



SPRINGER LABORATORY

W. Mächtle · L. Börger

Analytical Ultracentrifugation

of Polymers and Nanoparticles



Springer

TB383
M151

Walter Mächtle • Lars Börger

Analytical Ultracentrifugation of Polymers and Nanoparticles

125 Figures and 5 Tables



E200602243

 Springer

Dr. Walter Mächtle
BASF AG
Kunststofflaboratorium
67056 Ludwigshafen
Germany
e-mail: walter.maechtle@t-online.de

Dr. Lars Börger
BASF AG
Abt. Polymerphysik
GKP - G201
67056 Ludwigshafen
Germany
e-mail: lars.boerger@basf-ag.de

Library of Congress Control Number: 2005933893

ISBN-10 3-540-23432-2 **Springer Berlin Heidelberg New York**
ISBN-13 978-3-540-23432-6 **Springer Berlin Heidelberg New York**

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilm or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permissions for use must always be obtained from Springer. Violations are liable for prosecution under the German Copyright Law.

Springer is a part of Springer Science+Business Media
springer.com

© Springer-Verlag Berlin Heidelberg 2006
Printed in Germany

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Cover design: *design&production*, Heidelberg, Germany
Typesetting and production: LE-TEX Jelonek, Schmidt & Vöckler GbR, Leipzig, Germany

2/3141 YL 5 4 3 2 1 0 - Printed on acid-free paper

SPRINGER LABORATORY

Springer Laboratory Manuals in Polymer Science

Pasch, Trathnigg: HPLC of Polymers

ISBN: 3-540-61689-6 (hardcover)

ISBN: 3-540-65551-4 (softcover)

Mori, Barth: Size Exclusion Chromatography

ISBN: 3-540-65635-9

Pasch, Schrepp: MALDI-TOF Mass Spectrometry of Synthetic Polymers

ISBN: 3-540-44259-6

Kulicke, Clasen: Viscosimetry of Polymers and Polyelectrolytes

ISBN: 3-540-40760-X

Hatada, Kitayama: NMR Spectroscopy of Polymers

ISBN: 3-540-40220-9

Brummer, R.: Rheology Essentials of Cosmetics and Food Emulsions

ISBN: 3-540-25553-2

Mächtle, W., Börger, L.: Analytical Ultracentrifugation of Polymers and Nanoparticles

ISBN: 3-540-23432-2



Springer Laboratory Manuals in Polymer Science

Editors

Prof. Howard G. Barth
DuPont Company
P.O. box 80228
Wilmington, DE 19880-0228
USA
e-mail: Howard.G.Barth@usa.dupont.com

Priv.-Doz. Dr. Harald Pasch
Deutsches Kunststoff-Institut
Abt. Analytik
Schloßgartenstr. 6
64289 Darmstadt
Germany
e-mail: hpasch@dkl.tu-darmstadt.de

Editorial Board

PD Dr. Ingo Alig
Deutsches Kunststoff-Institut
Abt. Physik
Schloßgartenstr. 6
64289 Darmstadt
Germany
email: ialig@dkl.tu-darmstadt.de

Prof. Josef Janca
Université de La Rochelle
Pole Sciences et Technologie
Avenue Michel Crépeau
17042 La Rochelle Cedex 01
France
email: jjanca@univ-lr.fr

Prof. W.-M. Kulicke
Inst. f. Technische u. Makromol. Chemie
Universität Hamburg
Bundesstr. 45
20146 Hamburg
Germany
email: kulicke@chemie.uni-hamburg.de

Prof. H. W. Siesler
Physikalische Chemie
Universität Essen
Schützenbahn 70
45117 Essen
Germany
email: hw.siesler@uni-essen.de

Preface

In 1924, the Swedish chemist Theodor Svedberg invented the analytical ultracentrifuge (AUC) to characterize nanoparticles. He used it, for instance, to measure the particle size distribution of very small ($d = 2\text{ nm}$) gold colloid particles. Already in 1926, the Nobel Prize in Chemistry was awarded to Svedberg for his work. Later, he expanded his investigations to biochemistry, and determined the first molar masses of biopolymers, especially proteins, via AUC. In the following years, the AUC became the most important instrument for the characterization of biopolymers, culminating in the famous density gradient experiment of Meselson and Stahl in 1957, which proved that the DNA replication mechanism, proposed by the Nobel Prize winners Watson and Crick, was correct. Later, with the appearance of other new methods, the AUC lost this prominent position, also because there was no further instrumental development. Around 1980, only a few laboratories were still dealing with AUC. This changed with (1) the launch of a redesigned, fully computerized AUC, the Optima XL-A in 1991, and especially with the XL-A/I in 1997 by Beckman Instruments, Inc., Palo Alto, USA, and (2) some important instrumental developments in specialized AUC laboratories, in particular in the new field of synthetic polymers and colloids. A renaissance of AUC was starting.

In general, analytical ultracentrifugation is a powerful method for the characterization of polymers, biopolymers, polyelectrolytes, nanoparticles, dispersions, emulsions, and other colloid systems. The method is suited to determine the molar mass, particle size, particle density, and interaction parameters such as virial coefficients and association constants. Because AUC is also a fractionation method, the determination of the molar mass distribution, particle size distribution, and particle density distribution is possible as well. A special AUC technique, the density gradient method, allows us to fractionate heterogeneous samples according to their chemical nature, which means that the chemical heterogeneity of a sample can be detected.

The latest textbooks on AUC were published in 1992 and 1994. They deal mainly with biopolymers, theoretical considerations, and do not describe the new possibilities of the Optima XL-A/I. It is the intention of our book to fill this gap, and to demonstrate by means of carefully selected application examples that, especially in the field of synthetic polymers and organic and inorganic nanoparticles, the AUC is an excellent characterization tool for such species. Our book is written for beginners as well as for experienced chemists, physicists and material scientists. It allows the reader to become familiar with the actual status of instrumentation,

which means the latest state of the art and the different AUC techniques. All these techniques are described in a simple manner and by means of examples. Detailed instructions for conducting experiments and for their evaluation are given, including explanations of the theoretical background. In this laboratory manual, emphasis is laid more on practical aspects, rather than on details of centrifugation theory. The book is subdivided into seven chapters, concerning the history and basic theory, instrumentation, sedimentation velocity experiments, density gradient experiments, sedimentation equilibrium experiments, application examples, and possible future developments. In particular, the detailed application chapter demonstrates the versatility and power of AUC by means of many interesting and important practical industrial examples. Most of these examples stem from an industrial AUC research laboratory of a leading chemical company, where both authors have gained many years of experience in AUC instrumentation, and AUC characterization of complex polymer and nanoparticle systems.

The authors wish to express their gratitude and appreciation to all colleagues who provided experimental details and data, in particular M.D. Lechner (Os-nabrück), T.M. Laue (Durham, NH, USA) and H. Cölfen (Potsdam). The support of many colleagues from the Polymer Research Laboratory of BASF Aktiengesellschaft, Ludwigshafen, Germany, in providing devices, samples and advice, is gratefully acknowledged, above all U. Klodwig. We also would like to thank our coworkers M. Page, U. Gonnermann and M. Stadler for their great commitment in preparing the manuscript and the figures, and H. Roth, M. Kaiser, K. Vilsmeier and K.H. Zimmermann, too, who carried out nearly all measurements presented in this book in an accurate manner.

Carefully reviewing a book means lots of work but not much appreciation for the reviewers. Therefore, the authors wish to express their deep gratitude to Helmut Cölfen (Potsdam) and Karl-Clemens Peters (Bad Dürkheim) for taking on this difficult job.

Furthermore, the authors would like to thank the management of BASF Aktiengesellschaft for supporting the publication of this book.

Last but not least, we thank our families, in particular our spouses, for their indulgence and understanding during the long time of preparing the manuscript of this book, when we often did not adequately take part in family life.

Ludwigshafen, November 2005

*Walter Mächtle
Lars Börger*

List of Symbols and Abbreviations

β	Density gradient constant
ϵ	Specific decadic absorption coefficient
η_s	Viscosity of solvent
ϕ	Angle of sector-shaped AUC cell
λ	Wavelength
φ	Volume fraction
ρ_p	Particle or polymer density
ρ_s	Solvent density
σ	Standard deviation
τ	Turbidity
\bar{v}	Partial specific volume
ω	Angular velocity
a	Optical path length
a_{centr}	Gravitational field
A	Absorption
A_2	Second virial coefficient
AUC	Analytical ultracentrifuge
c	Sample concentration
c_0	Initial sample concentration
d_p	Particle or polymer diameter
D	Diffusion coefficient
DLS	Dynamic light scattering
EM	Electron microscopy
f	Frictional coefficient
F_b	Buoyant force
F_f	Frictional force
F_s	Gravitational force
FFF	Field flow fractionation
g	Acceleration due to gravity
$g(s)$	Differential sedimentation coefficient distribution
$G(s)$	Integral sedimentation coefficient distribution
I	Intensity of light
J	Interference fringe displacement
k_s	Concentration dependence coefficient
K	Kelvin

m	Mass of sample
M	Molar mass
M_n	Number-average molar mass
M_w	Weight-average molar mass
M_z	z-average molar mass
MMD	Molar mass distribution
n	Refractive index
n_p	Refractive index of particle or polymer
N	Rotor speed
N_A	Avogadro's number
P	Skewness parameter of the MMD
PSD	Particle size distribution
r	Radial distance from axis of rotation
r_b	Radial distance of the bottom of cell from axis of rotation
r_m	Radial distance of the meniscus from axis of rotation
R	Universal gas constant
RCF	Relative centrifugal field
rpm	Revolutions per minute
s	Sedimentation coefficient
s_0	Sedimentation coefficient at infinite dilution
S	Svedberg unit
SEC	Size exclusion chromatography
t	Experimental time
u	Sedimentation velocity
UV	Ultraviolet
V	Volume
$w(M)$	Differential molar mass distribution
$W(M)$	Integral molar mass distribution
X	Relative squared radial distance, $(r^2 - r_m^2) / (r_b^2 - r_m^2)$

Table of Contents

1	INTRODUCTION	1
1.1	Historic Examples of Ultracentrifugation	2
1.1.1	Investigations on Gold Colloids in 1924	3
1.1.2	Investigations on the Structure of DNA in 1957	5
1.2	Basic Theory of Ultracentrifugation	7
1.2.1	Svedberg's Simplified Theory	8
1.2.2	Derivation of Lamm's Equation	10
1.3	Basic Experiment Types of Ultracentrifugation	12
1.3.1	Sedimentation Velocity Experiment	12
1.3.2	Synthetic Boundary Experiment	13
1.3.3	Sedimentation Equilibrium	13
1.3.4	Density Gradient	14
1.3.5	Approach-to-Equilibrium (or Archibald) Method	15
1.4	Closing Remarks	15
	References	16
2	ANALYTICAL ULTRACENTRIFUGATION, INSTRUMENTATION	17
2.1	Ultracentrifuges	18
2.1.1	The Beckman-Coulter Optima XL-A/I	19
2.1.2	User-Made Centrifuges	21
2.2	Rotors	25
2.3	Measuring Cells	26
2.4	Detectors	29
2.4.1	Absorption Optics	31
2.4.2	Interference Optics	34
2.4.3	Schlieren Optics	37
2.4.4	Other Detectors	38
2.5	Multiplexer	43
2.6	Auxiliary Measurements	43
2.6.1	Measurement of the Solvent Density and the Partial Specific Volume	43
2.6.2	Measurement of the Refractive Index and the Specific Refractive Index Increment	45
	References	46

3	SEDIMENTATION VELOCITY	47
3.1	Introduction	47
3.2	Basic Example of Sedimentation Velocity	47
3.2.1	Determination of s	50
3.2.2	Standard Conditions for s Estimation	51
3.2.3	Radial Dilution and Thickening	52
3.2.4	Concentration Dependence	53
3.3	Advanced Theory of Sedimentation Velocity Runs	54
3.3.1	Johnston–Ogston Effect	54
3.3.2	Self-Sharpening of Boundaries	55
3.3.3	Pressure Dependence	55
3.3.4	Speed Dependence	56
3.3.5	Charge Effects	57
3.3.6	Separation of Sedimentation and Diffusion	58
3.3.7	Test of Homogeneity	61
3.4	Sedimentation Velocity Runs of Macromolecules to Measure Average M and MMD	62
3.4.1	Evaluation of the Average Molar Mass M by Sedimentation Velocity Runs via Scaling Laws	62
3.4.2	Evaluation of Molar Mass Distributions (MMD) by Sedimentation Velocity Runs via Scaling Laws	64
3.5	Sedimentation Velocity Runs on Particles to Measure Average d_p and PSD	69
3.5.1	Particle Size Distribution via AUC Turbidity Detector and Mie Theory	70
3.5.2	Coupling Technique to Measure very Broad PSD	77
3.5.3	H ₂ O–D ₂ O Density Variation Method to Measure Particle Densities via Sedimentation Velocity Runs	80
3.5.4	PSD Measurement of very Small Platinum Clusters Using UV Optics	84
3.6	Synthetic Boundary Experiments	86
3.6.1	Synthetic Boundary Crystallization Ultracentrifugation	93
	References	95
4	DENSITY GRADIENTS	97
4.1	Introduction	98
4.2	Static Density Gradients	101
4.2.1	Theory of Static Density Gradients	102
4.2.2	Gradient Materials	115
4.2.3	Experimental Procedure	117
4.2.4	Examples	118
4.3	Dynamic Density Gradients	120
4.4	Other Types of Density Gradients	125
4.4.1	Percoll Density Gradient	125
	References	129

5	SEDIMENTATION EQUILIBRIUM	131
5.1	Introduction	131
5.2	Experimental Procedure	133
5.3	Data Analysis	138
5.3.1	Basic Equations for Molar Mass Averages – Classical Evaluation of AUC Equilibrium Runs	138
5.3.2	Basic Equations for Molar Mass Distributions – Nonlinear Regression Evaluation (Lechner Method)	141
5.3.3	Application of the Basic Equations – Classical and Nonlinear Regression Evaluation	144
5.3.4	M-STAR, a Special Data Analysis to obtain M_w	149
5.4	Examples	151
5.4.1	Absorption Optics Examples of a Polyelectrolyte and Calibration Polystyrene NBS 706	152
5.4.2	Association Equilibrium Example and AUC Buoyant Density Method to Determine \bar{v}	155
5.4.3	The New Fluorescence Detector of Laue Used for Green Fluorescent Protein	158
5.5	Further AUC Methods to Measure Molar Masses	159
	References	162
6	PRACTICAL EXAMPLES OF COMBINATION OF METHODS	163
6.1	Combination of Different AUC Methods	163
6.1.1	Core/Shell Particles	163
6.1.2	Characterization of Microgels and Nanogels	170
6.1.3	Ion Exchange in Carboxylated Latices	179
6.2	Combination of AUC with Other Techniques	189
6.2.1	AUC and SFFF	190
6.2.2	AUC and EM	198
6.3	Literature Examples: AUC and Nanoparticles	212
	References	212
7	RECENT DEVELOPMENTS AND FUTURE OUTLOOK	215
7.1	New AUC Instrumentation and Detectors	216
7.2	New AUC Methods	222
7.3	New AUC Data Analysis	228
	References	233
8	SUBJECT INDEX	235

1 Introduction

Various questions have to be answered before writing a book about analytical ultracentrifugation, a topic that is nowadays not of wide interest, but more a specialized recess. These questions may be summarized as follows: is it worth putting a lot of work into a book on just one, not widely spread technique, and will there be any readers?

To answer the latter question first: as you, the reader, hold this book in your hands, there is obviously at least one interested reader. It is more difficult to answer the first question. If we had not answered this question with a clear yes, you would not be reading this introduction now. The motivation to invest this huge amount of time in our book arises mainly from three aspects:

Firstly, the authors simply do not understand why the powerful technique we are talking about, the analytical ultracentrifuge (AUC), is widely used in the field of biology and adjacent areas, but to our knowledge is not, or almost never applied to colloid and synthetic polymers (especially not in the measurement of particle size distribution in the range 1–5000 nm). One reason might be that simply nobody knows about AUC? Here, our book may be helpful.

Secondly, also the authors believe that there is a need for a book that takes into account the latest developments of the last decade, since the most recent books on AUC were published in 1992 [1] and 1994 [2]. There are some other well-known, older books dealing with analytical ultracentrifugation [3–10], starting with the first in 1940 by Svedberg and Pedersen. However, the focus of nearly all of these books lies on biological systems. In contrast, we would like to remind scientists of a technique they may know but may have forgotten, and put the focus of this book on how powerful the AUC can be, applied on synthetic polymers and colloids.

Thirdly, we would like to emphasize that in times where mega-trends such as nanotechnology, soft materials and biotechnology are en vogue, the need for accompanying analytical methods is increasing. By the end of this book, the reader should be convinced, if necessary, that AUC can be a helpful tool in these modern scientific fields.

The power of AUC is often underestimated. The reasons for this are not easy to address. Looking back to the very beginning of this technique, one is automatically confronted with the work of the Nobel Prize winner Theodor Svedberg [11, 12]. He invented the first practical, usable *analytical* ultracentrifuge (that is, an ultracentrifuge with an optical detecting system) in 1924, together with his coworker Rinde, with the motivation to learn about colloidal systems, and especially about

the size and size distribution of colloidal systems [13]; the first centrifuge that was equipped with an optical detecting system was built by Svedberg and Nichols in 1923 [14]. Hence, writing a book focusing more on the use of AUC in colloidal science takes us back to the origin of ultracentrifugation. In the years following his invention, Svedberg turned his attention more from colloidal toward biological questions, such as the determination of the molar mass of proteins [15]. The focus of the analytical ultracentrifugation community stayed on these biological and biochemical questions during the next decades. Still today, most publications containing AUC investigations deal with this field of science.

The AUC was the first instrument delivering reliable values for molar masses of biopolymers, and therefore had its outstanding place in biochemistry. To a certain degree, AUC lost this place with the upcoming of new methods such as the laser technique (allowing light scattering measurements), the development of electron microscopes (EM), the polyacrylamide gel electrophoresis (PAGE), and the size exclusion chromatography (SEC). SEC is today the dominating method to measure average molar masses M and molar mass distributions (MMD) of synthetic polymers. All this went along with a lack of improved instrumentation after the most successful AUC apparatus, the Model E by Beckman, became obsolete. Also several other companies that built ultracentrifuges turned their interest away from the AUC (see Chap. 2). The field of analytical ultracentrifugation was “starving” around 1980, with just a very few laboratories still dealing with the technique. This changed with the launch of a redesigned AUC, the Optima XL-A by Beckman in 1991, and nowadays there is a trend reversal.

Certainly, at present there is a demand for a *fractionating* measurement tool such as the AUC, which provides physicochemical information on a wide choice of topics. And this demand may increase due to the recent scientific mega-trends described above: nanotechnology and biotechnology.

1.1 Historic Examples of Ultracentrifugation

With respect to these mega-trends, two highlights from scientific history may be given in this introduction to illustrate the importance of AUC (and to serve as an appetizer to read the rest of the book that contains a lot of modern examples):

- (i) Investigations on gold colloids in 1924, and
- (ii) Investigations on the structure of DNA in 1957.

These two historical examples have also been selected because they illustrate two major principles of centrifugation: *sedimentation* velocity runs, and (density) *equilibrium* runs. Both examples reflect the variety of fields covered by analytical ultracentrifugation: while sedimentation velocity runs on colloids, first done by Svedberg, are representative for the field of inorganic nanoparticles and colloids in general (investigations on synthetic polymers may be implied here as well), the Meselson–Stahl density gradient experiment stands for biochemical or pure biological questions.

1.1.1 Investigations on Gold Colloids in 1924

In 1926, Svedberg won the Nobel Prize in chemistry for “his work on disperse systems”, just one year after the German chemist Zsigmondy received the prize “for his demonstration of the heterogeneous nature of colloid solutions and for the methods he used, which have since become fundamental in modern colloid chemistry”. Zsigmondy invented the *ultramicroscope*, and used it to prove the particle nature of colloids with particle diameters in the nanometer range. Later in his Nobel Prize lecture [16], Svedberg pointed out that in his opinion the ultramicroscope of Zsigmondy had a big disadvantage: “The *distribution* of the particle size cannot be determined”. And, in fact, Svedberg invented the analytical ultracentrifuge with the intention to determine particle size distributions of colloids by fractionation. Later, its value for the analysis of polymeric systems, both biopolymers and synthetic polymers, was discovered. In fact, Svedberg chose the name *ultracentrifuge* in analogy to Zsigmondy’s *ultramicroscope*.

The heart of any AUC is a rotor that contains parts called analytical cells (see Chap. 2). These cells house the samples to be investigated. By centrifuging the rotor at high speed, a centrifugal field is generated, and the reaction of the sample on the field can be studied with analytical detectors. As we will see below, one of the possible reactions of the sample to the centrifugal field is the sedimentation of the dispersed or dissolved particles with a characteristic velocity. The underlying principle that allows us to learn about particle size distributions from the ultracentrifuge is that the sedimentation velocity is in general well correlated to particle size, in that the larger a particle, the faster it sediments. This is a major subject of this book, and it will be discussed in detail below. Figure 1.1 shows the original data of the sedimentation experiment that Rinde and Svedberg performed

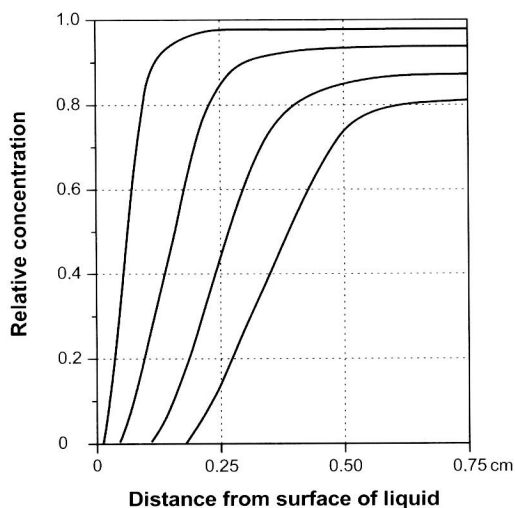


Fig. 1.1. Radial concentration distribution of a highly disperse gold colloid in an AUC cell, recorded 5, 10, 15, and 20 min after beginning of centrifugation (centrifugal field 28 800 times gravity; original work of The Svedberg in 1924; reprinted with permission from [13])

on a gold colloid sample in 1924. The presented radial concentration profile of the sedimenting colloidal gold particles inside the measuring cell, recorded every 5 min, was measured in the first AUC apparatus by Rinde and Svedberg (the details of these results will be the subject of Chaps. 2 and 3).

Each of these radial concentration profiles of gold colloids in the cell is a measure for the sedimentation velocity, or more precisely, for the sedimentation velocity distribution of the differently sized gold particles. In contrast to microscopic methods, not only a few but *all* particles of the sample contribute to the measuring signal in the example shown. Thus, a high statistical relevance is guaranteed. Each of the different radial concentration profiles in Fig. 1.1, recorded at different, well-defined times, can be converted by means of Stokes' law into a particle size distribution, abbreviated *PSD* (for details, see Chap. 3). The resulting (differential) *PSD* is given in Fig. 1.2. Within the errors of measurement, all these radial concentration profiles yield the same *PSD*.

The historical unit $\mu\mu$ on the axis of abscissas in Fig. 1.2 stands for millimicron (also $m\mu$), and that is what we call today a nanometer (nm). Hence, the maximum of the *PSD* given in Fig. 1.2 is close to 1.5 nm, and the whole diameter range lies between 0.7 and 2.2 nm. By means of these, and comparable AUC measurements, Rinde and Svedberg were able to demonstrate that the gold colloids observed by Zsigmondy in his "classic" work were in fact not as narrowly distributed as thought before. Obviously, Svedberg and coworkers were able to characterize colloids that would be named *nanoparticles* in today's terminology. In Chap. 3, we will demonstrate that the advantages of a nanoparticle analysis done by applying AUC, first performed by Svedberg, are still true today.

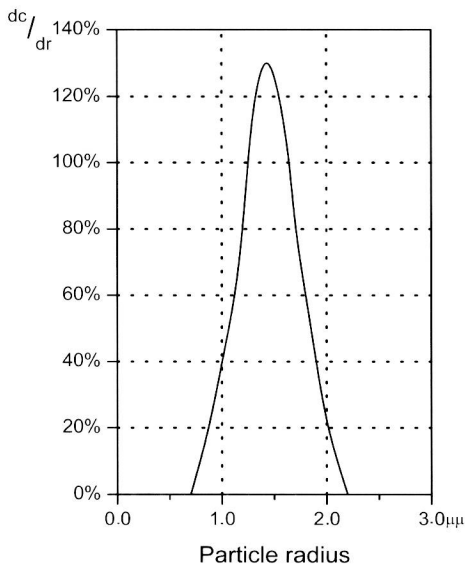


Fig. 1.2. Differential particle size distribution of a highly disperse gold colloid, calculated from Fig. 1.1, taken from the original Svedberg paper (reprinted with permission from [13])