INTERNATIONAL STANDARD

ISO 16564

First edition
2004-10-01

Rubber, raw natural — Determination of average molecular mass and molecular-mass distribution by size exclusion chromatography (SEC)

Caoutchouc naturel brut — Détermination de la masse moléculaire moyenne et de la répartition des masses moléculaires par chromatographie d'exclusion stérique (SEC)

Reference number
ISO 16564:2004(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 16564 was prepared by Technical Committee ISO/TC 45, *Rubber and rubber products*, Subcommittee SC 2, *Testing and analyses*.

Rubber, raw natural — Determination of average molecular mass and molecular-mass distribution by size exclusion chromatography (SEC)

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory limitations prior to use.

1 Scope

This International Standard specifies a method of determining the average molecular mass and the molecular-mass distribution of raw natural rubber dissolved in tetrahydrofuran. A set of polystyrene standards is used for calibration purposes (i.e. the method is a relative one).

An alternative method, using cyclohexane as solvent and polyisoprene standards, is included in an informative annex.

2 Principle

Dried natural rubber is dissolved in tetrahydrofuran at room temperature. The solution is filtered to remove "gel" (slightly crosslinked rubber) and other insoluble materials. The filtrate is used to determine the molecular mass by size exclusion chromatography [1]. From the chromatogram, the number-average molecular mass (M_n), the mass-average molecular mass (M_w) and a polydispersity value (M_w/M_n) are calculated.

3 Materials

3.1 The recommended solvent is HPLC-grade tetrahydrofuran (THF). If analytical-grade THF is used, 0,5 % of butylated hydroxytoluene (BHT) shall be added to the solvent as an antioxidant. The BHT is strongly retained and acts as a marker for the end of the chromatogram. It also allows minor variations in run time to be corrected, as well as indicating when a serious change in the column conditions has occurred.

3.2 The solvent is filtered through a polytetrafluoroethylene (PTFE) membrane (4.2) before use.

3.3 A set of polystyrene standards with proper traceability shall be used, typically covering an M_w range from $6{,}5 \times 10^3$ to $1{,}06 \times 10^7$. An example of a suitable set of polystyrene standards is given in Table 1.

Table 1 — Polystyrene standards

No. of standard	M_p	M_w/M_n
1	$3{,}55 \times 10^5$	1,08
2	$7{,}06 \times 10^5$	1,12
3	$2{,}89 \times 10^6$	1,36
4	$3{,}84 \times 10^6$	1,30
5	$4{,}48 \times 10^6$	1,47
6	$5{,}48 \times 10^6$	1,40
7	$6{,}77 \times 10^6$	1,37
8	$8{,}42 \times 10^6$	1,33

4 Apparatus

4.1 Size exclusion chromatograph: Suitable high-performance liquid chromatography equipment consisting of the components specified in 4.1.1 to 4.1.7.

4.1.1 Solvent reservoir, large enough to hold an adequate quantity of solvent (see 3.1). About 3 l of solvent is recommended for a complete analysis. Refilling can result in variations in dissolved air and the inclusion of impurities. When fresh solvent is added, it takes a long time to obtain a stable baseline.

4.1.2 Automatic on-line degassing system, to prevent the formation of bubbles in the mobile phase and to ensure a stabilized liquid feed flow.

4.1.3 Pump with temperature-control equipment, capable of delivering the solvent at a sufficient rate and a suitable temperature, for example 1,0 cm^3/min at 35 °C.

4.1.4 Injector.

4.1.5 Columns: Columns for the SEC of natural rubber are usually packed with rigid spheres of crosslinked polystyrene gel (prepared from styrene-divinylbenzene copolymer) having a range of pore sizes. Molecular masses in the range 2×10^3 to $> 10^{12}$ can be measured, depending on the particular set of columns used. Other column packings can be used providing they are capable of separating the polymers of different molecular mass in the range of interest and are not adversely affected by the solvent used. The columns shall be connected to the chromatograph in such a way that the sample is eluted through the column of greatest pore size first and the column of smallest pore size last.

4.1.6 Refractive index detector (or other type of detector), capable of giving an adequate response to natural rubber at the concentrations used.

4.1.7 Data-processing unit, capable of calculating the required molecular masses and molecular-mass distributions.

4.2 PTFE membrane filters, with a porosity of 0,45 µm to 1 µm. The actual porosity used will depend on the columns being used and the laboratory set-up.

4.3 Syringes, capacity 100 mm^3 and 200 mm^3 (or larger).

4.4 Syringe, capacity 10 cm^3.

4.5 Vortex shaker.

4.6 Auto-sampler, with vials (brown when available).

NOTE An auto-sampler with associated software may be used for sample introduction.

5 Procedure

5.1 Preparation of solvent

Filter the solvent through a PTFE filter (4.2) and degas under vacuum for 30 min. This shall be done 12 h prior to the analysis.

5.2 Setting up the SEC chromatograph

Condition the chromatograph, fitted with a suitable set of columns, with the solvent flowing at 0,6 cm^3/min until a stable baseline is obtained. The columns are normally kept at a constant temperature at least 10 °C above room temperature, i.e. between 30 °C and 45 °C. Sufficient degassed solvent for the complete set of analyses shall be stored in the solvent reservoir under an inert gas.

5.3 Setting up the data-processing unit

The data processor (4.1.7) converts the detector output into a digital signal, making measurements typically at a rate of 1 Hz to 5 Hz. If it is necessary to reduce noise, the measurements can be combined to give time slices with a width of 1 s to 2 s.

5.4 Preparation and injection of sample solution

5.2.1 Prepare a solution of the sample by placing 0,01 g or less of dried sample into a 20 cm^3 screw-cap vial (brown when available) containing 10 cm^3 of the previously prepared solvent (see 5.1).

5.2.2 Stir the vial, using a vortex shaker (4.5), for 1 min at room temperature and then keep warm at 37 °C for 24 h.

Take care not to expose the sample solution to light, e.g. by shielding the vial from light during warming, or by using a brown vial.

5.2.3 Filter the solution through a PTFE membrane (4.2) and introduce the filtrate into a syringe (4.3) or an auto-sampler vial.

Record the mass of the dried PTFE membrane (4.2) before and after filtration and report the percentage “gel” content together with the porosity of the filter used.

NOTE When the sample contains > 30 % “gel”, a significant fraction of the rubber is not being measured. This seriously affects the use of the molecular-mass distribution data for the prediction of the behaviour of the rubber.

5.2.4 Inject the sample solution and carry out the sample run in the normal way.

6 Calibration

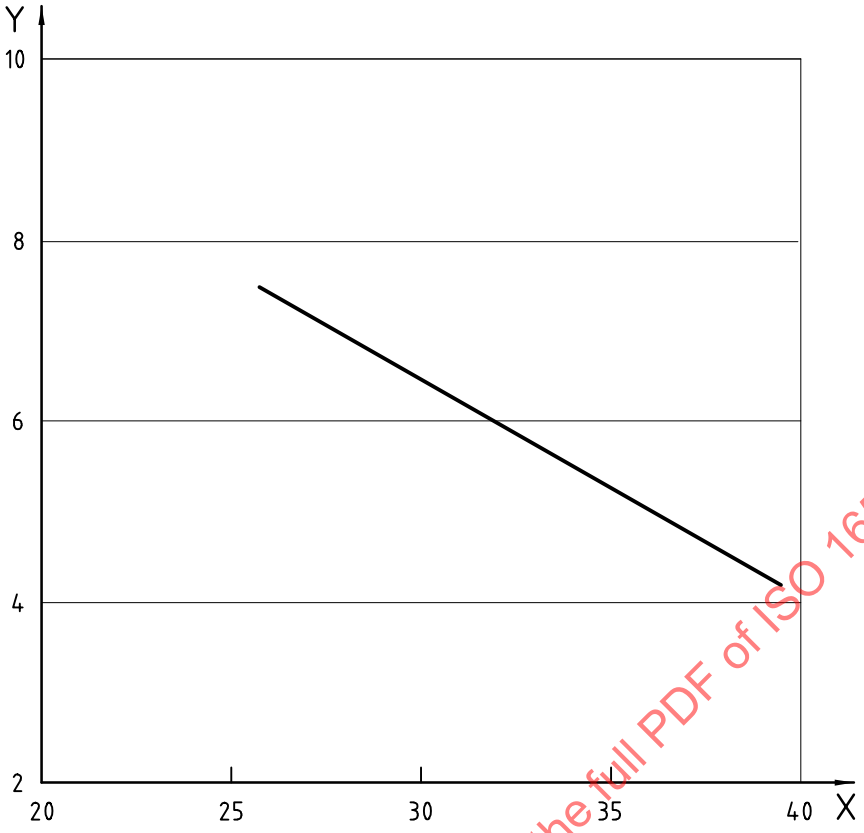
6.1 Polystyrene standards (see 3.3) dissolved in the prepared solvent (see 5.2) at a concentration of 0,1 g/l shall be used for calibration. The standards shall cover the entire molecular-mass range of the sample.

Prepare the standards by swelling them in solvent overnight without shaking. Shaking will reduce the molecular mass and broaden the peak width.

6.2 Run three 100 mm^3 injections of each standard and read the retention times of the peaks. Take the average of the three retention times for each standard.

6.3 Plot the average retention time against the molecular mass. A typical molecular-mass calibration curve for a set of polystyrene standards is shown in Figure 1.

6.4 The calibration curve is used by the instrument software to calculate the sample parameters to be determined.

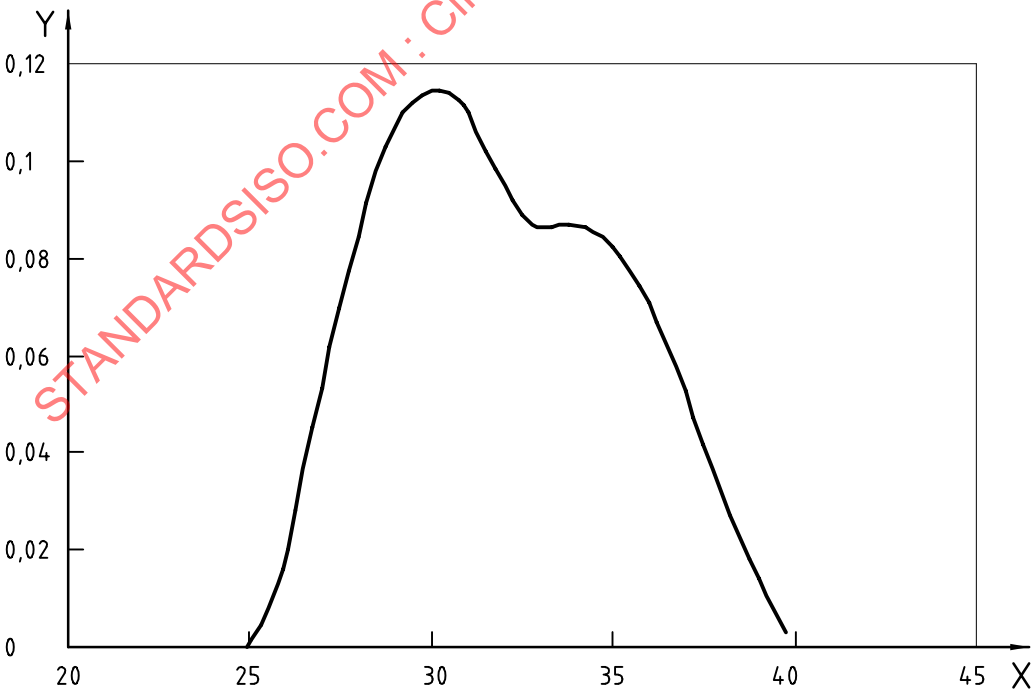


Key

X retention time, t_R (min)

Y $\log M_w$

Figure 1 — Typical calibration curve for a set of polystyrene standards



Key

X retention time, t_R (min)

Y mass fraction

Figure 2 — Example of an SEC chromatogram

7 Expression of results

7.1 For each sample solution, the following parameters are obtained automatically from the instrument:

— mass-average molecular mass (M_w);

— number-average molecular mass (M_n);

— z-average molecular mass (M_z);

— polydispersity (M_w/M_n);

— peak molecular mass (M_p).

In addition, a graphical representation of molecular-mass fraction vs. retention time is obtained.

7.2 An example of a chromatogram is shown in Figure 2.

8 Precision

The precision of this method has not yet been determined.

9 Test report

The test report shall include the following:

a) a reference to this International Standard;

b) all details necessary to identify the sample analysed;

c) the date and place of the analysis;

d) a graphical representation of the molecular-mass distribution (see 7.1);

e) the values obtained for the parameters M_n, M_w and M_w/M_n;

f) the percentage "gel" content;

g) the porosity of the filter used.