of the co-extraction constant determined from equilibrium experiments, •, Lewis cell experiments, ♦, and the extraction equilibrium model, —. Initial conditions were 35mM phenylalanine and 10 to 50mM hydroxide anions in the aqueous phase, and 200mM Aliquat 336 in the solvent phase, N_{AQ} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1.

8.6 Mass Transfer Modelling

This section details the modelling of the reactive extraction of phenylalanine by the ion exchange reagent Aliquat 336. The extraction system used in this study was modelled previously, and a brief review of these models, which were based on two-film theory, was given previously in Section 2.4. In those previous models, developed for use with experimental apparatus similar to that used in this study, the effect of co-extraction was not considered. The study of the effects of co-extraction on equilibrium extraction presented earlier revealed that it can have a considerable effect on solute extraction, therefore the effect of co-extraction on mass transfer must be considered. The objective of the modelling process was to allow for the determination of the film mass transfer

coefficient for the extraction system. The film mass transfer coefficient was then used to determine whether added compounds increased the resistance to mass transfer during the extraction of phenylalanine.

8.6.1 Interfacial Flux Balance

In the system under study, co-extraction occurs because the ion exchange reagent is not specific to phenylalanine. Hydroxide anions added to the aqueous phase to raise the pH, and allow phenylalanine to dissociate, will also be extracted. The co-extraction of buffer anions was identified by Scarpello and Stuckey (2000) to contribute significantly to mass balances used in the determination of equilibrium constants, and in mass transfer modelling. However, in that study and all other previous studies of phenylalanine extraction the fluxes of the transferring species were assumed to be equal, such that,

$$J_{Phe} = J_{QCl} = J_{QPhe} = J_{Cl} \qquad (8\text{-}24)$$

This assumption is not correct, as the effect of co-extraction should be considered when equating fluxes. Previously the co-extraction flux, and its relationship with the flux of the ion exchange reagent was ignored when defining the flux balance across the interface (Equation 8-24). In those studies only the primary extraction reaction species were considered (Haensel *et al.*, 1986; Uddin *et al.*, 1990; Chan and Wang, 1993; Coelhoso *et al.*, 1997; Scarpello and Stuckey, 2000).

When the co-extraction of hydroxide ions is taken into consideration (see Figure 2-4) the idea that the flux of Aliquat-chloride is equal only to the flux of Aliquat-phenylalanine becomes flawed. This is because a proportion of the flux of Aliquat-chloride will be due to the flux of Aliquat-hydroxide, so for the solvent phase the flux balance should be defined as,

$$J_{QCl} = J_{QPhe} + J_{QOH} \qquad (8\text{-}25)$$

Likewise, in the aqueous phase the flux of chloride will be balanced by the sum of the

fluxes of phenylalanine and hydroxide,

$$J_{Cl} = J_{Phe} + J_{OH} \tag{8-26}$$

The primary and co-extraction reaction mechanisms both have a 1:1 stoichiometry so the aqueous and solvent phase fluxes of individual species that cross the interface will be equal.

$$J_i = J_{Qi} \tag{8-27}$$

In Section 8.5 the changes in the concentration of phenylalanine and chloride in the aqueous phase were related by a constant coefficient, the co-extraction constant (α), and hence the fluxes will also be related by the same coefficient,

$$\alpha = \frac{J_{Cl}}{J_{Phe}} = \frac{J_{Phe} + J_{OH}}{J_{Phe}} \tag{8-28}$$

Balancing the fluxes in this way is logically correct, a fact supported by the experimental evidence given in Section 8.5 where the bulk aqueous phase concentrations are linearly related over time. This flux balance will be used in the next section as the basis for describing mass transfer and developing a model of the system.

8.6.2 Mass Transfer Mechanism

In this Section a mass transfer mechanism is proposed for the reactive liquid-liquid extraction process that occurs in the modified Lewis cell. The proposed mass transfer mechanism was developed for a situation that is analogous to the two-film model of interphase mass transfer (Whitman, 1923; see Section 3.3.4.1). In the two-film model steady state mass transfer occurs, and is controlled by diffusion through stagnant interfacial films. The interface is assumed to offer no resistance to mass transfer, there is no accumulation of mass at the interface, and concentrations either side of the

interface are related by equilibrium. The basis for elucidating the mass transfer mechanism is the flux balance described in the previous section, and the nature of the constant coefficient (α) used to relate the fluxes.

In Figure 8-13 it was shown that for the same initial conditions the co-extraction constants determined for equilibrium experiments and Lewis cell experiments were the same. This implies that the level of co-extraction remains constant from the moment the phases are contacted until equilibrium is reached. The interfacial condition of the extraction reaction will control the level of extraction and co-extraction, and as this level remains constant throughout the mass transfer process so then the interfacial concentrations will remain constant. This is predicted by the Two-film theory model where it is assumed that during mass transfer the interface is in a state of equilibrium and steady state transfer occurs, therefore no changes in the interfacial concentrations will occur. For the interface to produce a level of extraction and co-extraction equal to the final conditions of the system then the interfacial concentrations must be equal to the equilibrium concentrations of the system.

The situation described above can be physically envisaged by considering an interfacial region where the ion exchange reactions are occurring. The reactants and products present in this region are in equilibrium, as described by the reaction equilibrium constants, and have been since the two phases were contacted when the initial concentrations of the system set the equilibrium concentrations. Species enter and leave the interfacial region by diffusion, where the flux of the products and reactants are related according to the co-extraction constant and the total moles within the reaction region remains constant. The ion exchange reaction can be considered to be instantaneous, therefore, as soon as the reactants enter the reaction region they are converted to products and immediately diffuse away from the interface, this maintains the species concentrations at a constant value.

According to the film model (Section 3.3.3.1) the solute flux is equal to the mass transfer coefficient times the concentration difference. Therefore, the flux is affected by changes is the mass transfer properties of the thin film, as described by the mass transfer coefficient, and/or by changes in the concentration driving force across the film. Co-extraction has been shown to affect the equilibrium concentration of the solute, and

therefore will affect the flux by changing the concentration driving force. Increased levels of co-extraction will reduce the amount of solute extracted, and thereby increase the equilibrium concentration and reduce the driving force (and flux). Co-extraction is not expected to effect the mass transfer coefficient as the reaction is interfacial and should not interfere with the diffusion of species to the interface.

8.6.3 Determining Film Mass Transfer Coefficients

The description of the mass transfer process given in the previous section, and in particular the assumption that the interfacial concentrations are constant and determinable from initial conditions, will be used to allow the film mass transfer coefficients for the transferring species to be determined. In a similar manner to the method used previously for the physical extraction system, a differential mass balance was written using the species flux to describe the loss or gain of material from the system. However, in contrast to the previous method it is possible to define the interfacial concentration, and therefore a film mass transfer coefficient can be determined instead of an overall mass transfer coefficient.

Similar to that shown previously in Section 2.4 for chloramphenicol extraction, a mass balance for phenylalanine in the aqueous phase may be written as,

$$V\frac{dC_{Phe}}{dt} = -A\,k_{Phe}\left(C_{Phe} - C_{Phe,i}\right) \tag{8-29}$$

Taking the interfacial concentration to be constant, Equation 8-29 can be solved for the limits $C_{Phe} = C_{Phe,0}$ at $t = 0$, and $C_{Phe} = C_{Phe,t}$ at $t = t$, to give,

$$\frac{V}{A}\ln\left(\frac{C_{Phe,0} - C_{Phe,i}}{C_{Phe,t} - C_{Phe,i}}\right) = k_{Phe}\,t \tag{8-30}$$

Previously in Section 8.3 a model was developed that allowed prediction of the equilibrium concentrations and the extent of the reaction given the initial conditions for the system and the equilibrium constants. Using this model the interfacial concentration

may be predicted, or the value can be determined from an equilibrium extraction experiment. Having determined the interfacial concentration experimental concentration-time data can be plotted according to Equation 8-30 where the change in the left hand side with time should be constant and equal to the film mass transfer coefficient, k_{Phe}. This is shown in Figure 8-14 where a plot obtained for the extraction of phenylalanine is presented.

Also shown in Figure 8-14 is a plot obtained when a differential mass balance for the aqueous phase chloride is solved using an expression for the chloride flux. The procedure is similar to Equations 8-29 and 8-30, and the flux of chloride is given by $k_{Cl}(C_{Cl,i} - C_{Cl,b})$. It can be seen from Figure 8-14 that the phenylalanine and chloride plots are almost identical, indicating that k_{Phe} and k_{Cl} are equal.

Given the interrelationship of species involved in this extraction system, and the assumptions of two-film theory, it would be expected that this result would be observed, and can be shown by considering the flux balance (Equation 8-28) . The fluxes of these two species are related through the co-extraction constant, and therefore so will the driving forces for mass transfer.

$$\alpha = \frac{J_{Cl}}{J_{Phe}} = \frac{k_{Cl}\ \Delta C_{Cl}}{k_{Phe}\ \Delta C_{Phe}} = \frac{k_{Cl}}{k_{Phe}}\alpha \tag{8-31}$$

So $k_{Cl} = k_{Phe}$. Likewise, the mass transfer coefficient for Aliquat-phenylalanine (k_{QPhe}) will be equal to k_{Phe} because the driving force will be the same, as determined by the interfacial equilibrium condition, and in assuming steady state interphase mass transfer the fluxes (J_{Phe} and J_{QPhe}, Equation 8-27) will also be equal. Therefore, in this mass transfer system the fluxes of all the species are interrelated by the reactions occurring at the interface, and the mass transfer coefficient of each species is equal.

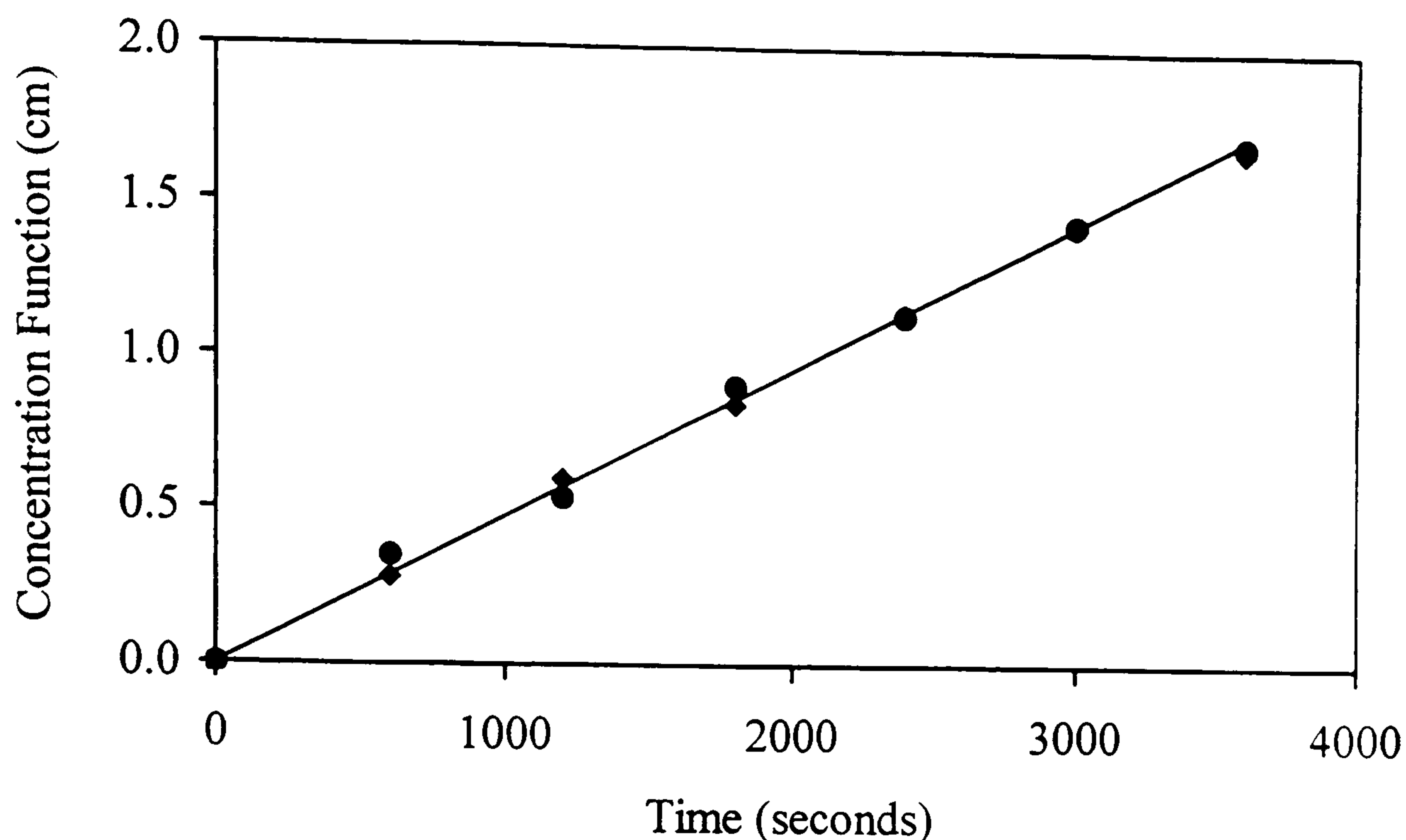


Figure 8-14 Example of plots used to determine the film mass transfer coefficient for phenylalanine (•) and chloride (♦). Initial phenylalanine concentration of 50mM, hydroxide anion concentration of 50mM, Aliquat 336 concentration of 200mM, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and PVR = 1.

8.7 The Effect of Co-Extraction on Mass Transfer

The model of mass transfer presented here postulates that the relationship between interfacial concentrations is governed by the equilibrium constants for the reactions, and the concentrations are equal to the final equilibrium values of the system. In Section 8.2 the effect of the initial composition on the extent of the extraction reactions was examined. In that section it was demonstrated that varying the concentration of any of the species involved in the reaction will result in a change in the extent of both reactions. It was also shown that concentration changes involving compounds with high equilibrium constants would have the greatest effect on the extent of solute extraction and co-extraction.

To examine the effect of the co-extraction reaction on mass transfer the previously studied system involving the addition of bromide anions was used. In Section 8.4.3, results were presented for equilibrium experiments where the effect of bromide co-extraction was examined. The equilibrium constant for bromide was found to be 2.658, and given the similarity to the value for phenylalanine (K^*_{Phe} = 2.804) it was found to have an appreciable effect on the equilibrium position of the extraction reactions.

Increasing the concentration of bromide over the range 0 to 70mM caused the extent of the phenylalanine reaction to decrease from 31.5 to 27.2 mM, but the combined extents of all reactions caused the chloride concentration to increase from 51.2 to 88.5 mM. In addition to the decrease in phenylalanine extraction, the large increase in the chloride concentration, caused by increased bromide co-extraction, lead to the value of the co-extraction constant increasing from 1.63 to 3.25. Based on these figures it should be possible to observe the effect of co-extraction on mass transfer, and by analysing the mechanism of mass transfer presented earlier the effects may be postulated.

Co-extraction will also cause an increase in total flux relative to phenylalanine, as indicated by the increase in the co-extraction constant. However, by inspection of the model it is not immediately obvious whether this will have an effect on the absolute flux of a species. Figure 8-15 shows the effect of co-extraction on the total flux of the chloride and Aliquat-phenylalanine, the products of the primary extraction reaction.

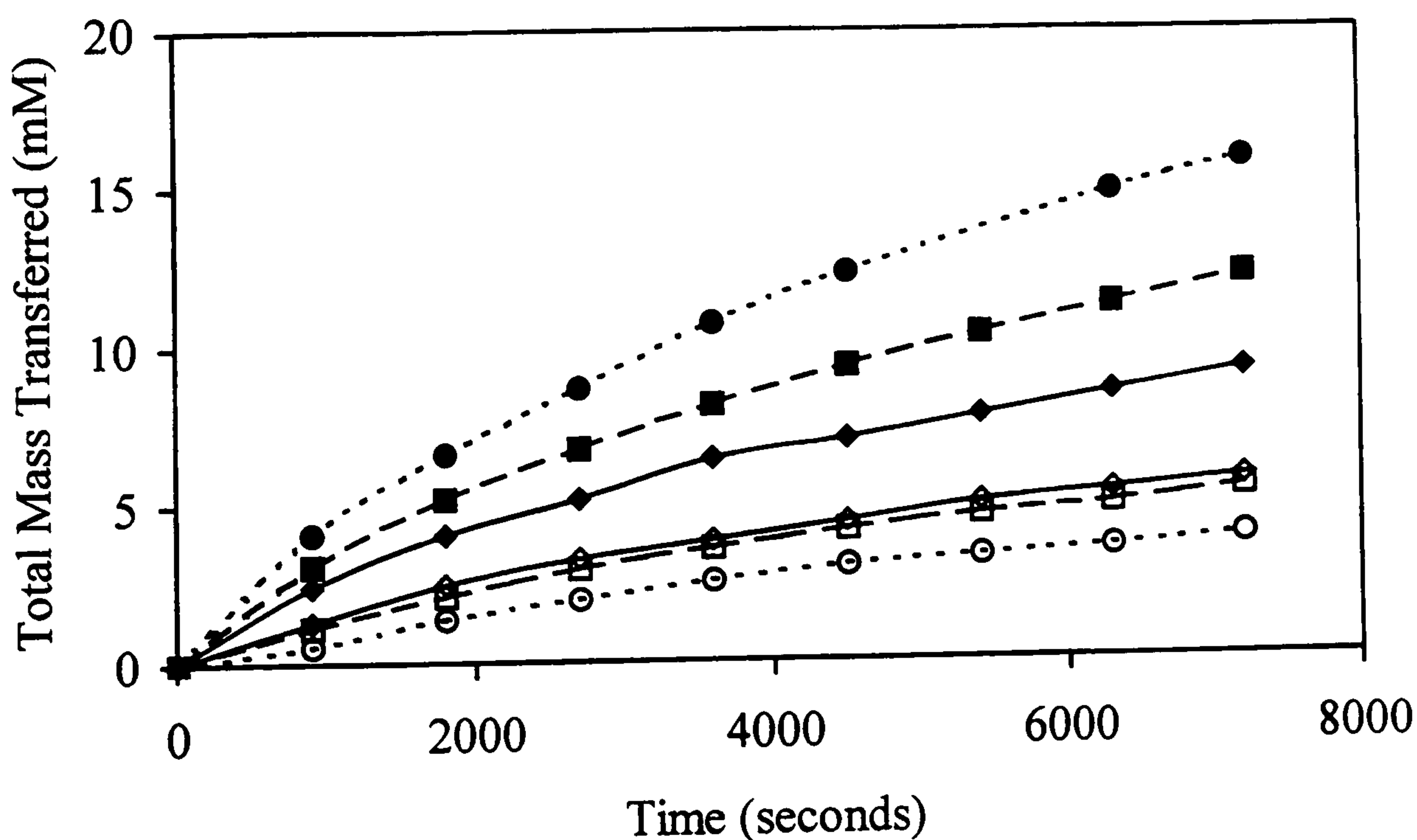


Figure 8-15 The effect of initial bromide concentration (10mM (♦), 35mM (■) and 70mM (•)), on the extent of the phenylalanine extraction reaction (hollow markers) and combined extents of all extraction reactions (filled markers). Initially: 35mM phenylalanine and 25mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1.

In Figure 8-15 it can be seen that with increasing initial bromide concentration. and hence an increase in the co-extraction constant, the flux of chloride increases and the flux of phenylalanine decreases. This effect is due to the change in the extents of reaction at equilibrium caused by a change in the initial composition of the extraction medium. With increased co-extraction the equilibrium concentration, and hence the interfacial concentration of phenylalanine, increases. This will reduce the driving force, and if the mass transfer coefficient is unaffected by co-extraction then the flux of phenylalanine will be reduced. In a similar manner the flux of chloride will increase due to a increase in the interfacial concentration of chloride, and hence an increase in the driving force.

The effect of co-extraction on the value of the mass transfer coefficient is shown in Figure 8-16, where it can be seen that the mass transfer coefficient is unaffected by the level of co-extraction. It is envisaged that this parameter would be unaffected by the reaction, as the reaction does not occur within the thin film, but instantaneously at the interface where it will not affect the diffusion process.

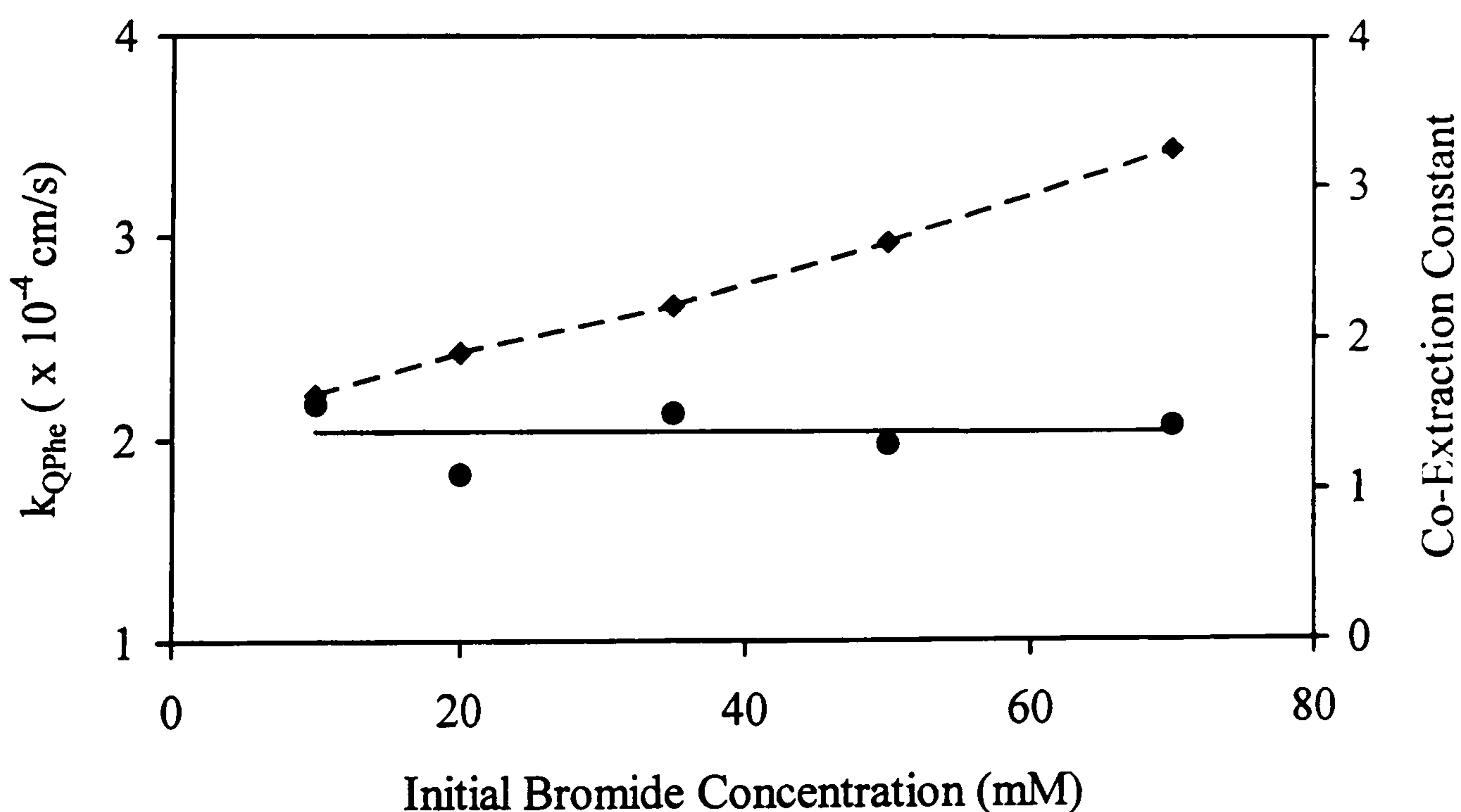


Figure 8-16 The effect of initial bromide concentration on the film mass transfer coefficient (•, 1st y-axis) and the co-extraction constant (♦, 2nd y-axis). Initially: 35mM phenylalanine, 25mM hydroxide anions and 10-70mM bromide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1.

Co-extraction will affect mass transfer by altering the interfacial concentration, and hence the driving force, however, it will not affect the transport properties of the system. Thus, increased co-extraction will cause the solute flux to decrease, but the mass transfer coefficient will remain constant. Therefore, the resistance to mass transfer is only due to transport through the liquid films, and is not influenced by the reaction at the interface. The effect of hydrodynamics on mass transfer was investigated by Scarpello (1998), who found that the flux of phenylalanine was unaffected by changing aqueous phase hydrodynamics but was affected by the solvent phase hydrodynamics. From this evidence the conclusion is drawn that, for the system under study here, diffusion of a solvent phase species controls the rate of interphase mass transfer.

In Section 8.2 it was shown that the phase volume ratio affected the value of the equilibrium constant. Although it is unclear why this occurs, it may have implications for the level of solute flux across the interface. Earlier, the situation was described where upon initial contact the interface attained equilibrium as described by the equilibrium constant. Given that the equilibrium constant varies with PVR then this interfacial region may behave in two ways as a result of changing PVR. The interface may attain the equilibrium described by the equilibrium system of similar PVR; or the interface may attain the equilibrium described by the equilibrium system where PVR is 1. In the later case this would indicate that the interfacial reaction region contains equal volumes of aqueous and solvent phases, however, if the former case prevails then this would suggest that interfacial equilibrium is not attained immediately upon contact and the interfacial condition is dictated by the whole system. Unfortunately no mass transfer experiments were conducted where the phase volume ratio was varied so no experimental evidence is provided to support these predictions.

8.8 Extraction from Fermentation Broth

The overall objective of this study was to investigate the effect of broth components on liquid-liquid extraction. Many anionic species will be present in fermentation broth and they are liable to be extracted by Aliquat 336. As seen in Section 8.5 co-extraction can have a substantial effect on phenylalanine extraction, so the effect of co-extraction occurring during solute extraction from fermentation broth should be investigated.

Throughout this investigation of phenylalanine extraction it was generally accepted that in order to assess the effects of broth components on mass transfer the change in concentration of phenylalanine would have to be measured. Unfortunately no suitable analytical technique was available to analyse phenylalanine, and an extensive investigation of the effect of fermentation broth was not conducted. The mechanism of mass transfer proposed in Section 8.6.1 only became apparent towards the end of the study. This model predicts that mass transfer coefficients for all species are interrelated, and thus, effects on the transfer of one species may be conferred on the other species present in the system. In light of this, experiments involving extraction from fermentation broth could have been conducted using only chloride measurements to assess effects on mass transfer. A limited number of experiments were conducted using fermentation broth where the chloride concentration was measured. Based on these experiments a brief assessment of the effect of filtered fermentation broth is presented in Section 8.8.1. The effect of biomass on mass transfer has also been examined; these results are presented in Section 8.8.2.

8.8.1 The Effect of Filtered Fermentation Broth

In Figure 8-17 the results of equilibrium and mass transfer experiments performed using different dilutions of fermentation broth are presented. It is seen that the concentration of chloride produced from the ion exchange reaction increases as the concentration of the broth is increased. This will be due to the increased concentrations of reactants, and the large amount of chloride suggests that the complete fermentation broth contains a large quantity of anionic compounds. The media used for the fermentation contained quantities of phosphate and sulphate and a proportion of these original amounts are expected to be present in the harvested fermentation broth. The ion chromatograph used to analyse for chloride concentration also detected phosphate and sulphate. From those measurements and by assuming complete extraction of the phenylalanine and hydroxide, 78% of the chloride generated in the reactions can be accounted for. Therefore, other compounds either initially present or produced during the fermentation are being co-extracted from the fermentation broth. For the complete filtered broth the minimum possible value of the co-extraction constant is 2.24, and if complete extraction of phenylalanine does not occur, which is probable, this value will be higher. In Section

8.3 a model was developed that allowed the level of co-extraction to be predicted for a two component system. This equilibrium extraction model may be extended for use on fermentation broth systems if the all the extraction reactions are characterised and the reaction constants determined. Given the complex nature of fermentation broth it may be impossible to achieve this, however, a model that neglects components of low concentration and/or those with low equilibrium constants would probably give a good approximation to the real situation.

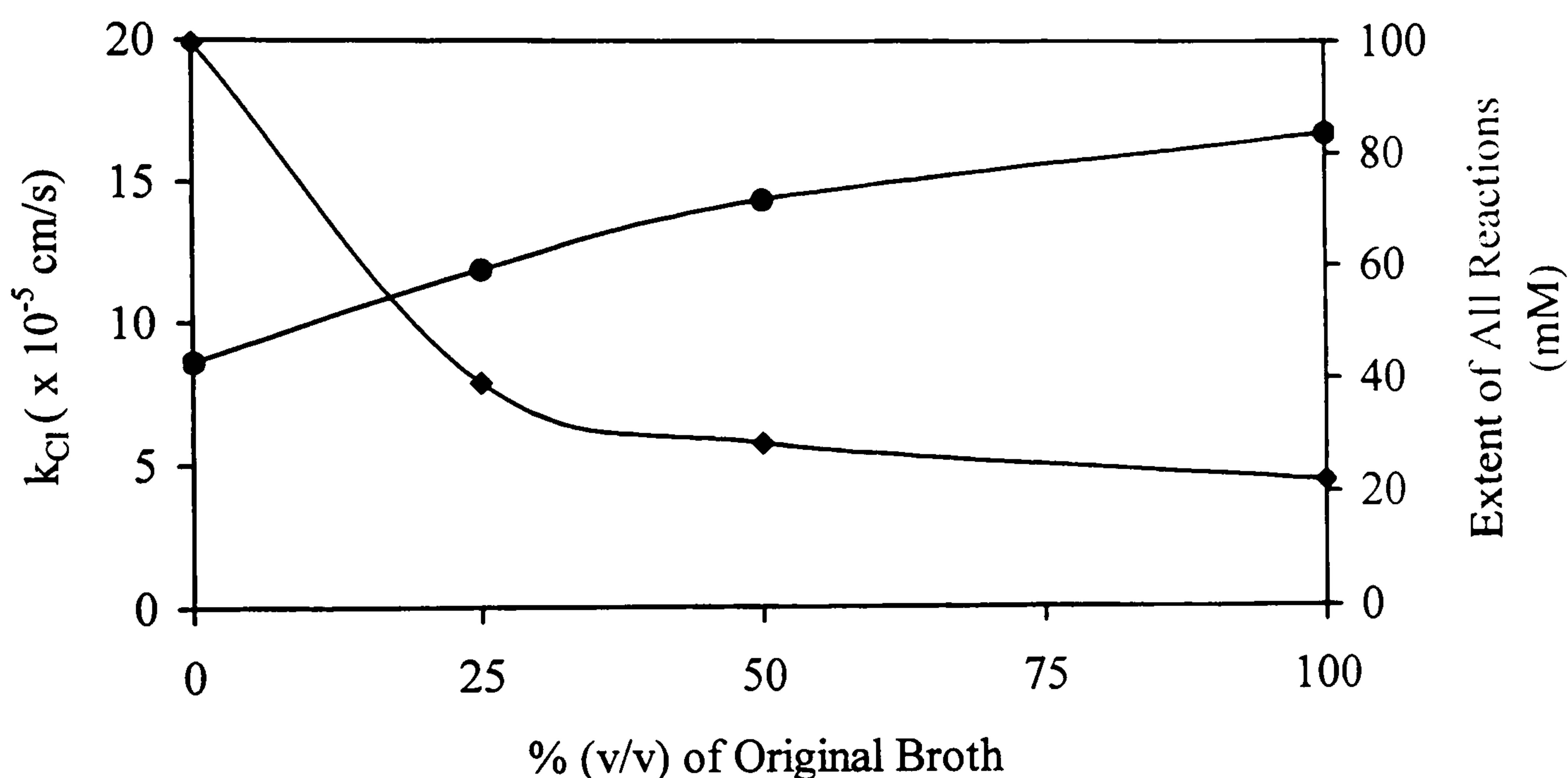


Figure 8-17 The effect of dilutions of filtered fermentation broth on the film mass transfer coefficient for phenylalanine extraction (♦) and the extent of all extraction reactions (•). Initially: 35mM phenylalanine and 25mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1.

Also shown in Figure 8-17 is the effect on the mass transfer coefficient of different dilutions of the filtered broth. It is seen that with increasing broth concentration the mass transfer coefficient is reduced, and the complete broth reduces the mass transfer coefficient by 75% compared to extraction from water. The trend is similar to that observed in the study of physical extraction (Figure 7-8) where the complete fermentation broth also reduced the mass transfer coefficient by 70%. In that work the surface tension was also found to decrease with increasing broth concentration (Figure 7-9), and from this the negative effect on mass transfer was attributed to increasing concentrations of soluble surface active compounds within the broth. For the reactive extraction system the complete filtered broth recorded a surface tension of 40.6 mN/m,

indicating the presence of adsorbed compounds at the interface, which like the case of the physical extraction system are considered to be responsible for the reduction in the mass transfer coefficient.

8.8.2 The Effect of Biomass

An investigation into the effect of biomass was conducted. The biomass was found to have no effect on the extent of any extraction reactions. However, it did affect the solute flux, and the results of these experiments are presented in Figure 8-18. It can be seen that for all biomass concentrations the mass transfer coefficient is below the value recorded for extraction from the biomass free system. Initially, increasing the biomass concentration caused the mass transfer coefficient to decrease slightly, but above 0.08 g/l of biomass no further effect was observed. This correlates with the effect observed on the surface tension of the biomass solutions (data not shown), where the maximum reduction in surface tension occurred at biomass concentrations above 0.2 g/l. From this it is concluded that biomass adsorbed to the interface where it reduced mass transfer. The nature of this effect could be due to blockage of the interfacial area, as discussed previously in Section 7.7.3.

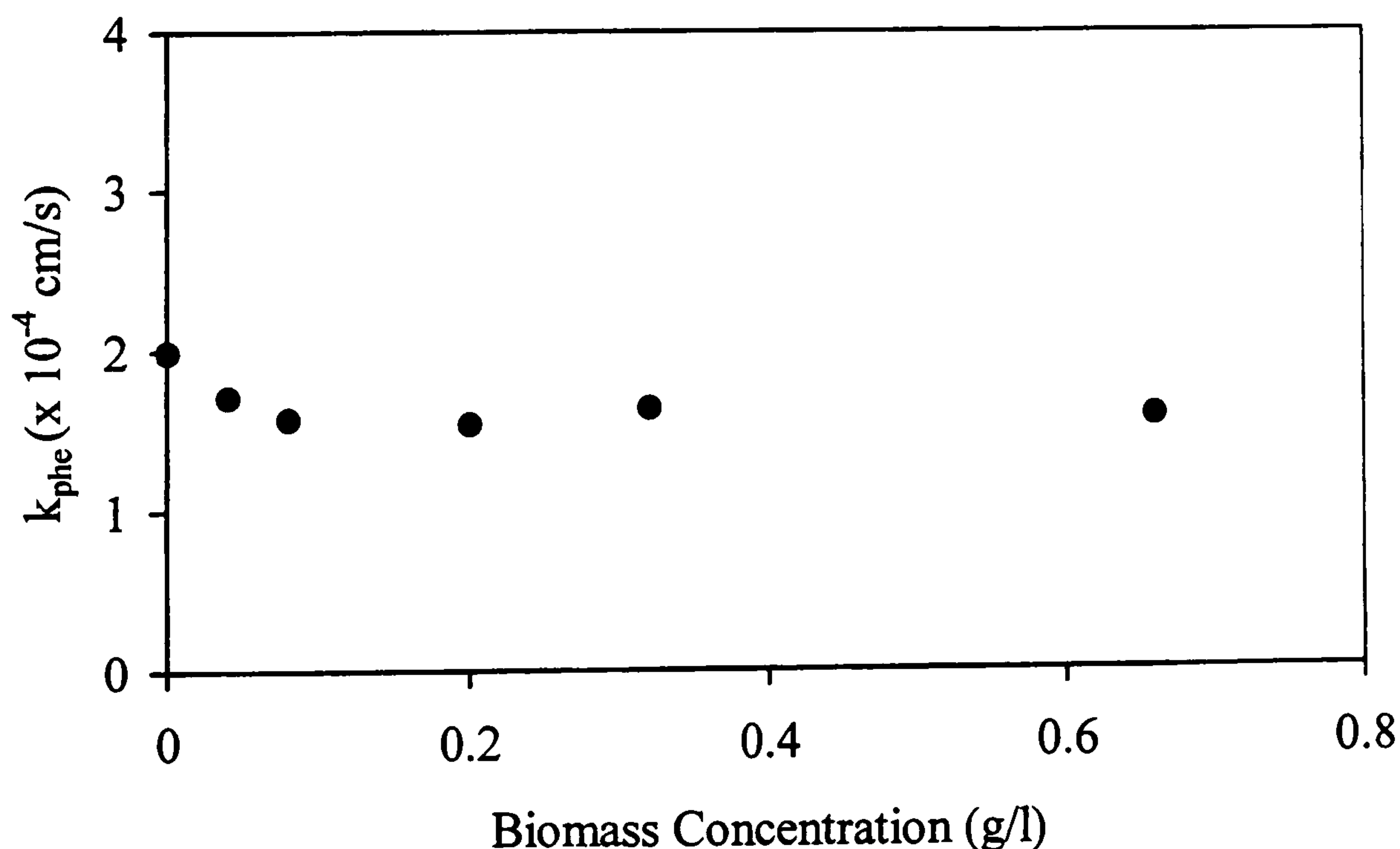


Figure 8-18 The effect of biomass concentration on the film mass transfer coefficient for phenylalanine extraction. Initially: 35mM phenylalanine and 25mM hydroxide anions in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1.

8.9 The Effect of Surfactants on Extraction

As discussed in Section 7.10 surfactant compounds may be used to improve fermenter performance or to aid in downstream product recovery. Therefore, these compounds will be present in the extraction medium, and due to their surface activity will be present at the interface where they may affect mass transfer. In this section the effect of two surfactants, DTAB (cationic) and Softanol 30 (non-ionic), were investigated. These surfactants may be used to formulate colloidal liquid aphrons, which have recently been studied using the phenylalanine extraction system (Scarpello and Stuckey, 1998).

The effect of the solvent phase concentration of Softanol 30 on the mass transfer coefficient and the co-extraction constant is shown in Figure 8-19. It can be seen that the addition of Softanol 30 has no effect on either of these parameters. As Softanol 30 is non-ionic it was not expected to affect the extraction reaction mechanism. However, interfacial tension measurements indicate that Softanol 30 did adsorb to the interface, but that its presence there would appear to have no effect on the mass transfer process.

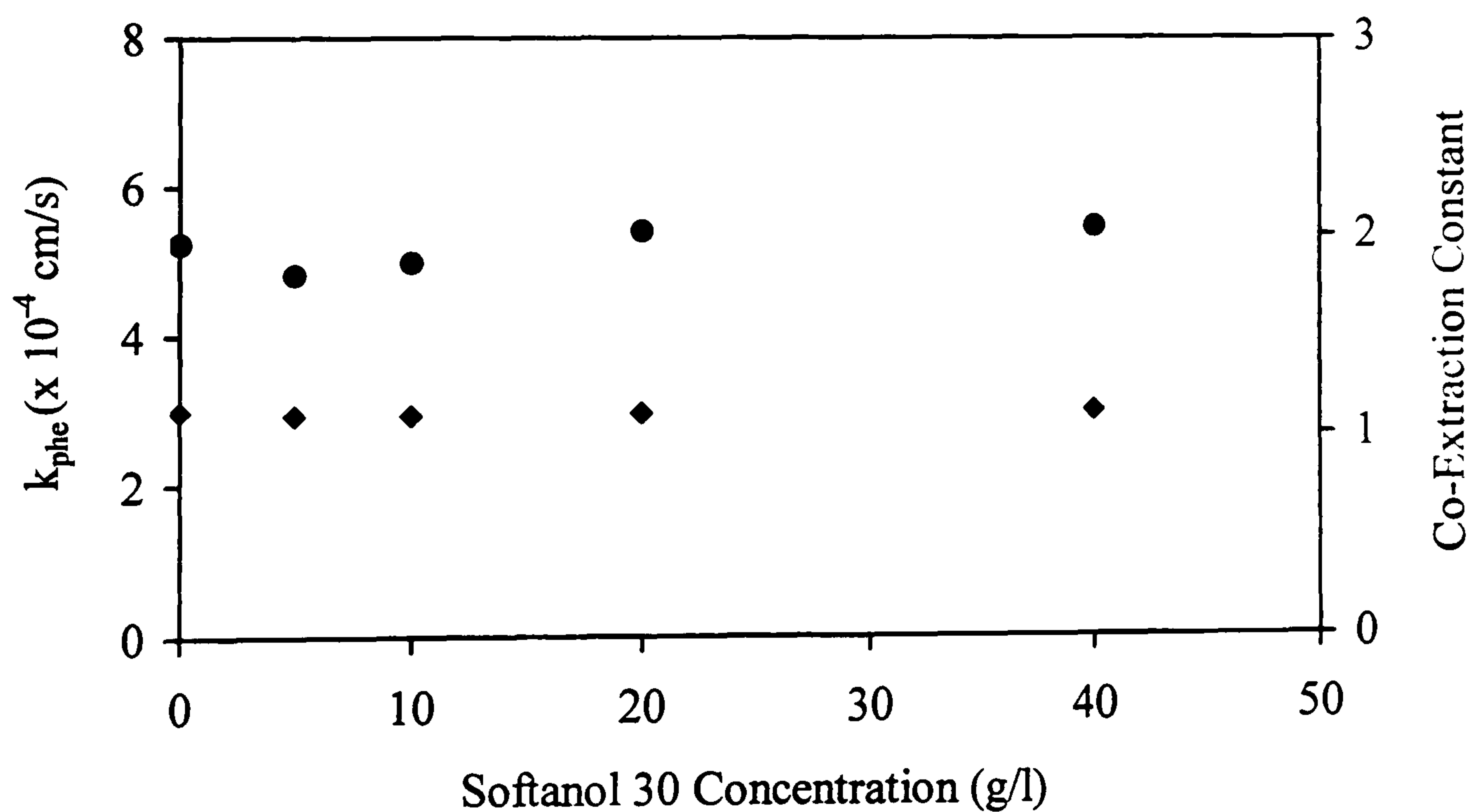


Figure 8-19 The effect of the addition of Softanol 30 to the solvent phase on the film mass transfer coefficient (•, 1st y-axis) and the co-extraction constant (♦, 2nd y-axis). Initially: 35mM phenylalanine, 25mM hydroxide anions and in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1.

Figure 8-20 presents results for the addition of DTAB to the aqueous phase of the extraction system. It is seen that the mass transfer coefficient is constant at up to 10 g l DTAB, but for the readings above this concentration the mass transfer coefficient was slightly increased but constant. It is believed that the increased values of the mass transfer coefficient do not represent a real effect on interfacial mass transfer. During these experiments the aqueous phase became cloudy, which indicates the presence of emulsified solvent droplets. The presence of solvent droplets in the aqueous phase would cause increased levels of extraction and give a distorted picture of mass transfer across the known interface. The reason for this spontaneous emulsification may be due to the transfer of DTAB across the interface. It is reported that the transfer of surface active compounds across the interface can cause spontaneous emulsification as a result of localised reductions in the interfacial tension (Davis and Rideal, 1963).

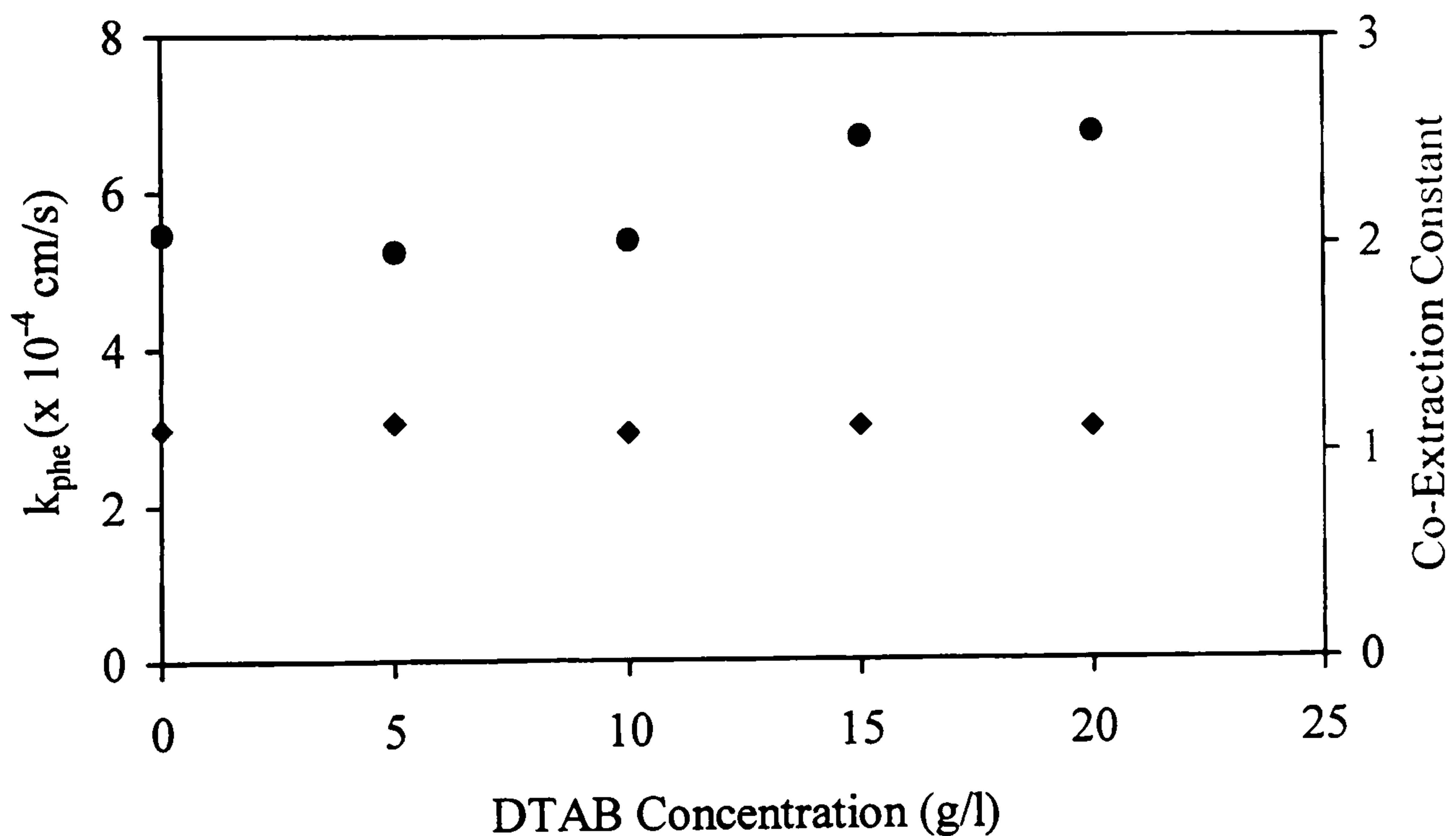


Figure 8-20 The effect of the addition of Softanol 30 to the solvent phase on the film mass transfer coefficient (•, 1st y-axis) and the co-extraction constant (♦, 2nd y-axis). Initially: 35mM phenylalanine, 25mM hydroxide anions and in the aqueous phase, 200 mM of Aliquat 336 in the solvent phase, N_{Aq} = 100 rpm, N_{Sol} = 250 rpm, temperature = 25°C, and a PVR of 1.

Measurements of the aqueous phase bromide concentration indicated that bromide was extracted into the solvent phase, however, the amount of bromide extracted was greater than the amount predicted from the difference between measurements of chloride and phenylalanine. The likely cause of this discrepancy was that bromide was partitioning into the solvent in the form of DTAB, and not taking part in the extraction reaction

process. This idea is supported by the evidence that DTAB had no effect on the co-extraction constant, as shown in Figure 8-19, and thus did not take part in any extraction reaction.

8.10 Summary of Results and Discussion

An experimental study has been conducted using the amino acid phenylalanine as the model solute to allow an investigation of solute extraction from fermentation broth using a reactive liquid-liquid extraction process. In fermentation broth there will be many anionic species available for co-extraction by Aliquat 336, and this will have an effect on solute extraction. Therefore, the work presented in this Chapter has focused on the role of co-extraction during solute extraction. Presented in Section 9.10.1 is a summary of the effect of co-extraction on the extent of equilibrium extraction. In Section 9.10.2 details of the proposed mass transfer mechanism for reactive extraction are summarised. Extractions were performed from fermentation broth and surfactant solutions; a summary of these experiments is given in Section 9.10.3.

8.10.1 Equilibrium Extraction

An investigation of phenylalanine extraction using the ion exchange reagent Aliquat 336 has been conducted. Aliquat 336 does not specifically extract phenylalanine, and it has been demonstrated that the co-extraction of other anionic compounds does occur. The extraction of a species can be described by the equilibrium constant for its extraction reaction and the values for phenylalanine, hydroxide and bromide were found to be 2.804, 0.313 and 2.658 respectively.

Based on the description given by the equilibrium constant a model was developed to describe the extent of extraction and co-extraction at equilibrium. Using this model the effect of initial conditions on extraction has been explored, and when possible the model predictions were confirmed by results obtained from extraction experiments.

The extent of extraction and co-extraction is influenced by the initial concentrations of all the reacting species. Increasing the initial concentration of phenylalanine lead to an increase in the amount of phenylalanine extracted. When the initial concentration of the co-extracting species was increased the extent of the phenylalanine extraction was reduced. This occurred because the co-extraction reaction produces chloride, which is also produced by the phenylalanine extraction reaction, thus an increase in one of the products would push the equilibrium of the phenylalanine reaction toward the reactants. Increasing the concentration of the ion exchange reagent lead to an increase in extraction of both anionic compounds present.

The effect of the equilibrium constant of the co-extracting species was also examined, it was found that increasing its value reduced the extent of phenylalanine extraction. Experimentally this was examined further by adding a second co-extractable anion, bromide, to the extraction system. The bromide's equilibrium constant is of a similar magnitude to phenylalanine, therefore it was found that increasing its initial concentration had a substantial effect on extraction and co-extraction.

8.10.2 Mechanism of Mass Transfer

An experimental study of mass transfer during reactive extraction was conducted using a Lewis cell. In this contactor a two-film theory model of mass transfer is assumed to apply, whereby the main resistance to mass transfer lies in stagnant films either side of the interface, and steady state solute transfer occurs through both films with no accumulation of mass at the interface.

Experimentally it was found that during mass transfer the flux of phenylalanine was linearly related to the flux of chloride, and the constant of proportionality is called the co-extraction constant. The co-extraction constant may be similarly defined for equilibrium conditions, where the extents of all reactions are proportional to the extent of the phenylalanine reaction. It was found that the value of the co-extraction constant determined from mass transfer experiments and those determined from equilibrium extraction experiments are identical for the same initial conditions. From this it was concluded that the relative fluxes of all reactants and products are governed by

equilibrium conditions occurring at the interface. For the co-extraction constant to remain constant throughout a mass transfer experiment then the interfacial condition must be fixed at the equilibrium position for the system, and thus the interfacial concentrations are equal to the equilibrium concentrations for the system.

Using this prediction of the interfacial concentration a simple model of mass transfer was derived, this allowed determination of the mass transfer coefficient, which can be used to assess the effects of added compounds on mass transfer.

8.10.3 Effects of Added Compounds

The effect of co-extraction on mass transfer was examined, and it was found that increased levels of co-extraction would cause a decrease in the solute flux. The decrease was caused by the effect of co-extraction on the equilibrium position of the phenylalanine reaction. Increased co-extraction would reduce the extent of the phenylalanine reaction, and therefore, the interfacial concentration of phenylalanine would increase. Thus, co-extraction reduces the driving force for mass transfer and hence the flux of phenylalanine. The mass transfer coefficient remained unchanged with increased co-extraction, therefore, co-extraction does not effect the transport properties of thin films adjacent to the interface.

A brief examination of the effect of the soluble and insoluble components of fermentation broth on phenylalanine extraction was conducted. The filtered fermentation broth was found to affect the value of the surface tension, from which it was concluded that the fermentation broth contained surface-active compounds. The effect of the concentration of these compounds on mass transfer was examined by performing extractions from dilutions of the filtered fermentation broth. Equilibrium experiments revealed that the broth contained large quantities of anionic compounds that were co-extracted. It is probable that this co-extraction caused a reduction in phenylalanine extraction, although it was not possible to measure the phenylalanine content of the fermentation broth. Mass transfer experiments were performed using chloride measurements to assess the effect of broth components on mass transfer. From these experiments it was seen that the mass transfer coefficient decreased as the

concentration of broth components increased, with a reduction in the mass transfer coefficient of 75% being recorded for extraction from undiluted filtered fermentation broth. The reduction in the mass transfer coefficient is attributed to the presence of surface-active compounds at the interface. The effect of the biomass obtained from the fermentation broth on phenylalanine extraction was also examined. The biomass was not found to affect the equilibrium position of extraction, and therefore took no part in the extraction mechanism. However, the biomass was found to adsorb to the interface, and a reduction in the mass transfer coefficient was recorded. The observed reduction in the mass transfer coefficient is believed to be due to a reduction in the interfacial area, and not due to an increased resistance to mass transfer.

The effects of two surfactants on extraction were also examined, they were the cationic compound DTAB and the non-ionic compound Softanol 30. Neither were found to affect extraction equilibrium or mass transfer.

Chapter 9

Conclusions and Recommendations for Future Work

9.1	Introduction		206
9.2	Conclusions		207
	9.2.1	Physical Extraction	207
	9.2.2	Reactive Extraction	208
9.3	Recommendations for Future Work		209
	9.3.1	Broth Characterisation	209
	9.3.2	Fermentation Conditions	210
	9.3.3	Characterisation of the Interface	210
	9.3.4	The Effect of Dynamic Adsorption on Mass Transfer	211
	9.3.5	Reactive Extraction from Fermentation Broth	211

9.1 Introduction

An investigation into the effect of fermentation broth components, and added compounds, on mass transfer during liquid-liquid extraction was conducted. In Section 2.5 the specific objectives of this study were outlined, and they are reiterated here for reference with the conclusions that have been drawn from this work, which are presented in Section 9.2. In Section 9.3 recommendations of future work are given.

The specific objectives of this study were to,

◊ Investigate the effect of soluble and non-soluble components of fermentation broth on mass transfer and equilibrium conditions during liquid-liquid extraction.

◊ Investigate the effect of surfactant and biosurfactant compounds on mass transfer and equilibrium conditions during liquid-liquid extraction.

◊ Study and characterise the role of co-extraction during reactive liquid-liquid extraction.

◊ Investigate the effect of soluble and insoluble broth components and surfactant compounds on reactive liquid-liquid extraction.

9.2 Conclusions

The main conclusions from the study of the effects of fermentation broth components and added compounds on liquid-liquid extraction are outlined in section 9.2.1 for physical extraction, and 9.2.2 for reactive extraction.

9.2.1 Physical Extraction

◊ Common media components and anti-foaming agents used in fermentation processes do not have an effect on solute extraction. However, the post fermentation medium contains both soluble and insoluble compounds that will adsorb to the liquid-liquid interface and affect solute extraction.

◊ In the fermentation broth used in this study the soluble components found in the weight range of 10000 to 30000 Daltons caused the greatest reduction in mass transfer, although all weight ranges were found to have a negative effect on mass transfer (Figures 7-11).

◊ Biologically produced amphiphilic compounds such as proteins and phospholipids will be adsorbed to the liquid-liquid interface and reduced mass transfer. The quantity of biosurfactants required to do this is very small, with noticeable effects being observed at concentrations as low as 0.01mg/l (Figures 7-16 to 7-19).

◊ The addition of the surfactants SDS and DTAB caused interfacial turbulence, which greatly enhanced mass transfer rates. The cause of this effect was probably due to the transfer of the surface active species through the interface, which created the microscopic interfacial tension gradients that are required to initiate interfacial turbulence.

◊ When added to a system containing biomass both SDS and DTAB preferentially adsorbed to the interface.

◊ The presence of biomass prevented DTAB from crossing the interface and causing interfacial turbulence. This possibly occurred due to adsorption of the positively charge surfactant onto the negatively charged cell wall, this prevented DTAB from being available to transfer across the interface and initiate interfacial turbulence.

9.2.2 Reactive Extraction

◊ Aliquat 336, the ion exchange reagent used in this study, is not specific to phenylalanine and will extract any anionic species present in the extraction medium. The extent of solute extraction and any co-extraction is controlled by the description of the reactions, as defined by the equilibrium constant for each reaction.

◊ Using the equilibrium constants to describe concentrations at equilibrium, a model has been developed that allows the extent of extraction and co-extraction reactions to be predicted for a given set of initial concentrations and equilibrium constants.

◊ The model was defined in this study for the situation where only one co-extraction reaction occurred, but it is anticipated that this model can be extended to include any number of co-extraction reactions provided that the equilibrium constant can be defined and determined.

◊ During mass transfer the relative fluxes of all species involved in any extraction reactions are related through the co-extraction constant, which describes the ratio of the flux of chloride (the common product of all reactions) to the flux of the phenylalanine. The value of the co-extraction constant found during mass transfer had the same value as the co-extraction constant determined from an equilibrium extraction for the same initial conditions. Therefore, the relative fluxes of species across the interface are controlled by the final equilibrium condition of the system.

◊ A mechanism of mass transfer in reactive extraction has been proposed where the interfacial concentrations are constant, and are defined by the equilibrium extents of the reactions at equilibrium for the same initial conditions. Based on this mechanism, solute mass transfer has been modelled to allow mass transfer

coefficients to be determined, this has allowed the effect of different compounds on mass transfer to be studied.

◊ The level of co-extraction occurring during solute extraction does not affect the resistance to mass transfer. However, increased co-extraction will reduce the total extent of solute extraction, and hence, the mass transfer driving force. Therefore, increased co-extraction will reduce the rate of solute transfer across the interface.

◊ Fermentation broth contains many co-extractable anionic compounds which are liable to be extracted, and thus, affect the extent and rate of solute extraction. Fermentation broth also contains soluble surface-active compounds that increase the resistance to mass transfer. The biomass produced during fermentation is also surface active and will adsorb to the interface where it will reduce the solute flux by reducing the interfacial area.

9.3 Recommendations for Future Work

During this study a number of areas have been identified for further investigation, and details of these are given in the following sections.

9.3.1 Broth Characterisation

In Sections 7.7 and 8.9 it was shown that soluble components of the fermentation broth adsorbed to the interface and affected mass transfer, however, the nature of these compounds is essentially unknown. Although it was shown that the greatest effect on interfacial processes occurred for compounds in the weight range 10000 to 30000 Daltons, the other weight fractions were also found to affect mass transfer and surface tension. Further work on broth extraction should include characterisation of the type and quantities of compounds produced during fermentation, and an assessment of their effect on mass transfer. This will allow the mass transfer performance of extraction operations to be predicted and lead to the efficient design of future processes.

9.3.2 Fermentation Conditions

It is reported in literature that the type and quantities of surface-active material that can be produced by micro-organisms is influenced by the fermentation conditions (Desai and Banat, 1997). Use of oil based carbon sources and limiting supplies of a nitrogen source have both been associated with increased production of biosurfactant compounds, and in addition to these other environmental conditions e.g. pH, pO_2, temperature and agitation have also been found to affect production. Therefore, a study of the effects of these factors on mass transfer during extraction would seem appropriate, if a fermentation system could be tailored to limit the production of biosurfactants then a large reduction in the cost of downstream processing may be achieved.

9.3.3 Characterisation of the Interface

The extraction experiments conducted in this study revealed that compounds present in the fermentation broth, or those added to the system can have a variety of effects on mass transfer. A common feature of these systems has been that adsorption to the liquid-liquid interface has occurred. It is supposed that effects on mass transfer have been due to adsorption of compounds at the interface. However, the exact mechanism(s) of the resistance to mass transfer is still uncertain. In recent years a number of new techniques have been developed that allow the molecular process occurring at interfaces to be examined, and these may help elucidate the mechanism of interfacial resistance. Techniques such as total internal reflection fluorescence microscopy and vibrational sum frequency spectroscopy can be used to determine the structure of monolayers at liquid-liquid interfaces. An in depth review of many surface characterisation techniques is given by Binks (1999). With a detailed knowledge of the mechanism occurring at the interface it may be possible to develop methods to prevent inhibition and/or promote enhancement of mass transfer.

9.3.4 The Effect of Dynamic Adsorption on Mass Transfer

The rate of adsorption of many macromolecular surface-active compounds can occur over a considerable period of time, and during this adsorption period any resistance to mass transfer may also be time-dependent. In situations where diffusion controls extraction and excessive mixing cannot be used to promote extraction (such as when stable emulsions occur) adsorption kinetics of surface active compounds may become important. During this investigation the Schlieren technique was used, as part of an optical set-up that also included a Mach-Zehnder interferometer, a technique that allows the interfacial flux to be determined for a diffusional mass transfer system. Using the Mach-Zehnder interferometer it is proposed that the effect of dynamic adsorption on mass transfer rates could be investigated. It may also be possible to adapt the Schlieren system for use as a pendant drop tensiometer; this technique can provide accurate and detailed information on adsorption kinetics. Furthermore, in conjunction with a micropump, the pendant drop tensiometer can be used to explore interfacial rheology by inducing small changes in interfacial area and measuring the response of the systems interfacial tension.

9.3.5 Reactive Extraction from Fermentation Broth

In Section 9.3 a model was developed that allowed the level of co-extraction to be predicted for a two component system. This equilibrium extraction model may be extended for use on fermentation broth systems if the all the extraction reactions are characterised and the reaction constants determined. Given the complex nature of fermentation broth it may be impossible to achieve this, however, a model that neglects components of low concentration and/or those with low equilibrium constants would probably give a good approximation to the real situation.

During this study the effects of surface active compounds on mass transfer was mainly examined using the physical extraction process. However, it was shown that fermentation broth components caused a resistance to mass transfer during reactive extraction. The effect of fermentation broth components on reactive extraction should be investigated further to assess whether the presence of the reaction occurring at the

interface has any influence on other interfacial processes, i.e. the resistance to mass transfer. Also during the study of reactive extraction it was found that the phase volume ratio affected the value of the equilibrium constant and thus the extent of extraction reactions, no explanation could be given for this effect and further investigation is required.

BIBL LONDON UNIV

References

Agble, D. K. (1998). Interfacial Mass Transfer in Binary Liquid-Liquid Systems. PhD Thesis, Imperial College, University of London.

Agble, D.K. and Mendes-Tatsis, M.A. (2000). The Effect of Surfactants on Interfacial Mass Transfer in Binary Liquid-Liquid Systems. *International Journal of Heat and Mass Transfer,* Vol. 43, pp1025-1034.

Anderson, D.W. and Lau, E.F (1955). Commercial Extraction of Unfiltered Fermentation Broth's in the Podbielniak Contactor, *Chemical Engineering Progress*, Vol. 51, pp507-512.

Andrew and Weiss (1959). Solubility of Antibiotics in Twenty-Four Solvent. II. *Antibiotics and Chemotherapy*, Vol. 9, pp277-279

Baird, M.H.I. (1991). Solvent Extraction - The Challenge of a Mature Technology, Canadian *Journal Of Chemical Engineering*, Vol. 69, pp1287-1301.

Bakker, C.A.P., van Buytenen, P.M. and Beek, W.J. (1966). Interfacial Phenomena and Mass Transfer, *Chemical Engineering Science*, Vol. 21, pp1039-1046.

Bakker, C.A.P., Fentener van Vlissingen, F.H. and Beek, W.J. (1967). The Influence of the Driving Force in Liquid-Liquid Extraction - A Study of Mass Transfer with and without Interfacial Turbulence Under Well Defined Conditions, *Chemical Engineering Science*, Vol. 22, pp1349-1355.

Behr, J.P., Lehn, J.M. (1973). Transport of Amino Acids Through Organic Liquid Membranes, *Journal of the American Chemical Society*, Vol. 95, pp6108-6110.

Belter, P.A., Cussler, E.L. and Hu, W-S. (1996). Bioseparation: Downstream Processing for Biotechnology, John Wiley and Sons, New York.

Binks, B.P, (1999). Modern Characterisation Methods of Surfactant Systems, Marcel Dekker, New York.

Blokker, P.C. (1957). On Mass Transfer Across Liquid-Liquid Interfaces in Systems With and Without Surface Active Agents, *Proceedings of the 2nd International Congress on Surface Activity*, 1957, pp503-511.

Bognolo, G. (1999). Biosurfactants as Emulsifying Agents for Hydrocarbons, *Colloids and Surfaces A: Physicochemical And Engineering Aspects*, Vol. 152, pp41-52.

Bora, M.M., Ghosh, A.C., Dutta, N.N., Mathur, R.K. (1997). Reactive Extraction of 6-Aminopenicillanic Acid with Aliquat-336: Equilibrium and Kinetics, *Canadian Journal of Chemical Engineering*, Vol. 75, pp520-526.

Bos, M.A. and Van Vliet, T., (2001). Interfacial Rheological Properties of Adsorbed Protein Layers and Surfactants: A Review, *Advances in Colloid and Interface Science*, Vol. 91, pp437-471.

Calvarin, L., Roche, B., Renon, H. (1992). Anion Exchange and Aggregation of Dicyanocobalamin with Quaternary Ammonium Salts in Apolar Environment, *Industrial and Engineering Chemistry Research*, Vol. 31, pp1705-1709.

Carslaw, H.S. and Jaeger, J.C. (1959). Conduction of Heat in Solids, 2nd Edition, Oxford University Press.

Chan, C.C, Wang, S.S. (1993). Kinetics of the Extraction of Phenylalanine and Glutamic Acid by Ion-Exchange Carriers, *Journal of Membrane Science*, Vol. 76, pp219-232.

Coelhoso, I.M., Crespo, J.P.S.G., and Carrondo, M.J.T., (1996). Modelling of Ion Pair Extraction with Quaternary Amines, *Separation Science and Technology*, Vol. 31, pp491-511.

Coelhoso, I.M., Crespo, J.P.S.G., and Carrondo, M.J.T.. (1997). Kinetics of Liquid Membrane Extraction Systems with Variable Distribution Coefficients. *Journal of Membrane Science*, Vol. 127, pp141-152.

Cooper, D.G. and Zajic, J.E (1980). Surface Active Compounds from Micro-organisms. *Advances in Applied Microbiology*, Vol. 26, pp229-253.

Coulson, J.M. and Richardson, J.F. (eds.), *Chemical Engineering*, Vol. 1, 1990, Pergammon Press.

Crabbe, P.G., Tse, C.W. and Munro, P.A. (1986). Effect of Microorganisms on Rate of Liquid Extraction of Ethanol From Fermentation Broth, *Biotechnology and Bioengineering*, Vol. 28, pp939-943.

Crank J. (1975). The Mathematics Of Diffusion, Oxford University Press.

Cussler, E.L. (1997). Diffusion: Mass Transfer in Fluid Systems, Cambridge University Press.

Dai, Y. and King, C.J. (1996). Selectivity Between Lactic Acid and Glucose during Recovery of Lactic Acid with Basic Extractants and Polymeric Sorbents, *Industrial and Engineering Chemistry Research*, Vol. 35, pp1215-1224.

Danckwerts, P.V. (1951). Significance of Liquid Film Coefficients in Gas Absorption *Industrial and Engineering Chemistry*, Vol. 43, pp1460-1467.

Davies, J.T. and Mayers, G.R.A. (1961). The Effect of Interfacial Films on Mass Transfer Rates in Liquid-Liquid Extraction, *Chemical Engineering Science*, Vol. 16, pp55-66.

Davis, J.T. and Rideal, E.K. (1963). Interfacial Phenomena, 2nd Edition, Academic Press, London

Desai, A.J., Patel, R.M., Desai, J.D. (1994). Advances in the Production of Biosurfactants and Their Commercial Applications, *Journal of Scientific Research*, Vol. 53, pp619-629.

Desai, J.D. (1997). Microbial Surfactants: Evaluation, Types, Production and Future Applications, *Journal of Scientific Research*, Vol. 46, pp440-449.

Desai, J.D. and Banat, I.M. (1997). Microbial Production of Surfactants and Their Applications, *Microbiology and Molecular Biology Reviews*, Vol. 61, pp47-64

Escalante, H., Alonso, A.I., Ortiz, I., and Irabien, A. (1998). Separation of l-phenylalanine by Nondispersive Extraction and Backextraction. Equilibrium and Kinetic Parameters, *Separation Science and Technology*, Vol. 33, pp119-139.

Fainerman, V.B., Lucassen-Reynders, E.H. and Miller, R. (1998). Adsorption of Surfactants and Proteins at Fluid Interfaces, *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* Vol. 143, pp141-165.

Garner, F. H. and Hale, A. R. (1953). The Effect of Surface Active Agents in Liquid Extraction Processes, *Chemical Engineering Science*, Vol. 2, pp157-163

Georgiou, G., Lin S-C., Sharma, M.M. (1992). Surface Active Compounds From Micro-organisms, *Bio/Technology*, Vol. 10, pp60-65

Gibbs, J.W. (1928). Collected Works, Vol. 1, 2nd Edition, Longmans, New York.

Gordon and Sherwood (1954). Mass Transfer Between Two Liquid Phases. *Chemical Engineering Progress*, Vol. 50, Symposium Series No. 10, p15

Gottlieb, D and Diamond, L. (1951). A Synthetic Medium For Chloromycetin, *Bull. Torrey Bot. Club*, Vol. 78, pp56-60

Graham, D.E. and Phillips, M.C. (1979). Proteins at Liquid Interfaces I. Kinetics of Adsorption and Surface Denaturation, Journal of Colloid and Interface Science, Vol. 70, p p403-414.

Habaki, H., Isobe, S., Egashira, R. and Kawasaki, J. (1998). Permeation and Concentration of Erythromycin by Supported and Emulsion Liquid Membranes *Journal of Chemical Engineering of Japan*, Vol. 31, pp47-54.

Haensel, R, Halwachs, W and Schugerl, K (1986). Reactive Extraction of *d.l*-phenylalanine with trioctylmethylammonium chloride (TOMAC) as a carrier – III Equilibrium and Mass Transfer Investigation, *Chemical Engineering Science*, Vol41, pp1811-1815.

Hamilton, D and Weatherley, L.R. (1995). The Effect of Biomass Concentration On Interfacial Tension in a Whole Broth Liquid-Liquid Extraction System, *IChemE Research Event*, 1995, p1139-1141.

Hano, T., Ohtake, T., Matsumoto, M., Kitayama, D., Hori, F. and Nakashio, F. (1991). Extraction Equilibria of Amino Acids with Quaternary Ammonium Salt, *Journal of Chemical Engineering of Japan*, Vol. 24, pp20-24.

Hariri, M.H. (1989). Bioseparations: Design and Engineering of Partitioning Systems, *Bio/Technology*, Vol.7, pp686-688.

Higbie, R (1935). The Rate of Absorption of a Pure Gas Into a Still Liquid During Short Periods of Exposure, *Transaction of the American Institute of Chemical Engineers*, Vol. 35, pp365-389.

Hildebrand, J.H., Prauznitz, J.M. and Scott, R.L. (1970). Regular and Related Solutions, Van Nostrand Reinhold, New York.

Hutchinson, E. (1948). Diffusion Across Oil-Water Interfaces, *Journal of Physical and Colloid Chemistry*, Vol. 52, p897-908.

Iwasaki, I, Utsumi, S, Hagino, K, Ozawa, T, (1956). A Spectrophotometric Method for the Determination of Small Amounts of Chloride using Mercuric Thiocyanate, *Journal of the Chemical Society of Japan*, Vol. 29, p860-864.

Jones, N.J. and Chapman, D.C. (1995). Micelles, Monolayers and Biomembranes. Wiley, New York

Kawasaki, J., Egashira, R., Kawai, T., Hara, H. and Boyadzhiev, L. (1996). Recovery of Erythromycin by a Liquid Membrane, *Journal of Membrane Science*, Vol. 112, pp209-217.

Kawase, Y and Moo-Young, M. (1987). Influence of Antifoam Agents on Gas Hold-up and Mass Transfer in Bubble Columns with Non-Newtonian Fluids, *Applied Microbiology and Biotechnology*, Vol. 27, pp159-167.

Kertes, A.S. and King, C.J. (1986). Extraction Chemistry of Fermentation Product Carboxylic Acids, *Biotechnology and Bioengineering*, Vol. 28, pp269-282.

King, C.J. (1980). Separation Processes, 2nd Edition, McGraw-Hill.

Laane, C, Boeren, S, Vos K, (1985). On Optimising Organic Solvents In Multi-Liquid-Phase Biocatalysis, *Trends in Biotechnology*, Vol. 3, p251

Lewis, J. B (1954). The Mechanism of Mass Transfer of Solutes across Liquid-Liquid Interfaces Part I: The Determination of Individual Mass Transfer Coefficients for Binary Systems, *Chemical Engineering Science*, Vol. 3, pp248-259.

Liddel, J.M. (1994). Solvent Extraction Process for Biological Processes, In "*Engineering Processes for Bioseparation*", Ed. Weatherley, L.R., Butterworth-Heinemann.

Lin, S-C., (1996). Biosurfactants: Recent Advances, *Journal of Chemical Technology and Biotechnology*, Vol. 66, pp109-120.

Lindland, K. P., and Terjesen, S. G. (1956). The Effect of a Surface Active Agent on Mass Transfer in Falling Drop Extraction, *Chemical Engineering Science*, Vol. 5, pp1-12

Lye, G.J. and Stuckey, D.C. (2000) Extraction of Erythromycin-A Using Colloidal Liquid Aphrons I. Equilibrium Partitioning, *Journal of Chemical Technology and Biotechnology*, Vol. 75, pp339-347.

Matsushita, K., Mollah, A.H., Stuckey, D.C., del Cerro, C. and Bailey, A.I (1992). Predispersed Solvent Extraction of Dilute Products Using Colloidal Gas Aphrons and Colloidal Liquid Aphrons – Aphron Preparation, Stability and Size, *Colloids and Surfaces,* Vol. 69, pp65-72.

Miller, R., Fainerman, V.B., Makievski, A.V., Kragel, J., Grigoriev, D.O., Kazakov, V.N., and Sinyachenko, O.V (2000). Dynamics of Protein and Mixed Protein / Surfactant Adsorption Layers At the Water/Fluid Interface, *Advances in Colloid and Interface Science*, Vol. 86, pp39-82.

Molinari, R., De Bartolo, L., Drioli, E. (1992). Coupled Transport of Amino Acids Through a Supported Liquid Membrane. 1. Experimental Optimisation, *Journal of Membrane Science*, Vol. 73, pp203-215.

Morao, A., Maia, C.I., Fonseca, M.M.R., Vasconcelos, J.M.T., and Alves, A.A., (1999). Effect of Antifoam on Gas-Liquid Mass Transfer in Stirred Fermenters, *Bioprocess Engineering*, Vol. 20, pp165-172.

Mudge, L. K. and Heideger, W. J. (1970). The Effect of Surface Active Agents on Liquid-Liquid Mass Transfer Rates, *Journal of the American Institute of Chemical Engineers*, Vol.16, pp602-608.

Nakayama, K., Sagamihara and Hagino, H. (1973). Process for Preparing l-phenylalanine. United States Patent Number 3,759,790

Orell, A. and Westwater, J.W. (1962). Spontaneous Interfacial Cellular Convection Accompanying Mass Transfer: Ethylene Glycol – Acetic Acid – Ethyl Acetate. *Journal of the American Institute of Chemical Engineers*. Vol. 8, pp350-356.

Perez de Ortiz, E.Z. (1992). Marangoni Phenomena, in Theory and Practice of Liquid-Liquid Extraction, Vol. 1, Ed. J.D. Thorton, Carendon, Oxford

Pertler, M., Blass, E. and Stevens, G.W. (1996). Fickian Diffusion in Binary Mixtures That Form Two Liquid Phases, *Journal of the American Institute of Chemical Engineers*, Vol. 42, pp910-920.

Pursell, M., Mendes-Tatsis, M.A., and Stuckey, D.C. (1999). The Effect of Surfactants During Solvent Extraction of Erythromycin-A from Buffer and Filtered Fermentation Broth", *Solvent Extraction for the 21st Century (Proceedings of ISEC'99)* Eds.: M. Cox, M. Hidalgo and M. Valiente, Society of Chemical Industry, London, Vol. 1, p155 (2000).

Ricker, N.L., Michaels, J.N., King, C.J. (1979). Solvent Properties of Organic Bases for Extraction of Acetic Acid From Water, *Journal of Separation and Process Technology*, Vol. 1, pp36-41.

Ricker, N.L., Pittman, E.F., King, C.J. (1980). Solvent Extraction With Amines for the Recovery of Acetic Acid From Dilute Aqueous Industrial Streams, *Journal of Separation and Process Technology*, Vol. 2, pp23-30.

Rosen, M.J. (1978). Surfactants and Interfacial Phenomena, 1st Edition, Wiley, New York.

Rosenberg, E. and Ron, E.Z. (1999). High and Low Molecular Mass Microbial Surfactants, *Applied Microbiology and Biotechnology*, Vol. 52, pp154-162

Sadana, A. (1997) Bioseparation Of Proteins: Unfolding-Folding and Validations, Separation Science and Technology Series, Vol. 1

Sawistowski, H. (1971). Interfacial Phenomena, in *Recent Advances in Liquid-Liquid Extraction*, (ed. C. Hanson) Pergammon Press, Oxford.

Scarpello, J.T. (1998). Solvent Extraction of a Polar Solute Using Colloidal Liquid Aphrons: Stability, Equilibrium and Mass Transfer, PhD Thesis. Imperial College, University of London

Scarpello, J.T., and Stuckey, D.C. (2000). The Reactive Extraction of Phenylalanine with Aliquat 336: Buffer Co-extraction Equilibrium and Mass Transfer Kinetics. *Biotechnology and Bioengineering*, Vol. 69, pp469-477.

Sherwood, T. K. and Wei, J. C. (1959). Interfacial Phenomena in Liquid Extraction. *Industrial and Engineering Chemistry*, Vol. 49, pp1030-1034.

Smith, C.G. and Hinman, J.W. (1963). Chloramphenicol, *Progress in Industrial Microbiology*, Vol. 4, pp139-163.

Smith, J.M. and Van Ness, H.C. (1987). Introduction to Chemical Engineering Thermodynamics, 4th Edition, McGraw-Hill, Singapore.

Sternling C.V. and Scriven L.E. (1959). Interfacial Turbulence: Hydrodynamic Instability and the Marangoni Effect, *Journal of the American Institute of Chemical Engineers,* Vol. 5, pp514-523.

Stuckey, D.C. (1996). Solvent Extraction In Biotechnology, Plenary Lecture at International Solvent Extraction Conference (ISEC '96)

Tamada, J.A., Kertes, A.S., King, C.J. (1990). Extraction of Carboxylic Acids With Amine Extractants. 1. Equilibria and Law of Mass Action Modelling, *Industrial and Engineering Chemistry Research*, Vol. 29, pp1319-1326.

Thien, M.P., Hatton, T.A., Wang, D.I.C., (1987). Separation and Concentration of Amino Acids Using Liquid Emulsion Membranes. *Biotechnology and Bioengineering*, Vol. 32, pp604-615.

Tong, Y., Hirata, M., Takanashi, H., Hano, T., Matsumoto, M., Miura, S. (1998). Solvent Screening for Production of Lactic Acid by Extractive Fermentation, *Separation Science and Technology*, Vol. 33, pp1439-1453.

Toor, H.L. and Marchello, J.M. (1958). Film Penetration Model for Heat and Mass Transfer, *Journal of the American Institute of Chemical Engineers*, Vol. 4, pp97-101.

Uddin, M.S., Hidajat, K, Lim, B-G, and Ching, C-B, (1990). Interfacial Mass Transfer in Extraction of Amino Acids by Aliquat 336 in Organic Phase, *Journal of Chemical Technology and Biotechnology*, Vol. 48, pp415-426.

Uddin, M.S., Hidajat, K, Lim, B-G, and Ching, C-B, (1992). Interfacial Mass Transfer in Stripping of Phenylalanine in Liquid Extraction Process, *Journal of Chemical Technology and Biotechnology*, Vol. 53, pp353-357.

Vining, L.C. and Westlake, D.W.S. (1984). Chloramphenicol: Properties, Biosynthesis and Fermentation, in *Biotechnology of Antibiotics*, Ed Van Damme, 1984.

Ward, A.F. and Tordai, L (1946). Time Dependence of Boundary Tension of Solutions I. The Role of Diffusion in Time-Effects, *Journal of Chemical Physics*, Vol. 14, pp453-

Weatherley, L.R. , Allen, G., Goodwin, J.A.S. and Haig, M.B. (1993). Interfacial Mass Transfer and Partition Studies During Whole Broth Extraction, *Proceedings of the International Solvent Extraction Conference (ISEC 1993)*, Eds. Logsdail, D.H and Slater, M.J, 1993, pp1003-1009.

Weatherley, L.R., Doherty-Speirs, E.A., Goodwin. J.A.S. and Slaughter. J.C. (1994). A Study of the Effects of Biomass Morphology Upon Partition in the System Propanol/Water/n-Decanol in the Presence of *Aureobasidium pullans. In "Separations for Biotechnology 3"*, Ed Pyle, D.L., SCI Publishing, pp539-545.

Weiss, P.J., Andrew, M.L. and Wright, W.W. (1957). Solubility of Antibiotics in Twenty-Four Solvents: Use in Analysis, *Antibiotics and Chemotherapy*, Vol. 7, pp374-377.

Wilke, C.R. and Chang, P. (1955). Correlation of Diffusion Coefficients in Dilute Solution, *Journal of the American Institute of Chemical Engineers*, Vol. 1. pp264-270.

Whitman, W.G. (1923). The Two-Film Theory of Adsorption, *Chemical and Metallurgical Engineering*, Vol. 29, p147

Yang, S-T., White, S.A., Hsu, S-T. (1991). Extraction of Carboxylic Acids with Tertiary and Quaternary Amine: Effect of pH, *Industrial and Engineering Chemistry Research*, Vol. 30, pp1335-1342.

Appendix A

Concentration Analysis Using UV-Visible Absorbance Measurements.

A.1 Chloramphenicol Analysis

Measurement of aqueous and solvent phase chloramphenicol concentration was determined by UV-visible spectrometry (Section 6.5.1). In this section the UV-visible spectrums obtained to identify the peak absorbances are presented, and details of the method used to construct the concentration-absorbance calibration curve is given.

In Figure A-1 the UV-visible spectrum of an aqueous chloramphenicol solution is shown, and also shown is the spectrum of the solvent phase arising from an equilibrium extraction experiment where chloramphenicol has partitioned into the solvent phase. It can be seen that chloramphenicol exhibits a peak absorbance at 272nm and 278nm for the aqueous and solvent phase respectively, and the magnitude of the peak was also found to vary directly with concentration. Therefore, the quantity of chloramphenicol present may be determined from UV absorbance measurements of either the aqueous or solvent phase.

However, compounds produced during the fermentation were found to interfere with aqueous phase measurements, as these compounds also absorbed light in the wavelength range of 250-300nm. This is shown in Figure A-2 where the UV-visible absorbance spectrum of a filtered fermentation broth solution is presented. It is seen that the solution absorbs light over the wavelength range of 250-320nm, and no peak due to chloramphenicol is identifiable. Thus, it will not be possible to determine changes in chloramphenicol concentration in the aqueous phase when these compounds are present. However, it is still possible to determine the concentration from solvent phase measurements, and shown in Figure A-2 is the spectrum for the solvent phase arising from an equilibrium extraction experiment using fermentation broth.

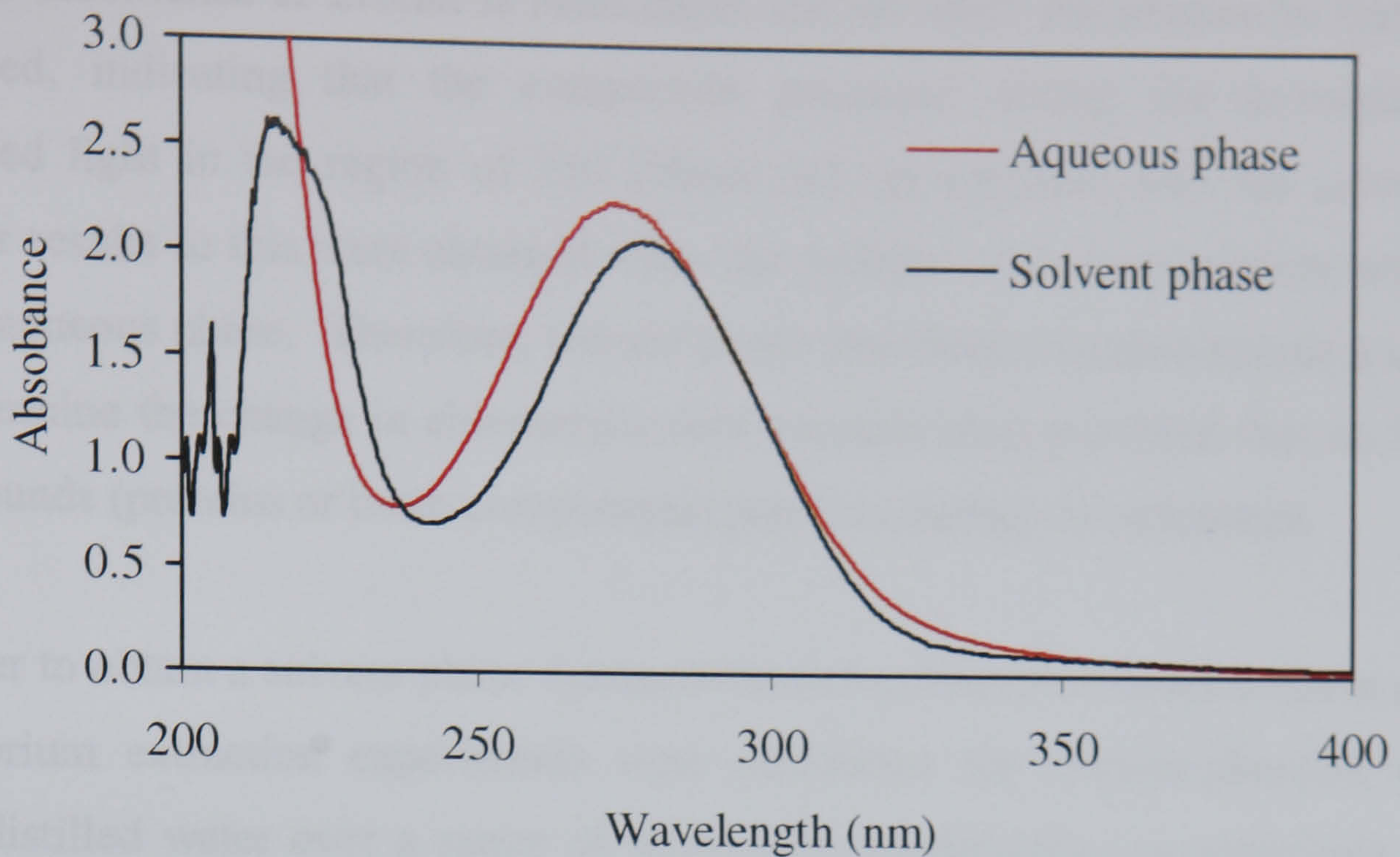


Figure A-1 UV-visible absorbance spectrum of the aqueous and solvent phases arising from a chloramphenicol equilibrium extraction experiment. Initially the aqueous phase was distilled water, pH 5.5, with a chloramphenicol concentration of 250µM, the solvent phase was 1-octanol, temperature = 25°C, and PVR = 1. De-ionised water and 1-octanol were used as the reference solutions for the aqueous and solvent phase scans, respectively. Cuvette path length = 10mm.

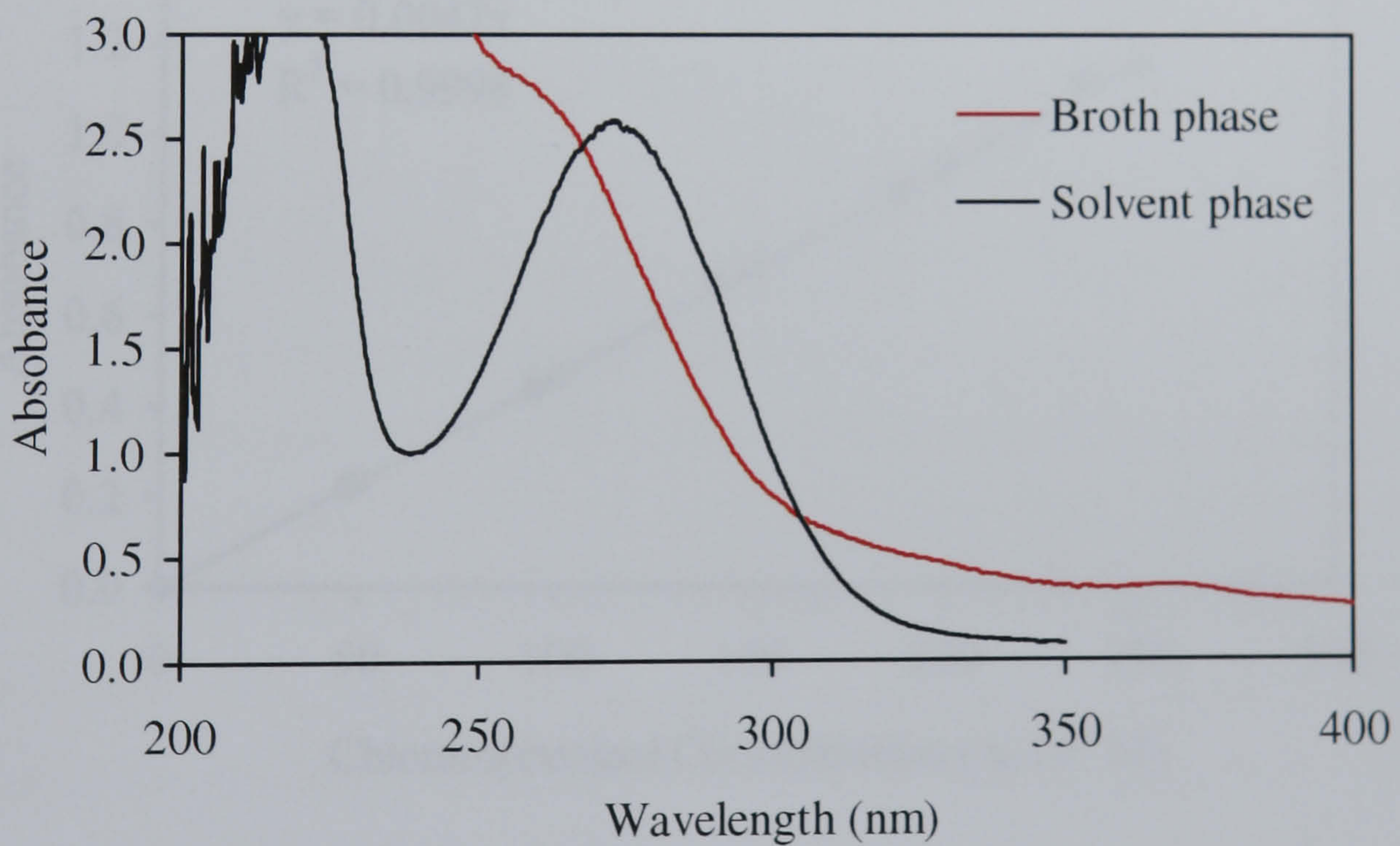


Figure A-2 UV-visible absorbance spectrum of the aqueous and solvent phases arising from a chloramphenicol equilibrium extraction experiment from filtered fermentation broth into 1-octanol. The initial aqueous phase was filtered fermentation broth with a chloramphenicol concentration of 250µm, pH 7, temperature = 25°C, and a PVR = 1. De-ionised water and 1-octanol were used as the reference solutions for the aqueous and solvent phase scans, respectively. Cuvette path length = 10mm.

A peak absorbance at 278nm is identifiable and no other absorbance in that region is observed, indicating that the compounds produced during the fermentation that absorbed light in the region of 250-300nm did not partition into the solvent phase. Similar results to this were obtained when the proteins and phospholipids were present in the aqueous phase. Therefore, solvent phase absorbance measurements may be used to determine the change in chloramphenicol concentration provided that no interfering compounds (proteins or broth components) partition during the extraction.

In order to obtain a solvent phase concentration-absorbance calibration curve a series of equilibrium extraction experiments were performed for chloramphenicol extraction from distilled water over a range of initial chloramphenicol concentrations. Prior to extraction the initial aqueous solutions were analysed for absorbance at a wavelength of 272nm. Using this data an aqueous phase calibration curve was constructed, which is shown in Figure A-3.

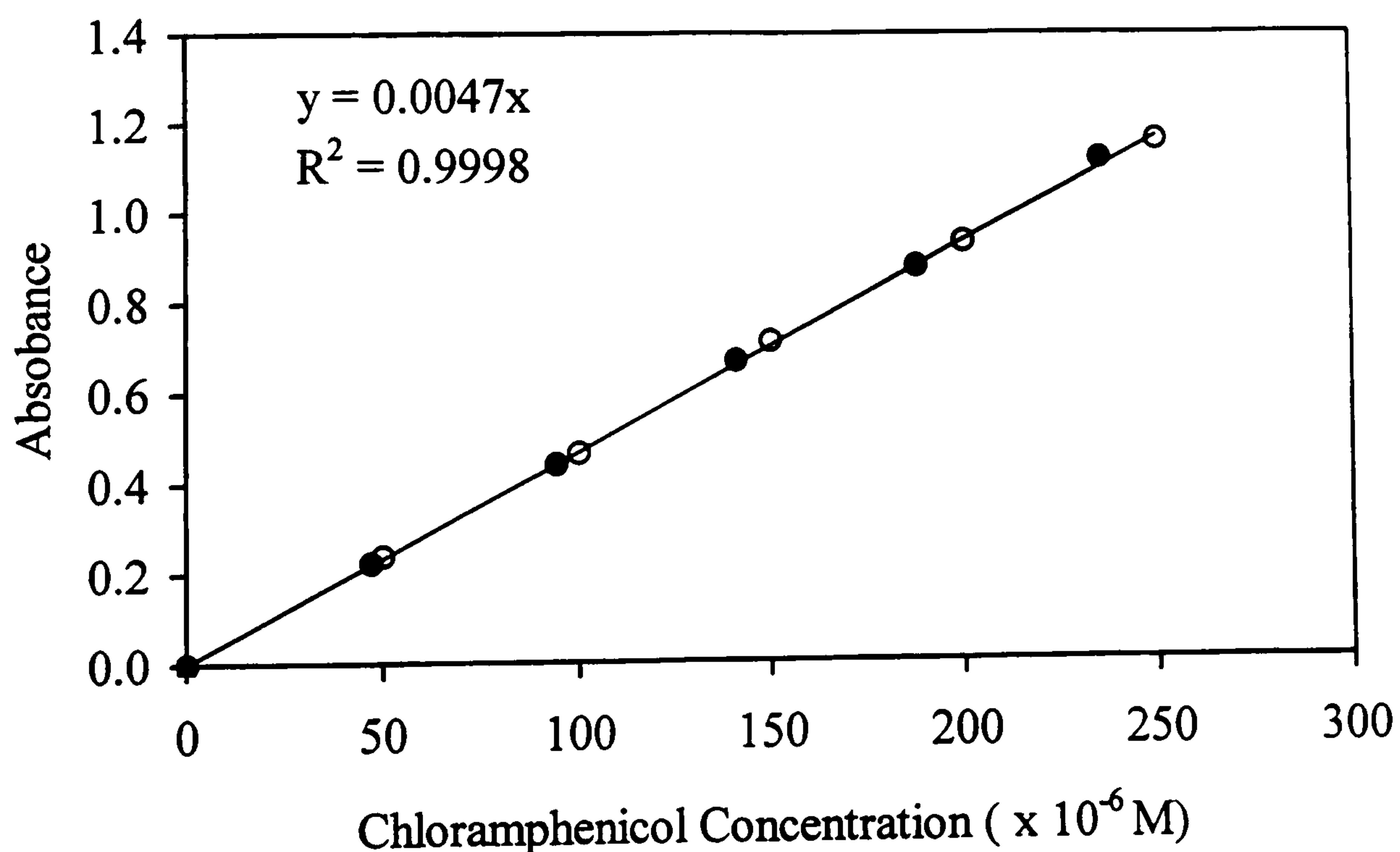


Figure A-3 Calibration plots obtained for chloramphenicol concentration-absorbance measurements: (a) aqueous phase measurements (o) for initial chloramphenicol concentrations of 0-250μM in distilled water, and (b) solvent phase measurements (•) after extraction with 1-octanol, PVR = 1. De-ionised water and 1-octanol were used as the reference solutions for the aqueous and solvent phase measurements, respectively. Cuvette path length = 10mm.

After the extractions had been performed the aqueous phases were re-analysed for absorbance and the concentration of chloramphenicol present was determined from the calibration curve described above. Thus, the solvent phase concentration could then be determined by mass balance, and after assaying the solvent phase for absorbance a calibration curve for solvent phase measurements was constructed, this calibration curve is also shown in Figure A-3, where the constant of proportionality was 0.0047. In subsequent experiments it was assumed that no compounds which adsorb light in the region of 250-300nm, other than chloramphenicol, partitioned into the solvent phase. Where possible this was verified by conducting blank extractions (no chloramphenicol present), and then scanning the UV-visible spectrum of the solvent phase. Therefore, any change in absorbance at 278nm in the solvent phase was due to changes in the concentration of chloramphenicol only, and the solvent phase calibration curve (Figure A-3) could be used to obtain concentration measurements.

A.2 Phenylalanine Analysis

Measurement of aqueous phenylalanine concentration was determined by UV-visible spectrometry (Section 6.5.2.1), and in this section the calibration curve used to determine the concentration from absorbance measurements is presented. At a wavelength of 257nm phenylalanine exhibits a peak absorbance in the aqueous phase (Haensel *et al.*, 1986; Uddin *et al.*, 1990 and 1992; and Scarpello and Stuckey, 2000) and this was confirmed by scanning the UV-visible absorbance spectrum of an aqueous solution of phenylalanine. In the solvent phase no peak was identifiable due to interference from Aliquat 336, the ion exchange reagent. The magnitude of absorbance was found to vary with the concentration of phenylalanine present, and in Figure A-4 the calibration plot obtained for a series of solutions of known phenylalanine concentration is presented. It is seen that absorbance varies directly with concentration, where the value of the constant of proportionality is 0.0348.

When fermentation media and broth components were present it was not possible to detect an absorbance peak due to phenylalanine in the aqueous phase. To separate these components from phenylalanine liquid phase chromatography (HPLC) was investigated using reverse phase C18 columns; however, no suitable separation was achieved.

Therefore, measurements from a fermentation medium were not possible using UV-visible spectrometry, and as no other suitable method was found an in depth investigation of phenylalanine extraction from fermentation broth could not be conducted.

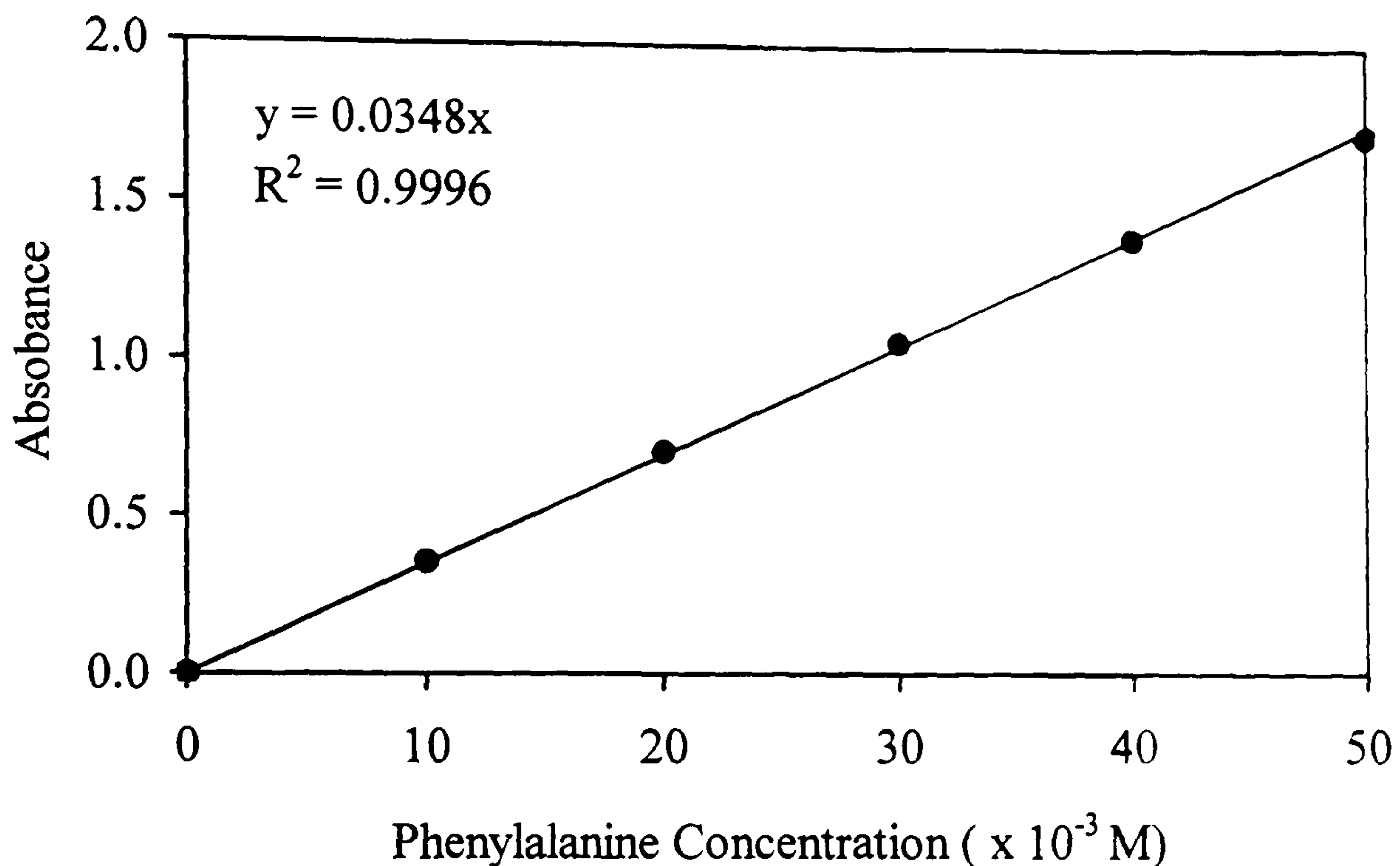


Figure A-4 Calibration plot obtained from the measurement of absorbance at a wavelength of 257nm for phenylalanine concentrations of 0-50mM, with a sodium hydroxide concentration of 100mM. Reference phase was de-ionised water and the cuvette had a path length of 5mm.

A.2 Chloride Analysis

Measurement of aqueous chloride concentration was determined using a colourimetric assay method (Iwasaki, 1956). Details of the chemical reagents and the experimental method where given previously in Section 6.5.2.2. The colour intensity of the resulting solution varied directly with chloride concentration and exhibited a peak absorbance at a wavelength of 460nM. An example of a calibration plot is given in Figure A-5, where the constant of proportionality is 0.0376. The intensity of colour development was also found to vary with reaction time and laboratory temperature, therefore, new calibration plots were determined for every analytical set. The accurate range of the assay was over the range chloride concentration range 0.1 to 3mM. However, chloride concentrations

encountered were in the range 10 to 30mM, therefore. sample where diluted accordingly. Due to interference of the colour development. when any other anions other than phenylalanine and hydroxide were present in the aqueous phase chloride analysis was performed using ion chromatography. as detailed in Section 6.5.2.3.

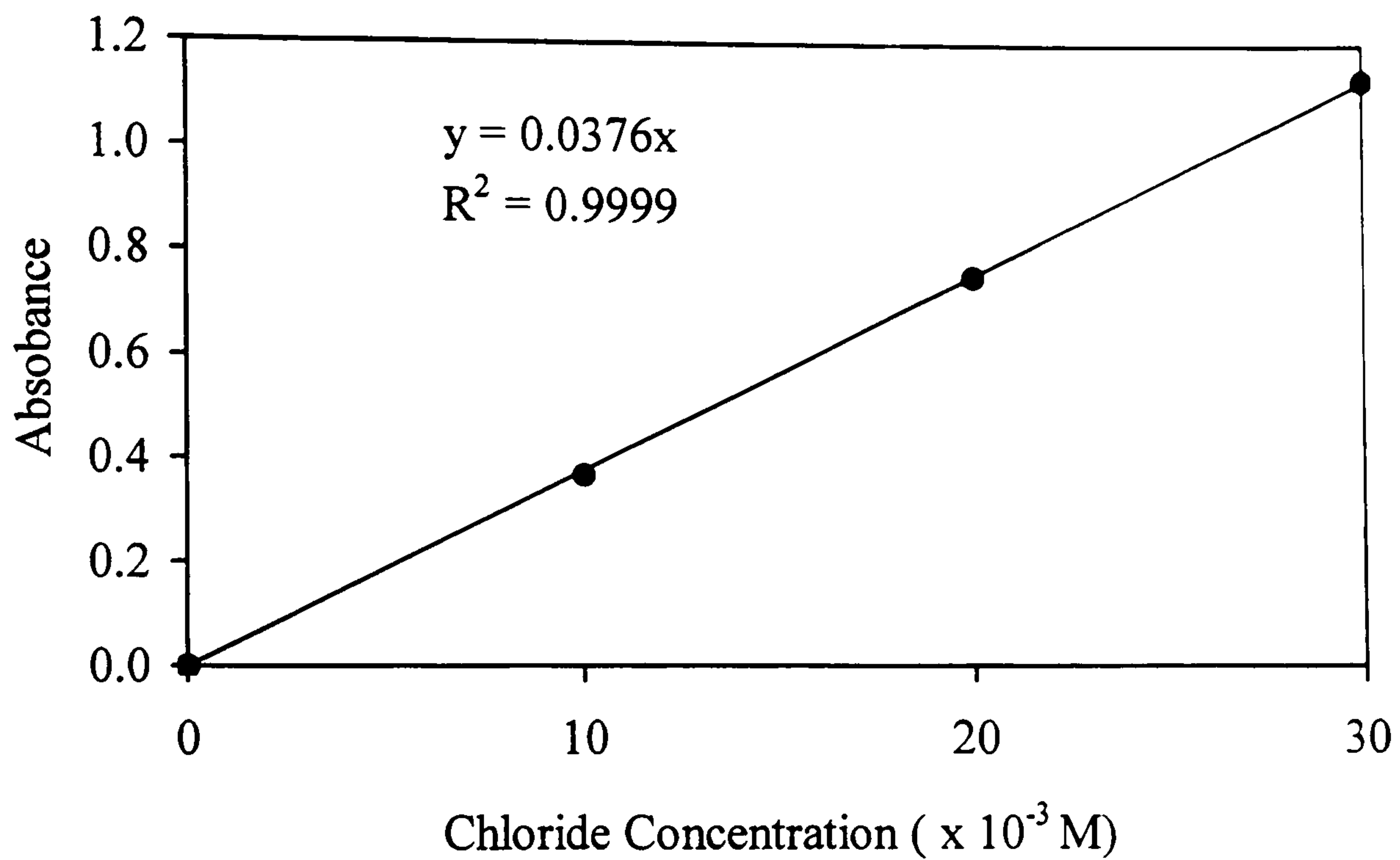


Figure A-5 Calibration plot obtained from the measurement of absorbance at a wavelength of 460nm for chloride concentrations of 0-50mM, with a phenylalanine concentration of 50mM, a sodium hydroxide concentration of 100mM, a sample dilution of 10 ($^V/_V$). Reference solution was a blank assay solution, and the cuvette had a path length of 10mm. Other experimental details are given in Section 6.5.2.2.

BIBL LONDON UNIV.