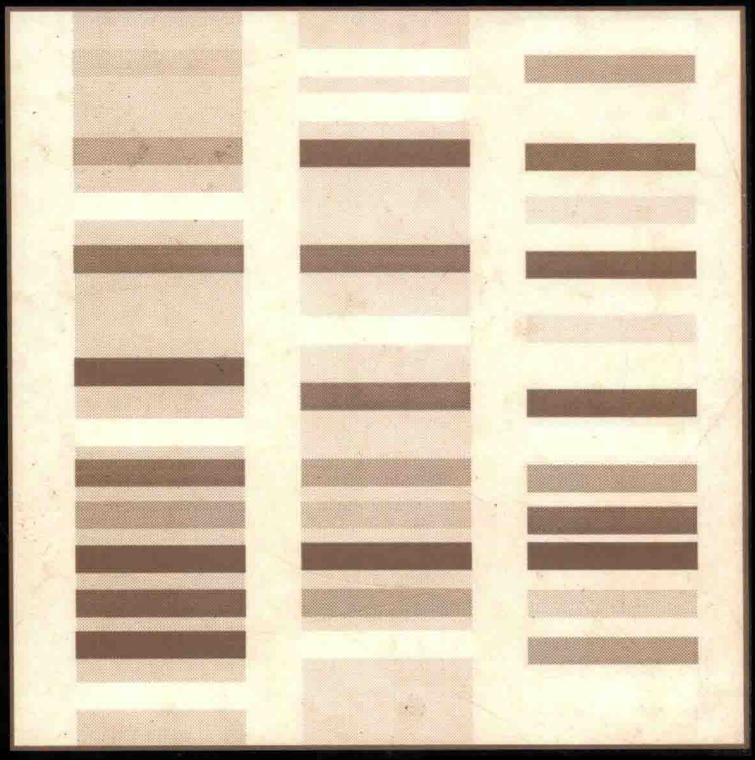
SEPARATIONS FOR BIOTECHNOLOGY 3



Separations for Biotechnology 3

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Papers presented at the Third International Symposium on 'Separations for Biotechnology' organised by the Biotechnology Group and Solvent Extraction and Ion Exchange Group of the Society of Chemical Industry, held on 12–15 September 1994, at The University of Reading.

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Preface

Bioseparations is the set of techniques by which the products of fermentations or biotransformations are recovered for subsequent use. The importance of recovery in high yield of pure biological products for process efficiency and economics as well as for product safety and quality cannot be overstated. Many of the technical problems posed in bioprocessing are new ones. A satisfactory solution must combine high yields and productivity with high levels of purity; it must also be predictable, reproducible and controllable. It is difficult to achieve these objectives at present, for a number of reasons. One is that the starting point of the downstream processing line is typically a dilute, but complex, impure and poorly characterised broth, often currently requiring several purification stages; secondly, many products are not robust, making the task of recovery difficult and one for which existing process technologies are not directly appropriate; thirdly, there are large gaps in fundamental understanding in areas of science and engineering relevant to separations technology for biomolecules; finally, extreme demands are imposed on product purity by regulatory requirements.

This book includes the papers - oral and poster - presented at the Third International Conference on Separations for Biotechnology. It includes papers on a very wide range of approaches and areas of biotechnology, reflecting the multidisciplinary nature of the problems. I believe that it contains many new and interesting contributions to the science, engineering and implementation of bioseparations technology, and that it will be of equal interest to researchers and to practising industrial biotechnologists.

The proceedings show that, whilst there are as yet few generally applicable solutions, and fewer obviously optimal ones, nonetheless, as a result of R&D, the range of available technologies is rapidly increasing. Research is also throwing up other exciting possibilities, although there still remain many problems for research and development. One of the most interesting features is the recognition that a variety of approaches may all have a contribution to make. This is certainly an area where multidisciplinary efforts are needed, and where molecular biology and process engineering must go hand in hand.

Whilst quality can never be absolutely assured, all the papers presented here have been refereed by experts. Although the conference itself was structured around a series of themes, I felt that to continue this into the book would have been to impose a rather artificial structure, and as a result these proceedings are presented alphabetically by author. The limited number of papers should, however, ensure that it will be rather easier for the reader to find their way around the text than to solve a typical sequencing problem.

I must acknowledge the help of the SCI and its staff and also of many individuals in putting this book together. I am very grateful to the conference organising committee for all their work, which was rewarded by a larger than average refereeing load. I am also very grateful to all those colleagues, acknowledged elsewhere, who helped with refereeing: such unsung and unpaid work is vital for the development of science and engineering. I wish to thank Amanda Wright and Chris Pyle for their help with editing and with the index and, last but by no means least, Jean Davis for all her efforts in handling the reams of paperwork associated with this volume.

Leo Pyle

Contents

Extraction Kinetics of Amino Acids to AOT Water-in-oil Microemulsion

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1. INTRODUCTION

The kinetics of the extraction with microemulsions(ME), which is important for separating biologically active substances, is very complicated because reorganization of ME droplets proceeds simultaneously with the extraction. Although several models have been proposed for elucidating the kinetic mechanism[1-8], the extraction rates have not yet been elucidated for the effects of the charged state of solute, the location of solute entrapment in ME droplets, the direction of extraction and the diameter of a ME droplet.

In this paper, we present the time evolution of the concentration profile of Trp for Trp transfer near an AOT-CCl_4/brine solution interface, from which the rate processes concerned with the transfer can be directly elucidated. The kinetic behaviour in the Lewis cell was examined for the extraction of Trp, I^- and K^+ between the aqueous phase and AOT in n-heptane solutions. We measured the rates of the forward and backward extractions of Trp in zwitterionic and anionic states. These solutes are suitable for investigating the effects of electrical charge and the location of solute taken up in a ME droplet on the extraction rate. In both systems, large interfacial resistance was observed. We propose a model for interpreting the interfacial resistance.

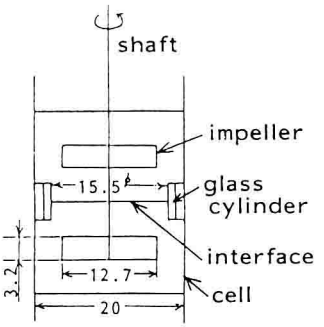

Fig. 1 Lewis-type cell.

2. EXPERIMENTAL

Materials AOT provided by Tokyo Kasei Co. Ltd. was used without further purification. Trp, sodium chloride, sodium iodide and potassium chloride of reagent grade, and n-heptane, carbon tetrachloride of spectrophotometric reagent-grade were used as supplied.

Method A Lewis-type cell(Fig.1) was used to measure the extraction rate. The ME phase was prepared by dissolving a desired amount of AOT in n-heptane, and then by equilibrating it with the brine solution in order to saturate the

ME phase with water. The two phases of equal volume were brought into contact with each other and then stirred at desired rates. The hydrodynamic condition of each phase is slightly different from each other. The concentrations of Trp in both phases were determined by a spectrophotometer (280nm) in situ. The concentrations of I^- and K^+ in the solutions sampled were measured by a spectroscopic method (Shimadzu UV-200) at 226 nm and an inductively coupled argon plasma emission spectrophotometer (Jourrel Ash, ICAP 500), respectively. The water extracted in the ME phase was analysed by the Karl-Fisher method.

We also measured the time-evolution of the Trp-concentration profiles in a static diffusion cell in the AOT-CCl_4 microemulsion/NaCl aqueous solution system with the help of the position-scanning spectrophotometer[9]. The geometry of the diffusion cell is 4 cm in height and 1 mm in thickness.

We measured the concentration profile of AOT in AOT-ME droplets/n-heptane solution with the aid of the position-scanning spectrophotometer at 250 nm, using the flow junction cell. The diffusion coefficient for the ME droplets in n-heptane was evaluated from the concentration profile for AOT.

3. RESULT AND DISCUSSION

Rate Processes concerned with Extraction of Trp by AOT microemulsion

The extraction of solute in the ME/aqueous solution system is generally a complicated process, because the reorganization of the droplets proceeds upon solute extraction. We first elucidate which rate processes play a key role in the solute transfer.

The time evolution of the concentration distribution of Trp in the static diffusion cell is shown in Fig.2. In this experiment, the Trp in the organic phase, which was prepared by bringing the AOT-ME in CCl_4 into contact with 1M brine solution containing Trp, was transferred to the 1M NaCl aqueous solution. The abscissa in Fig.2 represents the distances x from the interface normalized by $\sqrt{t}$ following Boltzmann's method[10]. These concentration distributions away from the interface are uniquely determined by the

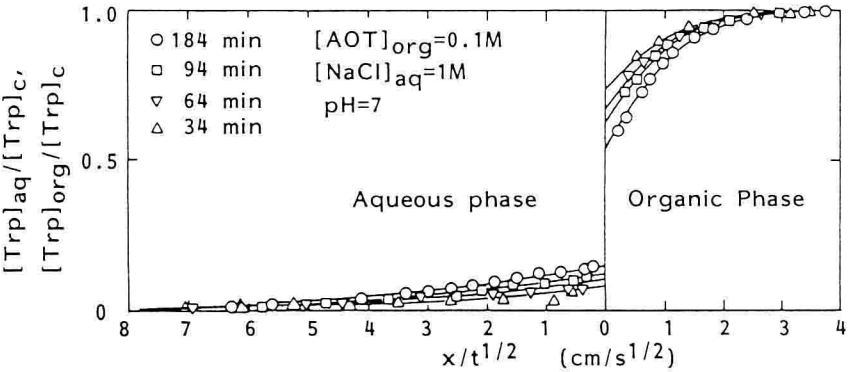

Fig. 2 Time evolution of the concentration distribution of tryptophan during extraction in the static diffusion cell. $[Trp]_o$:feed concentration.