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Liquid chromatography at critical conditions (LCCC) — Chemical heterogeneity of polyethylene oxides

Chromatographie liquide aux conditions critiques — Hétérogénéité chimique des oxydes de polyéthylène

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 35, *Paints and varnishes*.

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Introduction

Since the first description of liquid chromatography at critical conditions (LCCC) in 1986 (see Reference [1]), the method has been continuously refined and has proved itself to be indispensable for polymer characterisation. Separation is required not only for the quantitative analysis of the individual species. It also offers the preconditions for qualitative characterisation of the fractions by means of spectroscopic and spectrometric techniques. The key factor here is the reduction of the polydispersity/chemical heterogeneity within a fraction, which represents a large problem for mass-spectrometric investigations.

The method has been described extensively in professional circles over the last two decades for different polymer systems, see References [2] to [9].

Within the framework of the Technical Committee, the extent that the method supplies consistent results for a simple, chemically heterogeneous polymer mixture was clarified as part of interlaboratory testing.

At this time, necessary experience relating to the selection of the system (interaction between the polarities separation phase/eluent/sample) was not expected of any of the participating laboratories.

The interlaboratory testing has shown that, even with a well-characterized system and with specification of all pertinent system parameters, it has to date not been possible to classify the process as a routine method in laboratories with experience in polymer analytics.

The idea presents itself of offering a validation kit (polymer mixture with the expecting separation result).

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Liquid chromatography at critical conditions (LCCC) — Chemical heterogeneity of polyethylene oxides

1 Scope

This document establishes a valid method for separation of chemically heterogeneous polyethylene oxide (PEO) mixtures and for the determination the number and content of the chemically heterogeneous species in the overall sample.

The method presented in this document serves as a technical guideline and enables laboratories to learn the principle of "critical chromatography" on a validated system.

This method presented in this document with its stated system parameters is not applicable for other polymer classes, due to the diversity of the interactions between the polymer/mobile phase/stationary phase and the number of separation systems that are therefore available.

The evaluation of the interlaboratory testing has shown that many error sources relate to the technique of liquid chromatography in general. Possible error sources are described in Annex A.

Details on the evaluation of the interlaboratory testing are given in Annex B.

Elugrams of the participants (excerpts) are given in Annex C.

Investigations of the long-term stability of the test mixture are given in Annex D.

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2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
liquid chromatography at critical conditions
LCCC
special form of liquid chromatography of polymers at the point of adsorption, where chemically and structurally identical polymers with a certain repeat unit elute independently of the molar mass at the same retention time

Note 1 to entry: The individual monomer units do not contribute to the retention. Under these determined system parameters (defined combination of separation column/eluent mixture/temperature), a separation of polymer mixtures of the same repeat unit takes place based on chemical heterogeneity. Chemical heterogeneities can take the form of different functional groups, end groups, differences in the microstructure (e.g. copolymers and their composition) as well as topological differences (e.g. branching).

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4 Principle

In the following, the method for the separation of polyethylene oxides at critical conditions of adsorption is described for the ethylene oxide repeat unit.

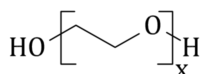
Three polyethylene glycols (PEG) with different molar masses (1 mg/ml to 2 mg/ml dissolved in the relevant eluent mixture (see 7.1) are measured, starting with a high proportion of the thermodynamically good solvent B (in this case acetonitrile). The eluent mixture is successively changed by increasing the proportion of component A (in this case water). This is followed by measurements in different compositions until all three standards elute independently of the molar mass at the same retention time. The determined critical solvent composition (csc) corresponds to the critical conditions.

Afterwards, the unknown mixtures are dissolved in this eluent mixture and measured.

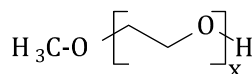
From the peaks of the resulting chromatograms, the number of species with different functionalities and their relative content (taking into account the detection properties) can be determined.

Species contained in the mixtures:

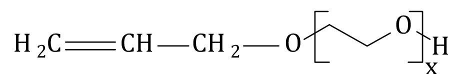
(1) Polyethylene glycol



(2) Methylene polyethylene glycol



(3) Polyethylene glycol monoallyl ether



5 Apparatus

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5.1 General

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The apparatus shall consist of the components shown in Figure 1, which are described in more detail below.

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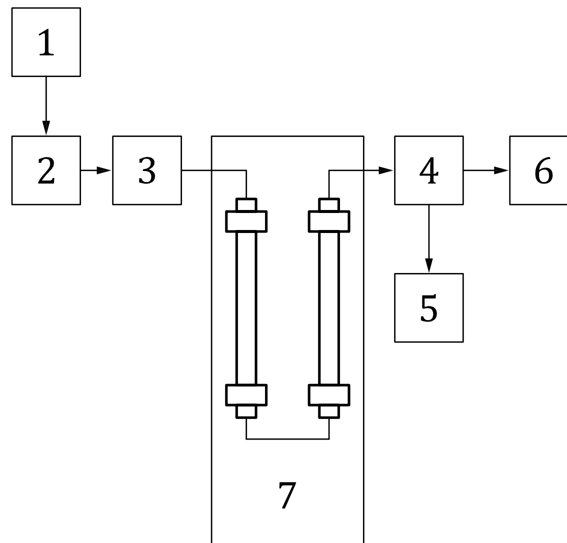

Key

1 LCCC mobile phase
2 pump, 0,1 ml/min to 2 ml/min
3 injection valve, autosampler
4 detectors: RI, ELSD, corona
5 data processing
6 waste
7 separation columns/column temperature control

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Figure 1 — LCCC apparatus

All components shall come into contact with the eluent or the sample solution are resistant to them and do not exhibit any adsorption or memory effects. The individual modules should be generally connected with steel capillary tubes for polymer analytics.

5.2 Eluent supply

The eluent reservoir shall adequately protect the eluent against external influences such as the atmosphere and light, if necessary by means of a blanket of inert gas above the liquid level.

The eluent reservoir shall contain a sufficient quantity of the eluent to bring the apparatus to equilibrium and to carry out several repeat analyses.

The eluent shall be degassed either before it is introduced into the reservoir, or by use of a device fitted between the reservoir and the pump, to prevent malfunctions of the pump or the formation of bubbles in the detector. The method of degassing used (e.g. bubble trap, online purging with helium or vacuum degassing) is open to choice, but shall be stated in the test report.

For polymer mixtures that contain chromophoric groups, other detectors may be used, e.g. UV or IR detectors.

5.3 Pump

The pump ensures that the eluent flow through the separation columns is as smooth and pulse-free as possible. The flow rate shall be 0,5 ml/min to 1 ml/min, depending on the dimensions of the columns used. To fulfil these requirements, the pump shall operate at optimum efficiency at this flow rate and at the counterpressure established in the process. The variation in the flow rate of the pump used may have a variation of max. 0,1 %.

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5.4 Injection system

The injection system serves to introduce into the chromatographic separation system an exactly specified amount of the sample solution (5 µl to 25 µl) in an eluent interval that is delimited as accurately as possible. This introduction may be carried out either manually or automatically.

When filling the sample loop with sample solution and subsequently introducing the sample solution into the eluent stream, the volume of liquid used shall be great enough to ensure that, even if laminar-flow effects occur, the sample loop is completely filled with the sample solution and subsequently completely flushed out.

Memory effects from the previous sample solution in the injection system shall be avoided by design or by adequate flushing.

5.5 Separation columns

The separation system consists of one or more columns connected in series, which are packed with a spherical (d < 10 µm) and porous separation material, the diameter of the pores corresponding to the size of the polymer molecules being analysed (80 Å to 300 Å for this method).

Depending on the molar mass of the samples under investigation, the pore size of the separation material may need to be adjusted accordingly.

At this point in the method, the separation material consists of a nonpolar/hydrophobic stationary phase (octadecyl-modified silica gel, reversed phase, C18). The hydrophobic properties of the material should have been further boosted by end-capping with trimethyl chlorosilane. The C-content thus obtained is ≫10 %.

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To meet the objective of this document of obtaining results that agree as well as possible in different laboratories using different LC apparatus with the same sample, it is necessary to adhere to the minimum requirements specified below with regard to peak broadening (expressed in terms of a number of theoretical plates) and separation efficiency; the actually obtained values shall be stated in the test report.

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Number of theoretical plates

The number of theoretical plates N shall be determined, for the apparatus used per metre of column used, from the peak width at half height. Inject up to 20 µl of a solution of the PEG 1 500 standard (min. mass concentration 1 mg/ml) on to the column, and evaluate the chromatogram obtained under the same conditions as are used for analysing polymers, using Formula (1):

$$N = 5{,}54\left(\frac{t_R}{w_{0,5}}\right)^2 \frac{100}{L} \quad (1)$$

where

t_R is the retention time at the peak maximum (the same units shall be used, e.g. min);

$w_{0,5}$ is the peak width at half height (see Figure 2);

L is the length of the column (column combination), in cm.

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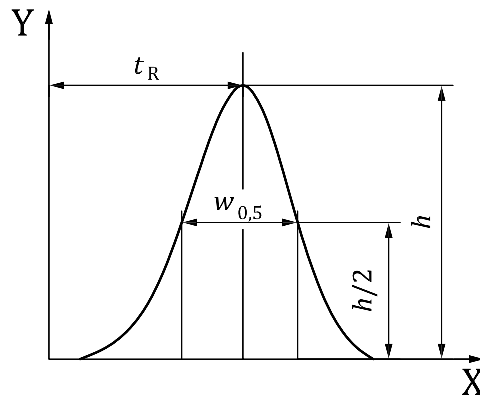

Key

X retention time, in minutes

Y detection signal

t_R retention time at the peak maximum, in minutes

$w_{0,5}$ peak width at half height of the peak

h maximum peak height

$h/2$ half peak height

Figure 2 — Determination of the number of theoretical plates by the half-height method

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The result is expressed as the number of theoretical plates per metre of column length. A column (column combination) should be used that achieves at least 5 000 theoretical plates per column (column combination) used.

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5.6 Column temperature control

The analyses can be performed in a temperature range from 20 °C to 45 °C. The temperature of the column shall not change by more than 0,5 °C during the analysis. All analyses shall be performed at the same temperature, as the LCCC is an enthalpic process and therefore temperature-dependent[1].

5.7 Detectors

The following can be used as detectors: RID (refractive index detector), ELSD (evaporative light scattering detector) or Corona CAD (charged aerosol detector).

5.8 Eluent

All eluents that are used should have the purity "HPLC grade". The eluents should not be returned to the eluent reservoir.

5.9 Data acquisition

The signals from the detector are recorded by means of an electronic data system.

6 Sample preparation

Dissolve the samples (1 mg/ml to 2 mg/ml) in the relevant eluent. To rule out changes to the polymers in solution as a function of the time as a possible source of error, the samples should be analysed within 6 h of production of the solution.

Fill up the validation kit (mixture 2) with 2 ml of the critical solvent composition.

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7 Performance of the measurements

7.1 Determination of the critical conditions

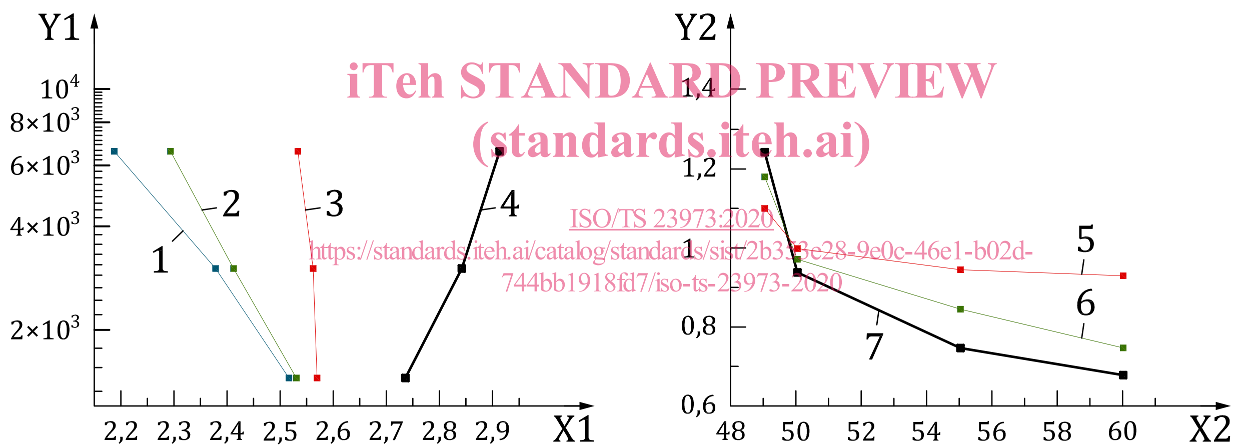
The critical conditions can be set up either by changing the temperature or by changing the composition of the mobile phase.

In the following procedure, keep the temperature strictly constant, and successively change the solvent composition with decreasing acetonitrile content. This can be done via separate mixing of the components or with a binary pump.

Measure the three standards with different molar masses in the relevant chosen solvent composition. At this point this this method, it is very important to make sure that the solvent in which the standard is dissolved also complies with the eluent composition.

The critical solvent composition (ACN : H_2O) is attained when all molecules of the same chemical structure (in this case PEG standards) elute at the same retention time. This means that, under these conditions, the chromatographic behaviour is no longer determined by the size of the molecule, but by chemical inhomogeneity.

To determine the critical solvent composition, plot lgM_p, in g/mol, over the retention time, in min, (Figure 3, left), or the retention factor k, over the content of component B in the eluent, in % (Figure 3, right).



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a) Logarithm of molar mass over retention time **b) Retention factor over content of component B in the eluent**

Key

X1 retention time, in minutes

Y1 lg M_p, in grams per mole

X2 ACN, as volume fraction in per cent

Y2 retention factor k

1 ACN / H_2O: 60 % / 40 %

2 ACN / H_2O: 55 % / 45 %

3 ACN / H_2O: 50 % / 50 %

4 ACN / H_2O: 49 % / 51 %

5 PEG 1460

6 PEG 3020

7 PEG 6600

Figure 3 — Schematic representation of the determination of the critical solvent composition

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The retention factor k is calculated using Formula (2):

$$k = \frac{(t_R - t_0)}{t_0} \quad (2)$$

where

t_R is the retention time of the standard in the relevant system;

t_0 is the retention time of a non-delayed substance.

7.2 Analysis of the validation kit

Mix the sample with 2 ml of the critical solvent mixture and dissolve it at room temperature under shaking.

To rule out changes to the polymers in solution as a function of the time as a possible source of error, the samples should be analysed within 6 h of production of the solution. Ensure that the temperature in the column remains constant.

The injection volume shall be matched to the set of columns used and can therefore be varied between 5 µl and 25 µl.

It is recommended to conduct a blind test (injection of the eluent/the pure sample solvent) before measuring the sample in order to identify solvent signals or peaks that are caused by the injection.

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The measurement of the sample ends after complete elution of the last system peak and after the signal returns to the base line.

Conduct three repeat measurements and calculate the mean values of the retention time for the separated peaks.

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Calculate the chromatographic resolution of two peaks R as a quality criterion for the separation using Formula (3):

$$R = 1{,}18 \times \frac{(t_{R2} - t_{R1})}{w_{f,hm,1} + w_{f,hm2}} \quad (3)$$

where

t_R is the retention time;

$w_{f,hm}$ is the full width of the peak at half maximum.

8 Test report

The test report shall contain at least the following information:

a) all details necessary for complete identification of the product tested;

b) a reference to this document (ISO/TS 23973:2020);

c) all information about the apparatus (pump, degasser, injection system, column oven, detectors, evaluation software);

d) all information about the separation columns (manufacturer, separation material, pore size type, grain size, number, dimensions, C-content);

e) the number of theoretical plates per column combination in the LC apparatus used;

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f) the column temperature;

g) the flow rate;

h) the critical solvent composition, in per cent, (mass fraction or volume fraction) with statement of the method of mixing (gradient pump or separate production of the mixtures);

i) the concentration of the injected solution, in milligrams per millilitre (for samples to be weighed in by yourself);

j) the injection volume, in microlitres;

k) the overlay of the elugrams of the three PEGs with different molar masses;

l) the validation kit surface percentage report, including chromatogram;

m) a statement of the mean values of the retention time;

n) a statement of the chromatographic resolution of the peaks R_{AB}, R_{AC} and R_{BC} according to Reference [3];

o) the result of the test;

p) any deviation from the method specified;

q) any unusual features (anomalies) observed during the test;

r) the date of the test.

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Annex A
(informative)

Error sources

The evaluation of the interlaboratory testing has shown that many error sources can not only be attributed specifically to a lack of experience in terms of the chromatography of polymers, but that they relate to the technique of liquid chromatography in general.

As is well known, the resolution R depends on the separation factor α (for selectivity, measure of the separation capability of the chromatographic system), the retention factor k (for capacity, measure of the strength of the interactions) and on the number of plates N (for efficiency, measure of the band broadening of the substance zone).

If the result does not correspond to the expectations, then the following aspects should be considered:

a) Have the column dimensions and packaging material been ideally chosen?

Improvement of the resolution via the number of plates:

— column as long as possible;

— particles as small as possible;

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— optimal flow rates;

— determination of the adsorption range and exclusion rate for the selected column;

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— determination of the number of plates (>25 000/m);

— adjustment of the pore size to the largest molar mass to be analysed (e.g. >100 Å for molar masses above 6 000 g/mol)

b) Is the column contaminated/changed due to previous injections, is the specification no longer met?

This can alter the selectivity, and this influences the resolution more strongly than the number of plates (separation factor α).

c) Are the solvent signals and system peaks overlaying the peaks of the analyte (particularly when using RI [and UV] detectors)?

d) Have the rinsing times been complied with during the change in rinsing to new solvent compositions (reference cell rinsing!)?

e) Do the internal diameters and lengths of the capillary tubes between the column and detector meet the requirements (maximum internal diameter of 0,12 mm)?

f) Are there dead volumes (particularly in the case of detectors connected in series)?

g) Has the sample actually been analysed in the current eluent mixture?

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