'LASER APPLICATIONS TO ANALYTICAL ULTRACENTRIFUGATION'

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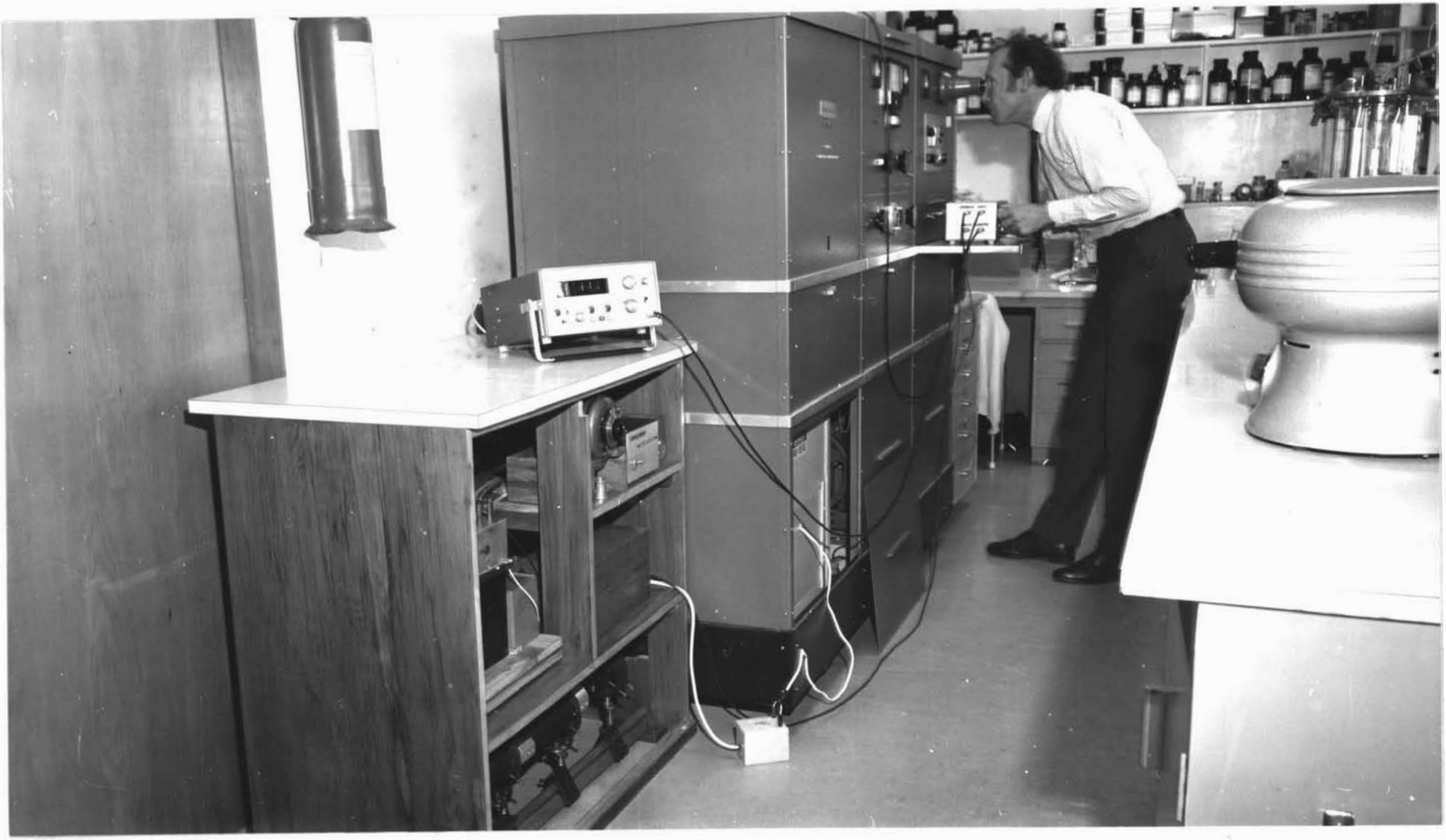

FRONTISPIECE: A GENERAL VIEW OF THE ANALYTICAL ULTRACENTRIFUGE WITH THE LASER AND ITS ASSOCIATED MODULATION EQUIPMENT.

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TABLE OF CONTENTS

CHAPTER Page

LIST OF ILLUSTRATIONS

FIGURE PAGE

FIGURE PAGE

PREFACE.

PREFACE

The work of Svedberg[1] and his collaborators in the early 1920's heralded the use of centrifugal fields for the study of macromolecular systems. Following this work the developments in both theoretical and experimental aspects have been dramatic, so much so that the majority of current researchers take the use of the analytical ultracentrifuge for granted as a basic tool for the determination of molecular weights of macromolecules. The latter is justified in view of the theoretical and experimental evidence to date, and reviews citing original references and covering the state of the art from its inception to the present day are available.[2-8]

Physically, the problem associated with a study involving the analytical ultracentrifuge is to obtain measurements that will enable a specific solution of the Lamm[9] equation to be obtained. For an ideal two-component system in which the partial specific volumes $\bar{v}_o$ (solvent) and $\bar{v}_1$ (solute) are constant the Lamm equation may be written[5]

$$\frac{\partial c_1}{\partial t} = \frac{1}{x}\frac{\partial}{\partial x}\left[xD\left(\frac{\partial c_1}{\partial x}\right) - s\,\omega^2 x^2 c_1\right] \qquad \text{(P1)}$$

where c_1 represents the concentration of solute (component 1) with respect to solvent (component o), at a radial position x in a centrifuge cell. D and s represent the diffusion and sedimentation coefficients respectively for component 1, and ω is the angular frequency of the rotor. Lamm's[9] original derivation of this second order partial differential equation was non-rigorous, but the work of Goldberg[10] using the concepts of irreversible thermodynamics has established clearly the conditions under which the equation applies. Similar equations exist for more complex molecular systems, but exact analytical solutions are difficult since the coefficients D and s are not constant but functions of the variables. Thus workers with the ultracentrifuge are confronted with the all too familiar problem in science of attempting the solution of partial differential equations with non-constant coefficients. Naturally, attempts have been made to derive analytical solutions to the Lamm equations under specified conditions, and the monograph by Fujita[5] contains a useful summary of work in this area.

Of particular interest both theoretically and in the context of this present study is the concentration distribution in a centrifuge cell at sedimentation-diffusion equilibrium. This is a thermodynamic equilibrium state and is defined as the state in which the total potential of any component in a solution being centrifuged is constant, and where the temperature is uniform throughout the centrifuge cell. Experimentally, this state is achieved under isothermal conditions when the concentration distribution no longer changes with time within the accuracy of the experiment. Obviously, this allows the time dependent terms in the solution of the relevant equations to be equated to zero. The latter condition greatly simplifies the analysis and the solutions then obtained are the most rigorous theoretically.

It is shown by Creeth and Pain[6] and elsewhere[3-5] that the ideal two component system considered in equation (P1) is distributed at sedimentation equilibrium according to the expression

$$M(1-\bar{v}\rho)\omega^2 x = \frac{\partial\mu}{\partial c_1}\left(\frac{\partial c_1}{\partial x}\right) \qquad \text{(P2)}$$

where M is the molecular weight of the solute (component 1) and μ and $\bar{v}$ the chemical potential and partial specific volume respectively of the solute at concentration c_1 (measured on a scale mass/ unit volume). The standard relationships between μ and c lead to the fundamental expression that is used in all experiments at sedimentation equilibrium

i.e. $$\frac{1}{xc_1}\,\frac{dc_1}{dx} = \frac{M(1-\bar{v}\rho)\omega^2}{RT(1+\underset{k}{c}\,d\ell n y/d\ell n c_1)} = \frac{Mapp(1-\bar{v}\rho)\omega^2}{RT} \qquad \text{(P3)}$$

Here R is the universal gas constant, T is the absolute temperature, y is the activity coefficient on the c scale, and M_{app} is the apparent molecular weight of the solute. The latter may be related to the true molecular weight by use of the thermodynamic non-ideality

$$\ell n\, y = BMc + \dots\dots\dots \qquad (P4)$$

in which B is an empirical coefficient and where higher terms in the series can frequently be neglected. Using equations (P3) and (P4) we note that

$$M_{app} = \frac{M}{1 + BMc} \qquad (P5)$$

The importance of equations (P3) and (P5) is that the molecular weight is determinable from measurements of $c(x)$ at equilibrium.

The ultracentrifuge operator has the choice of three separate optical techniques in the modern instrument for the evaluation of the concentration distribution in the centrifuge cell. Firstly, there is the Schlieren system that relies on the refractometric properties of the solution and gives a record of refractive index gradient versus radial position for a cell. Here it is assumed that the incremental solution refractive index is proportional to the solute concentration. Secondly, there is the Rayleigh interference system which again relies on the refractometric properties of the solution but gives a photographic record of difference in refractive index between the solution and solvent (solute concentration) versus the radial position in the cell. The interference and Schlieren systems commonly share the same optical components in the ultracentrifuge. Finally, there is the ultraviolet absorption system that utilises the specific absorption of radiation at 254 nm by macromolecules such as nucleic acids and proteins as the sensing phenomena for determining the solute concentration.

The comparative merits of the three systems have been evaluated by Schachman[4,11,23] but the interference system is the primary concern of the present study. It has been demonstrated adequately[12] that the interference system is capable of giving simple and accurate data that is suited particularly for sedimentation diffusion equilibrium work. To a physicist this is not surprising, since interferometry has provided the means of making some of the most precise measurements available to man. However, with the advent of the first operational gas laser by Javan et al[13] in 1961, the whole field of interferometry has undergone a revolution. This thesis is the culmination of a study initiated by the author in late 1967 into the possible applications of a gas laser to interference studies in the analytical ultracentrifuge.

Dr. J. W. Lyttleton of the Applied Biochemistry Division, D.S.I.R., Palmerston North, was kind enough to allow the use of his Beckman Model 'E' analytical ultracentrifuge to be used for this work, and also agreed to act as one of the supervisors for the author when the work was deemed appropriate as a staff Ph.D. project by Massey University. Obviously, the ultracentrifuge was in routine laboratory use, and any modifications to the instrument had to be compatible with this usage.

In chapter 1, a theoretical study of the Rayleigh interference optics in the analytical ultracentrifuge is undertaken. Extensive use is made of the Fourier theory of optics, and the basis upon which the refractometric measurements are made in the instrument is firmly established.

A discussion of coherence theory is included in chapter 2. The theoretical findings are applied to the conventional mercury source and a helium-neon gas laser employed in the work, allowing a definitive comparison between the two light sources to be made.

Chapter 3 includes a description of the actual instrumentation that was designed and constructed by the author, for use in the ultracentrifuge. A discussion of the optical alignment procedure is given and experimental results obtained both with the modulated laser and mercury sources are compared and discussed.

The design and construction of a new type of interference cell is the concern of chapter 4, together with the theory upon which the measurements using this cell are based. Ancilliary equipment necessary for the use of the new cell in the ultracentrifuge is described, together with a critical analysis of the results so far obtained.

Chapter 5 is a detailed theoretical analysis of the effects of the refractive index gradients on the experimental data derived from the interference system in the analytical ultracentrifuge. The study indicates that the effects are small for the assumptions made, but the analysis does provide correction procedures based on the raw experimental data that can be applied in practice.

Finally, in chapter 6 the effects of cell distortion in the centrifugal field are evaluated mathematically. Its importance in the context of this thesis is that sedimentation equilibrium studies frequently require a precise knowledge of the radial positions of the meniscus and base of the solution column. The theoretical and experimental work shows that the laser gives sharper definition of the menisci than does the mercury source, and this increased precision gained by the laser could well be lost if the menisci positions vary owing to extraneous unknown factors. The work in this chapter has been published[14] by the author in conjunction with Professor N.F. Barber who is acting as a second supervisor for this project. A reprint of the publication is included in an appendix to this work.