Characterization and Identification of Lipids by Their Critical Solution Temperatures

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Abstract

The mutual solubility of two liquids which are not miscible in all proportions is essentially a function of the temp. Raising temp usually increases the solubility, possibly reaching a point, i.e., the upper critical solution temp (CST), at which the two components become miscible in all proportions. The CST is characteristic for the two liquids involved.

A microtechnique developed by Fischer simplifies rapid and accurate determinations of the upper critical solution temp. This technique is described and its application to the characterization and identification of pure lipids and lipid mixtures is demonstrated. The significance of this simple laboratory procedure and its relation to complementary analytical methods is discussed.

It is well known that the solubility properties of organic compounds can be utilized for the separation of mixtures and for the characterization of their constituents. The solubility of a compound in a liquid is dependent upon the temp. Two liquids which are not completely miscible at room temp may dissolve in each other to a higher degree at either increasing or decreasing temp. The temps at which complete solubility are achieved are, therefore, the maximum or minimum temps at which this pair of liquids can exist in two liquid phases. These upper and lower critical solution temps are characteristic for each of the two liquids. Occasionally, both the upper and lower critical solution temps of a pair of liquids can be realized. In the majority of cases, a rise in temp increases the mutual solubility. Hence, determinations of the upper critical solution temp (CST) offer a means for characterizing a system of two liquids. With a suitable test liquid, many compounds can be characterized by their CST in the same sense as by their melting points and boiling points.

Initial studies of liquid-liquid systems were reported by Alexejew (1) and Rothmund (42). Various aspects of the solubility phenomena of "immiscible" liquids have since been extensively investigated (30, 35). The standard monograph by Hildebrand and Scott (24), and a volume of the Discussions of the Faraday Society (14) summarize the problems of phase equilibria and solubilities. Data on solubility characteristics of organic compounds have been compiled by Seidell (48), and physicochemical constants of binary systems have been collected by Timmermans (49). Francis (21) recently published a volume dealing exclusively with critical solution temps.

Generally, the total miscibility of two liquids at the upper critical solution temp is achieved instantly (Fig. 1), i.e., little temp change results in a significant change of the mutual solubility. This effect facilitates CST observations with great accuracy (6).

The percentage of palmitic acid dissolved in nitromethane at various temps is depicted in Figure 1, according to data reported by Broughton and Jones (6) and Hoerr et al. (28). The two solubility curves do not exactly merge. It can be assumed that the substances used were of different qualities.

Determinations of upper and lower critical solution temps are used mainly in petroleum chemistry for characterizing hydrocarbons, studying the properties of selective solvents, estimating impurities, and analyzing multiple mixtures. The method usually employed is to place at least 1 ml of each of the two liquids together in a test tube, and to observe the temp at which the two liquids just mix on heating or just cloud on cooling (21). The temp readings can be rendered difficult due to color, the formation of precipitations or the occurrence of isooptical mixtures (the refractive indices of both liquids become identical) (22). Refinements in observation of CST-values or methods of expediting the procedure have been described by numerous authors (3,7,21). In contrast to melting point determinations, super cooling does not occur. Therefore, the temp readings at the CST are the same during the heating and the cooling processes, both of which may be repeated several times.

In the lipid field several procedures have been proposed for assessing the quality of fatty raw material by determining their solubility characteristics. For example, the Crisman number represents the temp at which a homogeneous mixture of fat and solvent starts to separate into two phases (9-11). A modification of this procedure has been adapted as an AOCS Official Method (2). Related techniques have been proposed by Valenta (50,51), Fachini and Dorta (15), and many others (32). As all of these procedures require rather large amts of sample, they are hardly applicable for the characterization of highly purified compounds.

R. W. Fischer devised a method for the determination of upper critical solution temps, which permits the rapid and accurate analysis of as little as 0.5-2 µl sample sealed into a glass capillary together with a suitable test substance (16-20,23). This capillary is heated on a hot stage, and the interface between the two liquids is observed under a microscope. Fischer and co-workers applied this microtechnique to characterize petroleum products (20), fats and oils (17,19,31), and other naturally occurring substances. The small amts of sample required for a CST de-
termination can be isolated easily by chromatographic techniques.

Critical solution temp values determined with the micromethod agree in many cases with data reported by other authors who had obtained these values through solubility studies (6,25-28,47). Deviations can be due to differences in the purity of the preparations analyzed and/or the purity of the test substances used.

We have applied the micromethod of Fischer to characterize a large number of chromatographically pure aliphatic compounds. CST-values of homologous series and vinylogen fatty acids and methyl esters (44), triglycerides (45), alcohols, dialkyl ethers and wax esters (46), α-glyceryl ethers (4) and hydrocarbons (5) have been reported.

The purpose of the present communication is to describe the micromethod of Fischer as used in our laboratory, to review and discuss present knowledge about critical solution temps of lipids, and to present the results of some new studies.

The Micromethod of Fischer

The apparatus, the amount of sample used, the speed and accuracy of CST determinations are similar to the microdetermination of melting points as described by Kofler (34,40). Following will be a description of the equipment and procedure used, and the test substances employed will be specified.

Equipment

1) Microscope. The Reichert "RCH" (Reichert Optische Werke A. G., Wien 17, Austria) is especially designed for use in connection with a heating stage. It is equipped with an objective 10X and an Huygenian eyepiece 10X, thus yielding a total magnification of 100X. However, any upright transmitted light microscope can be used provided that the stage top contains two plug holes to accept clips for mounting a micro heating stage. The stage top of the microscope should be all metal and non-rotary in order to resist higher temps.

When using the Reichert-Kofler micro heating stage, the objective used in the microscope must have at least a free working distance of 6 mm (e.g., the Reichert Dry Achromatic Objective with a magnification of 10X, a numerical aperture of 0.55 and a working distance of 7.1 mm).

2) Micro heating stage. Suitable micro heating stages are available from Reichert Optische Werke, No. 4060 and 4065, and Arthur H. Thomas Co. (Philadelphia) No. 6886-A and 6886-B. These heating stages are equipped with exchangeable mercury in glass thermometers. The Reichert thermometers range from 20 to 230°C and 120 to 350°C, whereas the Thomas thermometers have ranges from 30 to 230°C and 60 to 350°C.

3) Slide and shifting device. All determinations of CST-values listed in this communication have been carried out using the adapted microscope slide illustrated in Figure 2.

Figure 2 shows a sealed capillary containing sample and a test substance (a), an adapted microscope slide (b) and the shifting device supplied with the Reichert micro heating stage (c).

A 38 x 26 x 1 mm microscope slide was adapted by gluing two glass strips (38 x 4 x 1 mm) onto it, leaving a channel of 0.5 mm in width. Recent experiments with brass slides and aluminum slides proved their suitability. A shifting device (Cat. No. 6887 M) with an aluminum slide (Cat. No. 6888 Q) for determination of CST-values by the micromethod is available from A. H. Thomas Company.

4) Capillaries. Glass capillaries of 0.2-0.3 inner diam. and about 15-20 cm length may be drawn from glass tubing (7 mm diam). They are also available commercially from A. H. Thomas Company.

Test Substances

The use of CST determinations as an analytical procedure requires standard test substances, in order to obtain reproducible results. Valuable is their CST determination with pure standard liquids such as benzene or hexadecane (see Table I).

The following requirements have to be considered when selecting a suitable test substance.

General Standards. The substance should be available in high purity and storeable without the use of special precaution (e.g., against autoxidation, etc.). It must be chemically uniform at different temps, or at least its solubility characteristics must be reproducible at any specific temp used. Furthermore, the test substance should not show a tendency to react or decompose and preferably be a liquid at room temp.

Special Requirements. The test substance should yield a CST with the sample in a temp range from 20 to 230°C.

At the CST the refractive indices of sample and test substance must be sufficiently different to allow one to see the interface between them.

Approaching the CST total miscibility should occur instantly making possible exact observations of the disappearing interface.

The following five liquids (see Table I) have been selected as test substances for characterizing various lipid classes. They proved to meet most of the requirements specified above. Only the 1,3-butanediol (Matheson, Coleman and Bell, Norwood, Ohio) was purified by vacuum distillation. All other liquids were used as obtained in order to facilitate their application.

By selecting a suitable test substance the CST-values of a series of compounds may be determined at various temp ranges.

Procedure (16,44)

The test substance and the liquid sample are introduced into the capillary, the sample being introduced in such a manner that the interface disappears instantaneously. The test substance and the liquid sample are introduced into the capillary, the sample being introduced in such a manner that the interface disappears instantaneously.

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<table>
<thead>
<tr>
<th>Substance</th>
<th>Supplied by</th>
<th>No.</th>
<th>CST (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-Ethanediol (ethylene glycol)</td>
<td>Fisher* (certified No. K-178, Baker (reagent, No. 2900), Fluka* (puriss., e.g., No. A 50297)</td>
<td>1.4016</td>
<td>1.112</td>
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<tr>
<td>1,3-Butanediol</td>
<td>M&amp;G® (No. BX 16401 (dist.), Fluka (puriss., No. A 51581)</td>
<td>1.4402</td>
<td>1.006</td>
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<tr>
<td>Nitromethane</td>
<td>Fisher (certified No. N-041, M&amp;G (spectroscopic, No. NX 613)</td>
<td>1.3520</td>
<td>1.134</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>Baker (reagent, No. 9011), M&amp;G (chromato-quality, No. 1445, Fluka (No. A 50057)</td>
<td>1.3455</td>
<td>0.780</td>
</tr>
<tr>
<td>Acetic anhydride</td>
<td>Baker (reagent, No. 9018, M&amp;G (puriss., No. A 63856)</td>
<td>1.3910</td>
<td>1.076</td>
</tr>
</tbody>
</table>

* Fairlawn, N.J.; b Phillipsburg, N.J.; c Switzerland; d Norwood, Ohio; e Chicago, Ill.