THERMOMICROSCOPY IN THE ANALYSIS OF PHARMACEUTICALS

BY

MARIA KUHNERT - BRANDSTÄTTER
DR. RER. NAT., MAG. PHARM.
PROFESSOR OF PHARMACOGNOSY
UNIVERSITY OF INNSBRUCK

PERGAMON PRESS
Oxford · New York · Toronto
Sydney · Braunschweig

Pergamon Press Ltd., Headington Hill Hall, Oxford
Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523
Pergamon of Canada Ltd., 207 Queen's Quay West, Toronto 1
Pergamon Press (Aust.) Pty. Ltd., 19a Boundary Street,
Rushcutters Bay, N.S.W. 2011, Australia
Vieweg & Sohn GmbH, Burgplatz 1, Braunschweig

First edition 1971
Library of Congress Catalog Card No. 70–127061

Printed in Great Britain by W. & G. Baird Ltd., Belfast

08 006990 8

INTERNATIONAL SERIES OF MONOGRAPHS IN
ANALYTICAL CHEMISTRY
General Editors: R. BELCHER AND M. FREISER

VOLUME 45

THERMOMICROSCOPY IN THE ANALYSIS OF PHARMACEUTICALS

OTHER TITLES IN THE SERIES IN ANALYTICAL CHEMISTRY

Vol. 1. WEISZ—Microanalysis by the Ring Oven Technique.
Vol. 2. CROUTHAMEL—Applied Gamma-ray Spectrometry.
Vol. 3. VICKERY—The Analytical Chemistry of the Rare Earths.
Vol. 4. HEADRIDGE—Photometric Titrations.
Vol. 5. BUSEV—The Analytical Chemistry of Indium.
Vol. 6. ELWELL AND GIDLEY—Atomic absorption spectrophotometry.
Vol. 7. ERDEY—Gravimetric Analysis Parts I-III.
Vol. 8. CRITCHFIELD—Organic Functional Group Analysis.
Vol. 9. MOSES—Analytical Chemistry of the Actinide Elements.
Vol. 10. RYABCHIKOV and GOL'BRAIKH—The Analytical Chemistry of Thorium.
Vol. 11. CALI—Trace Analysis for Semiconductor Materials.
Vol. 12. ZUMAN—Organic Polarographic Analysis.
Vol. 13. RECHNITZ—Controlled-potential Analysis.
Vol. 14. MILNER—Analysis of Petroleum for Trace Elements.
Vol. 15. ALIMARIN and PETRIKOVA—Inorganic Ultramicroanalysis.
Vol. 16. MOSHIER—Analytical Chemistry of Niobium and Tantalum.
Vol. 17. JEFFERY and KIPPING—Gas Analysis by Gas Chromatography.
Vol. 18. NIELSEN—Kinetics of Precipitation.
Vol. 19. CALEY—Analysis of Ancient Metals.
Vol. 20. MOSES—Nuclear Techniques in Analytical Chemistry.
Vol. 21. PUNGOR—Oscillometry and Conductometry.
Vol. 22. J. ZYKA—Newer Redox Titrants.
Vol. 23. MOSHIER and SIEVERS—Gas Chromatography of Metal Chelates.
Vol. 24. BEAMISH—The Analytical Chemistry of the Noble Metals.
Vol. 25. YATSIMIRSKII—Kinetic Methods of Analysis.
Vol. 26. SZABADVÁRY—History of Analytical Chemistry.
Vol. 27. YOUNG—The Analytical Chemistry of Cobalt.
Vol. 28. LEWIS, OTT and SINE—The Analysis of Nickel.
Vol. 29. BRAUN and TÖLGYESSY—Radiometric Titrations.
Vol. 30. RÙŽIČKA and STARÝ—Substoichiometry in Radiochemical Analysis.
Vol. 31. CROMPTON—Analysis of Organoaluminium and Organozine Compounds.
Vol. 32. SCHILT—Analytical Applications of 1,10—Phananthroline and Related Compounds.
Vol. 33. BARK and BARK—Thermometric Titrimetry.
Vol. 34. GUILBAULT—Enzymatic Methods of Analysis.
Vol. 35. WAINERDI—Analytical Chemistry in Space.
Vol. 36. JEFFERY—Chemical Methods of Rock Analysis.
Vol. 37. WEISZ—Microanalysis by the Ring Oven Technique (2nd Edition—large and revised.)
Vol. 38. RIEMAN and WALTON—Ion Exchange in Analytical Chemistry.
Vol. 39. GORSUCH—The Destruction of Organic Matter.
Vol. 40. MUKHERJI—Analytical Chemistry of Zirconium and Hafnium.
Vol. 41. ADAMS and DAMS—Applied Gamma Ray Spectrometry (2nd Edition).
Vol. 42. BECKEY—Ionization Mass Spectrometry.
Vol. 43. LEWIS and OTT—Analytical Chemistry of Nickel.
Vol. 44. SILVERMAN—Determination of Impurities in Nuclear Grade Sodium Metal.

Dedicated in gratitude to
ADELHEID KOFLER
the widow of my highly esteemed teacher
LUDWIG KOFLER

PREFACE

THE hot-stage microscope has found increasingly widespread use in the course of the last two decades. This is largely due to the work of Ludwig Kofler, who, together with his wife Adelheid, developed methods which have raised thermomicroscopy to an independent branch of the investigation of organic substances. Ludwig Kofler regarded it as his life's task to gain for the microscope, which, before his lifetime, had been treated rather neglectfully in chemistry, the position that it merited. A further aim of his efforts was to introduce the hot-stage microscope into pharmacy for testing organic compounds with respect to identity and purity.

In this field the starting position appeared particularly favourable, since in his course of studies the pharmacist is made substantially better acquainted with the microscope through botanical and pharmacognostic practical work than the chemist.

As a pupil and former colleague of Ludwig Kofler, after his death I regarded it as an obligation bequeathed to me to pursue his work relating to pharmacy. I was therefore very glad to comply with the request to write a book on thermomicroscopy especially directed to the requirements of pharmacy. This limitation enabled me to renounce a comprehensive account of the methods of thermomicroscopy and to rely on the experience gathered in a practical education lasting more than twenty years.

Although the individual thermomicroscopic determinations are easy to learn, it is impossible to do without this learning. If a person can use the microscope, this does not mean that he also understands thermomicroscopy. The microscopic performance of processes and the interpretation will be practised and learnt in exactly the same way as is obvious with other methods. We devote two or more terms to an introduction to qualitative chemical analysis, but where this involves the use of thermomicroscopic methods many believe that they need only to sit at the microscope and turn up the heating. This may suffice simply to determine the melting point, but never to exhaust all the possibilities that the use of the microscope offers. Thus, Walter McCrone (Chicago) has done great service in innumerable courses on the introduction of thermomicroscopy in the USA. Robert Fischer (Graz), a pupil of Kofler, has also been advocating thermomicroscopy in education and research for decades.

In the present book also, examples are given in individual cases which are intended to serve as an introduction to the method. The characteristics necessary for a meaningful use of thermomicroscopy for the identification of pharmaceuticals and a description of the processes observed are summarized in the "Identification Table for the Hot Stage". The characteristics for the Kofler hot bench are also arranged in tables. A description of the microscope and its operation has deliberately been renounced, since there are adequate handbooks or monographs in which this subject is treated in detail for anyone not accustomed to its use.

I heartily thank Frau Dr. Adelheid Kofler (Innsbruck) for her excellent co-operation in the investigation of the bulk of the pharmaceuticals contained in the identification tables.

I am also greatly obliged to Frl. G. Obruschka, med.techn. Ass. (Innsbruck), for providing the UV data and the hot bench data and also for her valuable assistance in reading the proofs. Finally, my thanks are due to the editors and publishers for the careful translation and for making it possible to reproduce numerous photographs in colour.

CONTENTS

CHAPTER 1

INTRODUCTION

BECAUSE of the extraordinary development of synthetic organic chemistry in recent years, the number of organic compounds used in medicine has risen to an enormous extent. Where previously three or four similar compounds were offered, today there are frequently thirty or forty. This means a substantially larger expenditure on methods and apparatus for the analyst. The new pharmacopoeias are therefore including to an increasing extent methods which go beyond the possibilities of a pharmacy. Nevertheless, the pharmacist is obliged to identify reliably drugs that are not in a form ready for use in order to exclude mistakes. The pharmacopoeia commissions of the individual countries face the difficult task of, on the one hand, giving all the new methods of analysis with their expensive apparatus for the testing of drugs that are to be mandatory on the drug manufacturers and, on the other hand, taking into account the fact that the purchase of this apparatus cannot be demanded of the individual pharmacist.

L. Kofler's thermomicroscopic method for identifying organic substances provides the possibility of closing this gap. Of the substances treated in this book, 65% can be identified on the hot-stage microscope without any other test. The remainder can be identified with the aid of chemical reactions or by means of the UV spectrophotometer even if no authentic sample for determining the mixed melting point is available.

The basis of this method is the microscopic determination of the melting point. It combines the advantage of a minimum consumption of the substance with the possibility of observing without difficulty the individual crystals under about 100-fold magnification during the heating process and of determining a series of characteristic phenomena that contribute to the identification of a substance. The circumstance that the 24-hour drying in a desiccator prescribed for determining melting points in capillaries is usually superfluous, since the crystals are heated in a loose distribution between the microscope slide and the cover-slip, must not be underestimated. Moreover, the microscopic determination of the melting point simultaneously includes a non-specific test for purity, which is particularly desirable in the study of pharmaceuticals.

In addition to this, on the hot stage the eutectic temperature with a reference substance and the refractive index of the melt can be determined, these being two constants which, in combination with the melting point, form a reliable means of identification. This does not mean only the possibility of identification in relation to the question: Does the package really contain the substance given on the label?, but, by means of a tabular arrangement of substances in order of increasing melting point, it gives an "analytical procedure" for the detection and identification of an unknown drug. This goes beyond the scope of the identification provided in the pharmacopoeias and is, in particular, extremely valuable for the solution of the problems that face the analyst in the field of toxicology.

Furthermore, the hot-stage microscope is suitable for investigating mixtures of substances and for various special investigations, which can be referred to only briefly within the framework of this book.

The Kofler hot bench can also be used in many cases as a very fast and simple identity control. In contrast to the hot stage, this is not a microscopic method, so that it involves the use of a somewhat larger amount of material. Moreover, the determination of the melting point on the Kofler hot bench does not offer the other advantages of the microscopic method. However, it has proved advantageous particularly for testing the identity of labelled substances in the pharmaceutical laboratory.

CHAPTER 2

APPARATUS

OF THE numerous hot stages that have been constructed since the introduction of thermomicroscopy by O. Lehmann,[1] only two will be described here, the Kofler hot stage,[2] since all the investigations that will be reported here were carried out on it, and the Mettler hot stage, since at the present time it forms the last phase of development in the field of thermomicroscopic apparatus.

(a) **Kofler hot stage**

The apparatus consists of a box-shaped metal housing (Fig. 1) which is divided by a horizontal plate into two chambers. In the lower chamber there is the heating coil which is

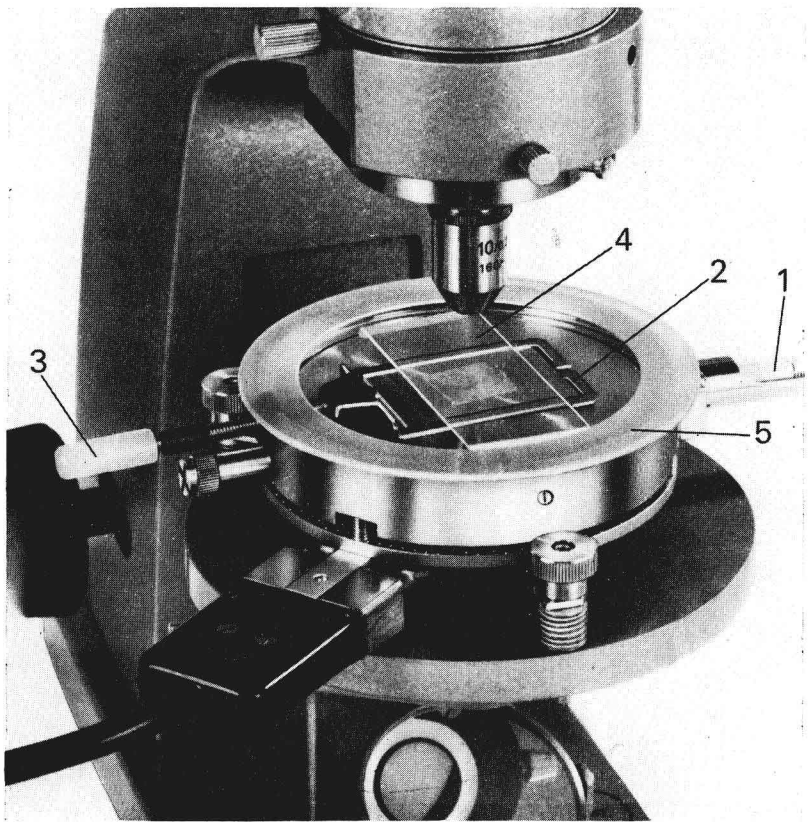

FIG. 1. Kofler hot stage.

fed through an adjustable transformer. In the centre of the heating plate there is a hole for the passage of the light. A lateral hole in the hot chamber takes the thermometer (1) which is in contact with the lower part of the metal plate which separates the hot chamber from the object chamber. The object chamber contains the framework (2) of the sample guide, which enables the sample to be moved while the hot stage is closed. The sample guide (3) can be rotated laterally about its perpendicular axis and can be displaced along the axis by operating the spindle. Transversely over the microscope slide there is a glass bridge (4) which has the object of ensuring a uniform temperature within the sample and also, in particular, in the case of highly volatile materials, of preventing the formation of non-transparent sublimates in the centre of the glass plate. The seal proper is formed by this round glass plate with a ground joint (5). In order to cool the hot stage rapidly between the individual determinations, after the removal of the glass plate, the glass bridge, the sample, and the sample guide framework, an aluminium plate about 2 cm high is placed on the hot stage.

The adjustable transformer (Fig. 2) possesses on its rotating knob (1) a temperature scale (2) by means of which the rate of rise can be adjusted. If, for example, a melting point of about 150°C is expected, the rotating knob of the transformer is turned until the scale mark "150°" is opposite the fixed mark on the housing (3). If the substance is one which melts without volatilization and without decomposition, the lower edge of the broad marking

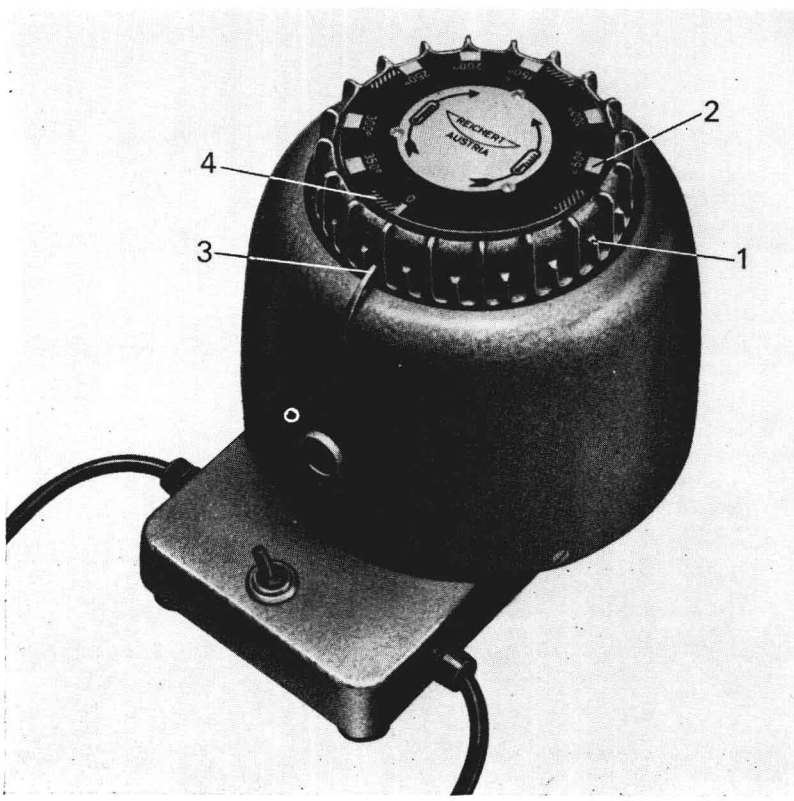

FIG. 2. Adjustable transformer for the Kofler hot stage.

is chosen. Heating takes place rapidly at first and then in the melting region it amounts to about 2°C per minute. If, on the other hand, more rapid heating is desired, the right-hand end of the scale mark is taken. The hatched segments of the scale (4) denote the positions at which a movement of the knob causes no change in the rate of heating. Since the mains voltage undergoes variations and the temperature of the room is not without influence, it is recommended to check the agreement of the actual rate of heating with the temperature figures on the adjustable transformer.

If the hot stage has not been used for a long time, it is desirable before use to heat it without a glass plate in order to dry it. Likewise, it may be recommended to make sure that the apparatus is in order by determining a melting point with a test substance. (Example: phenacetin, mp 134·5°C.) Errors occasionally arise because the thermometer is not pushed into the apparatus far enough to make contact. Another source of error is that a microscope slide which is not accurately standardized under certain circumstances becomes fixed in the framework of the sample guide and therefore does not lie flat on the hot plate. The cushion of air between the hot plate and the microscope slide leads to the situation that the temperature found is too high. The microscope slide must therefore fit loosely in the frame. Its measurements, 26 × 38 mm, correspond to half the usual microscope slides of the international format. The Kofler hot stage is provided with two thermometers which together range from room temperature to 350°C. They permit the recording of corrected figures, since they are specially calibrated for the hot stage. In contrast to older models of the hot stage, however, they are interchangeable, so that replacements are always available.

Basically, the hot stage can be used with any microscope available that has a suitable object stage and an objective with a free working distance of more than 6 mm. However,

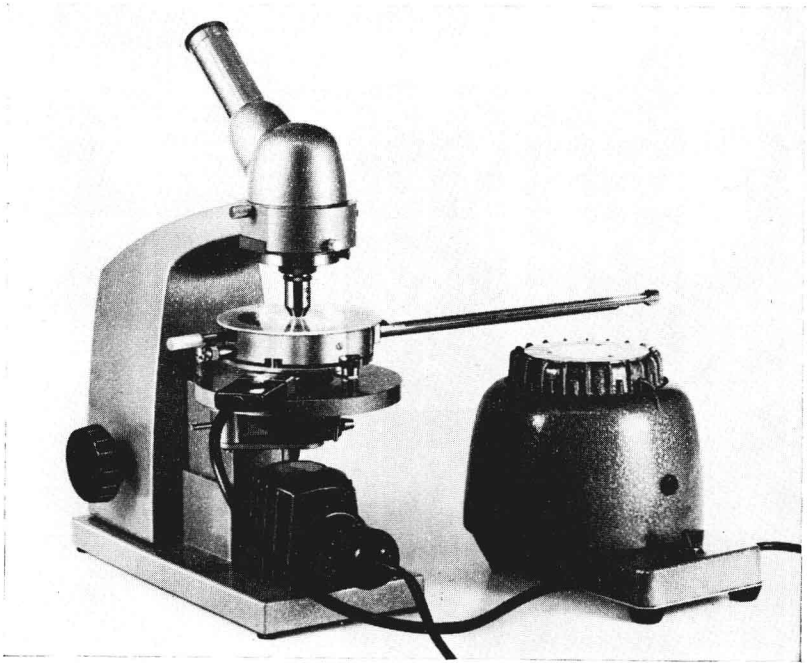

FIG. 3. Thermopan with Kofler hot stage.

the use of the Thermopan, designed specially for the Kofler hot stage, with a fixed hot stage, polarization device, and suitable filter holder, has proved to be particularly advantageous (Fig. 3).

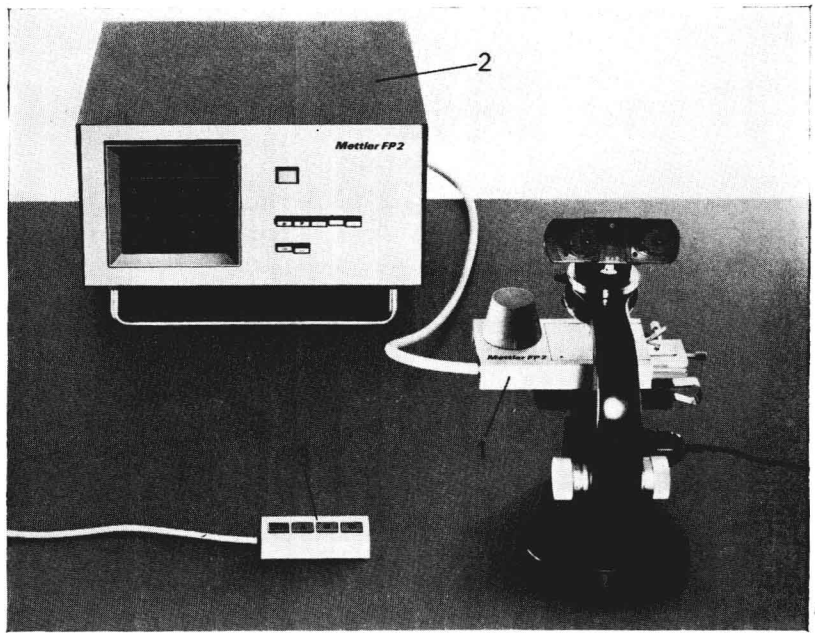

FIG. 4. Mettler FP2 hot stage with control device.

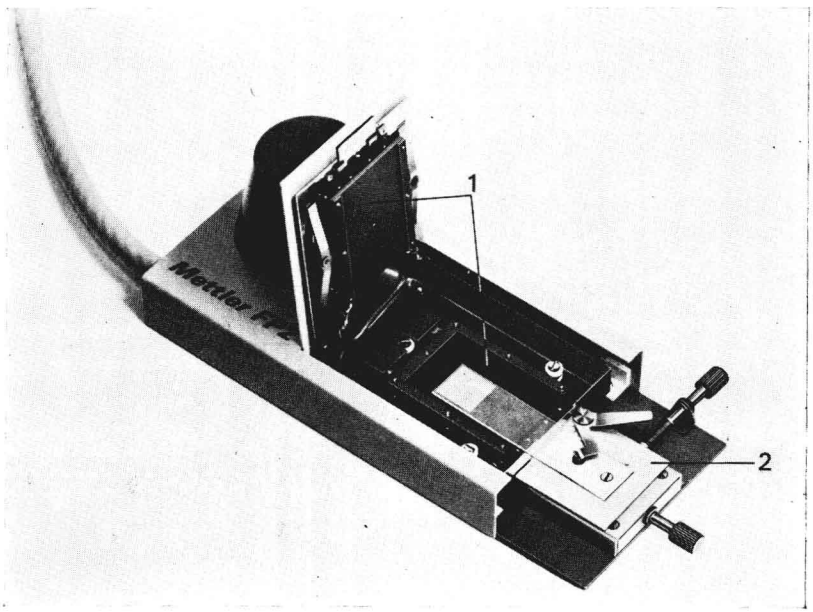

FIG. 5. Mettler hot stage opened.

(b) **Mettler FP2**

The Mettler FP2 melting point apparatus (Fig. 4) consists of a rectangular micro hot stage (1), an electronic control unit (2) and a key block (3). The hot stage (Fig. 5) contains a flat oven (1), which is heated electrically on both sides. The temperature is measured by means of a platinum resistance thermometer which is embedded in the lower part of the oven near the observation aperture. The temperature shown corresponds to the temperature of the sample, so that no calibration is necessary. The microscope slides are narrower than the international norm and the movement of the sample is carried out by a small cross-table (2) the range of action of which is, however, relatively small. The electronic control device permits the choice of the rate of rise of temperature which in this case, in contrast to the Kofler hot stage, is linear. Rates of 0·2°C, 2°C and 10°C per minute are provided in the standard embodiment, and instruments with other rates can be supplied on request. The digital readings of the temperature figures with data storage permit the uninterrupted observation of the sample during the critical phases. While the top digital display (Fig. 4 (2)) gives continuously the temperature prevailing in the hot stage, by operating the three keys A, B, and C (Fig. 4(3)), three prominent temperature values with readings in tenths of a degree can be stored. At a temperature rise of 0·2°C per minute, the standard deviation is only ±0·1°C, a precision which has not previously been achieved with a hot stage. Melting point determinations with an accuracy of a tenth of a degree are of particular interest when thermomicroscopy is to be used for purity testing. For the identification of materials the accuracy of ±0·5°C of the simple apparatus is quite satisfactory.

The Mettler FP2 covers the range from room temperature to 300°C and the possibility of extending it to lower temperatures down to −20°C is provided. The Kofler instruments are provided with their own micro cold stage which can be used from +80°C to −50°C. However, we give no further information about it here, since too few characteristic data exist for liquids for them to be tabulated.

References

1. LEHMANN, O., *Molekularphysik*, Engelmann, Leipzig, 1888; *Das Kristallisationsmikroskop*, Vieweg, Braunschweig, 1910.
2. KOFLER, L. and KOFLER, A., *Thermomikromethoden*, Wagner, Innsbruck, 1954.