
**Natural rubber latex concentrate —
Determination of total phosphate
content by spectrophotometric method**

*Concentré de latex de caoutchouc naturel — Détermination de la
teneur totale en phosphate par méthode spectrophotométrique*

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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.

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 45, *Rubber and rubber products*, Subcommittee SC 3, *Raw materials (including latex) for use in the rubber industry*.

Natural rubber latex concentrate — Determination of total phosphate content by spectrophotometric method

1 Scope

This International Standard specifies a method for the determination of total phosphate content of natural rubber latex concentrate. This method is not necessarily suitable for latex from natural sources other than the *Hevea brasiliensis*.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 124, *Latex, rubber — Determination of total solids content*

ISO 648, *Laboratory glassware — Single-volume pipettes*

3 Principle

Approximately 20 g of concentrated latex, of which the total solids content has been determined, is coagulated with hydrochloric acid.

The coagulated latex is removed and the serum filtered through filter paper.

The residual phosphate present in a known volume of the serum is determined by measuring absorbance with a spectrophotometer at wavelength 470 nm.

4 Apparatus

4.1 Balance, accurate to 0,1 mg.

4.2 Volumetric pipettes, of capacity 10 cm³ and 25 cm³, complying with the requirements of ISO 648, class A.

5 Reagents

Use reagents of recognized analytical grade and deionized water or water of equivalent purity.

5.1 Hydrochloric acid 37 %.

5.2 Hydrochloric acid 1:24.

Mix 40 ml of 37 % hydrochloric acid (5.1) with deionized water and make up to 1 000 ml.

5.3 Vanadate molybdate.

a) Dissolve 25 g ammonium molybdate in 300 ml deionized water.

- b) Dissolve 1,25 g ammonium metavanadate in 300 ml deionized water. Heat to dissolve completely. Cool to room temperature before mixing with 330 ml of 37 % hydrochloric acid and leave to cool.
- c) Mix solutions [5.3 a\)](#) and [5.3 b\)](#) and make up to 1 000 ml with deionized water.

5.4 Potassium dihydrogen phosphate (stock solution).

Dissolve 2,196 8 g potassium dihydrogen phosphate and make up to 1 000 ml with deionized water. The phosphorous concentration of this solution will be 500 ppm.

6 Procedure

6.1 General

Carry out the procedure in duplicate, using separate test portions obtained from the same homogenized sample.

The difference between two readings shall not exceed 30 mg/kg.

6.2 Determination of total solids content

Determine the total solids content of the concentrated latex in accordance with ISO 124.

6.3 Preparation of standard phosphate solutions

6.3.1 From the 500 ppm stock solution ([5.4](#)), pipette 0 ml, 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 10 ml then dilute with deionized water and make up to 50 ml to make the first dilution of concentrations 0 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, 100 ppm, respectively.

6.3.2 Pipette 10 ml of each solution ([6.3.1](#)) into 50 ml volumetric flasks. Into each flask add 10 ml vanadate molybdate ([5.3](#)). Then make up the volume to 50 ml with hydrochloric acid solution ([5.2](#)). The final concentrations will be 0 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm and 20 ppm.

6.3.3 Leave the solutions ([6.3.2](#)) for 20 min and filter the total 50 ml solutions through an 8 µm pore size filter paper¹⁾ and measure absorbance with a spectrophotometer at wavelength 470 nm. Measure in duplicate with different portion and take the average values.

Use 0 ppm solution as a blank.

Prepare a calibration curve by plotting the concentration of potassium dihydrogen phosphate at 0 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm and 20 ppm ([6.3.2](#)) against the absorbance after measuring absorbance with a spectrophotometer at wavelength 470 nm. Determine the slope from the calibration curve (S).

6.4 Determination of phosphate content

Weigh about 20 g (W) of homogenous concentrated latex to the nearest 0,1 mg in a 100 ml beaker. Coagulate with 25 ml hydrochloric acid solution ([5.2](#)) and warm in water bath without stirring at 70 °C for 5 min to coagulate the latex completely.

Squeeze coagulum with a glass rod to obtain the serum as much as possible and filter the serum through the 8 µm pore size filter paper.

1) Whatman No.40 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Pipette 10 cm³ of the filtered serum (V_{pipette}) into 50 ml volumetric flask and add 10 cm³ of vanadate molybdate (5.2), then dilute with HCl solution (5.1) to 50 cm³.

Wait for 20 min. Filter the solution through a filter paper.

Measure absorbance with a spectrophotometer at wavelength 470 nm against the blank (without latex).

7 Expression of results

The phosphate content, P , expressed in mg/kg, is calculated using the following equation:

$$P = \frac{0,05 \times Abs \times V_{\text{total}} \times 3,066 \times 1\,000}{S \times A \times V_{\text{pipette}}}$$

where

Abs is the absorbance with spectrophotometer at wavelength 470 nm,

S is the calibration curve slope versus the phosphate concentration;

V_{pipette} is the volume of serum pipetted from total serum to volumetric flask;

3,066 is the constant of conversion of P to PO_4^{3-} .

with:

$V_{\text{total}} = 25 + (W - A)$ assuming that the density of serum = 1 g/ml;

$$A = \frac{W \times TSC}{100}$$

8 Precision data

See [Annex A](#).

9 Test report

The test report shall include the following:

- a reference to this International Standard;
- all details necessary for the identification of the sample;
- all details necessary for the complete identification of the product tested;
- the results and the units in which they have been expressed;
- the date of the test;
- any unusual features noted during the determination;
- any operations not included in this International Standard to which reference is made, as well as any incident which might have affected the results.

Annex A (informative)

Precision data

A.1 The precision of the test method was determined in accordance with ISO/TR 9272[1]. Refer to this document for terminology and other statistical details.

A.2 The precision data are given in [Table A.1](#). The precision parameters should not be used for acceptance or rejection of any group of materials without documentation that the parameters are applicable to those particular materials and specific test protocols of the test method. The precision is expressed on the basis of a 95 % confidence level for the values established for repeatability r and reproducibility R .

A.3 The results contained in [Table A.1](#) are average values and give an estimate of the precision of this test method. The results were obtained from an interlaboratory test programme (ITP) carried out in 2014 where six laboratories took part in performing duplicate analyses on three samples which were low, medium and high concentration of phosphate content.

A.4 A Type 1 precision was evaluated based on the method of sampling used for the ITP.

A.5 Repeatability: the repeatability r (in measurement units) of the test method has been established as the appropriate value tabulated in [Table A.1](#). Two single test results, obtained in the same laboratory under normal test method procedures that differ by more than the tabulated r (for any given level) should be considered to have come from different or non-identical, sample populations.

A.6 Reproducibility: the reproducibility R (in measurement units) of the test method has been established as the appropriate value tabulated in [Table A.1](#). Two single test results, obtained in the same laboratory under normal test method procedures that differ by more than the tabulated R (for any given level) should be considered to have come from different, or non-identical, sample populations.

A.7 Bias: in test method terminology, bias is the difference between an average test value and the reference (or true) test property value. Reference values do not exist for this test method since the value (of the test property) is exclusively defined by the test method. Bias cannot therefore be determined for this particular method.

Table A.1 — Precision for phosphate content in latex

Material	Mean	Within laboratory			Between laboratories			Number of laboratories
		s_r	r	(r)	s_R	R	(R)	
Low content	162	21	58	35,98	28	78	48,31	6
Medium content	235	24	67	28,56	44	125	53,32	6
High content	298	65	65	22,83	53	152	51,14	6
s_r is the within-laboratory standard deviation (in measurement units); r is the repeatability (in measurement units); (r) is the relative repeatability; s_R is the between-laboratory standard deviation (for total between-laboratory variation in measurement units); R is the reproducibility (in measurement units); (R) is the relative reproducibility.								