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METHODS FOR DETERMINING THE DESIZING EFFICIENCY AND THE RELATIVE EFFICIENCY OF AMYLOLYTIC ENZYMES

(Revised)

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(Revised)

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Indian Standard

METHODS FOR DETERMINING THE DESIZING EFFICIENCY AND THE RELATIVE EFFICIENCY OF AMYLOLYTIC ENZYMES

(Revised)

0. FOREWORD

0.1 This Indian Standard (Revised) was adopted by the Indian Standards Institution on 19 June 1965, after the draft finalized by the Textile Chemistry Sectional Committee had been approved by the Textile Division Council.

0.2 This standard was published in 1954 as a tentative standard. The Sectional Committee concerned decided to revise the standard in the light of experience gained since its publication. It was felt that lower concentrations of enzymes than those stipulated in the first method of the standard would serve the purpose and also that the tests should be carried out at two temperatures, namely, 50°C and 70°C instead of only 50°C, taking into consideration the optimum temperatures of the enzymes used. The second method is deleted in this revision because it determined only the reducing groups produced in the hydrolysis of starch but would not indicate the extent of liquifying action and, therefore, it did not give real indication of the desizing efficiency of an enzyme. The third method is also deleted as it was not practicable and workable. A new method based on viscosity of starch paste containing enzyme is included in this revision as the second method.

0.3 Desizing by ' rot steeping ' has now become obsolete mainly because it takes long time and there are difficulties in controlling the fermentation reaction. Cloth desized by steeping in mineral acids, such as sulphuric acid or hydrochloric acid has low residual mineral contents. However, the process suffers from a serious risk of cellulose degradation during the action of acids if not properly controllled. As against these two methods, desizing with enzymes is very effective and economical. It can be easily controlled. Due to the specific nature of the action of enzymes, cellulose is not liable to attack at any stage.

0.4 Amylolytic enzymes are water soluble colloidal organic catalysts which hydrolyse starch into water soluble products. Amongst them, three

types, namely, malt extracts, pancreatic diastases and bacterial diastases are of use to the textile industry. However, chemically there are only two different forms of these enzymes, α-amylase and β-amylase, which split the starch in different ways. Both bacterial and certain pancreatic enzymes contain only α-amylase, whereas those of malt origin contain α and β in proportions which vary from sample to sample. It has been reported that malt amylases contain β-amylase to the extent of two to five times more than α-amylase.

0.4.1 The α- and β-amylases are readily distinguished by their initial mode of action. α-amylases are dextrinising enzymes, rapidly splitting the starch molecules into low molecular weight dextrin, the reaction being physically manifested by rapid liquefaction. β-amylases on the other hand are saccharifying enzymes and their action results in formation of large molecules of sugars without any considerable liquefaction of starch. When the starch is not sufficiently liquefied, it will not be effectively removed in subsequent washing. Thus β-amylase alone has no practical value in desizing.

0.4.2 The characteristics that distinguish the three different types of enzymes mentioned in **0.4** are variations in their reaction conditions as time, temperature and *p*H. Enzymes of the same general type also differ from one another. The optimum *p*H and temperature of each of the three types of enzymes are as under:

	*Optimum p*H	*Optimum Temperature*
Malt preparations	4·5 to 5·5	55° to 65°C
Pancreatic preparations	7 to 8	45° to 55°C
Bacterial preparations	6·0 to 7·5	65° to 85°C

These enzymes are largely used in the cotton textile industry for desizing. If the starch is not removed from the fabric, it would interfere with the efficiency of kiering operation and also produce degradation products of a reducing nature which would act detrimentally on coloured yarn under treatment.

0.5 Two methods of evaluating desizing efficiency of enzymes have been prescribed in this standard.

0.5.1 The first method is suitable for determining the desizing efficiency as well as the relative efficiency of different enzymes. It is simple in operation and quick in evaluation. This is mostly suitable for evaluation of different enzymes for their desizing efficiency under the practical working conditions in desizing of fabrics which are normally adopted in textile industry. But it suffers from the defect that it is an indirect method and the test results have to be corrected for the hydrolysed starch removed by rot steeping and the unhydrolysed starch removed mechanically along with hydrolysed starch by the squeezing roller through which the cloth is passed.

0.5.2 The second method determines only the relative efficiency of different enzymes. Further, it helps to give optimum working conditions of a particular enzyme to get the required results. In this method, the quantity of enzyme required to give definite flow time is determined.

0.6 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

1. SCOPE

1.1 This standard prescribes two methods for evaluating the desizing efficiency and the relative efficiency of amylolytic enzymes. In the first method conditions stipulated are similar to actual working conditions in mills. In the second method the amount of enzyme required to give a definite flow time is determined.

2. TERMINOLOGY

2.0 For the purpose of this standard, the following definitions shall apply.

2.1 Desizing Efficiency — The desizing efficiency of an amylolytic enzyme is a measure of its effectiveness to hydrolyse starch into products which are water soluble and as such mechanically removable from cloth. It is expressed in terms of percentage loss of starch (that is, starch hydrolysed) from a fabric calculated on the total weight of starch in the fabric.

2.2 Relative Efficiency — The relative efficiency of enzyme is its desizing efficiency as compared with that of other enzymes. It is expressed in qualitative terms, such as ' more ' and ' less ' or ' higher ' and ' lower '.

3. QUALITY OF REAGENTS

3.1 Unless otherwise specified, pure chemicals shall be employed in the tests and distilled water (*see* IS : 1070-1960†) shall be used where the use of water or distilled water as a reagent is intended.

NOTE — ' Pure chemicals ' shall mean chemicals that do not contain impurities which affect the experimental results.

4. FIRST METHOD

4.1 Procedure

4.1.1 Take about 5 g or more of good quality bleached long cloth free from residual sizing and finishing materials. Remove five threads from

*Rules for rounding off numerical values (*revised*).

†Specification for water, distilled quality (*revised*).

each side of the piece in order to avoid any loss of warp or weft threads in the subsequent processes. Dry the piece in a stoppered weighing bottle at 100° to 105°C to constant weight and weigh it accurately. Starch the piece by passage through a 10 ± 1 percent (*w/v*) mucilage of good quality maize starch previously adjusted to *p*H between 6·5 and 7·5. Squeeze the starched pieces through a pair of squeezing rollers set to about 100 percent squeeze. Dry the pieces first in hot air and then in the weighing bottle at 100° to 105°C to constant weight and weigh it accurately.

4.1.2 Similarly treat three more pieces for the test.

4.1.3 Calculate on oven-dry basis the starch present on each piece by the following formula:

$$d = W_2 - W_1$$

where

d = amount of starch, in g, present on the piece;

W_2 = weight, in g, on oven-dry basis, of the starched piece; and

W_1 = weight, in g, on oven-dry basis, of the unstarched piece.

4.1.4 Dissolve 1 g of enzyme under test in sufficient amount of water and make the volume to 1 litre.

4.1.5 Dissolve 1 g of sodium chloride in sufficient amount of water and make the volume to 1 litre.

4.1.6 Take two beakers and add 80 ml of water in each. Add to each beaker required amounts of enzyme solution (*see* **4.1.4**) and sodium chloride solution (*see* **4.1.5**) so that the amount of enzyme and sodium chloride taken for the test shall be 0·02 percent of the amount of starch present on the piece under test. Add the necessary amount of water to make the material-to-liquor ratio of 1 : 20. Treat two starched pieces (*see* **4.1.1**) separately for five minutes one at 50°C and another at 70°C with enzyme solutions. Pass the two pieces separately through the squeezing rollers without changing their settings and leave them in separate stopper bottles at 38° ± 2°C for 18 to 20 hours. Wash each of the two pieces 6 times giving two dips each time in 400 ml of fresh water at room temperature, passing the piece after every wash through the squeezing rollers set as previously. Dry the two pieces at 100° to 105°C to constant weight and weigh them accurately.

4.1.7 Calculate, on oven-dry basis, the percentage loss in weight of the pieces treated as in **4.1.6** by the formula given below:

$$a = \frac{W_2 - W_3}{d} \times 100$$

where

a = loss in weight, percent, of starch;

W_2 = oven-dry weight, in g, of the undesized starched piece;

W_3 = oven-dry weight, in g, of the desized starched piece; and

d = amount of starch present on the piece (*see* **4.1.3**).

4.1.8 Similarly treat the other two pieces by substituting water in place of the diluted enzyme solution.

NOTE — The material-to-liquor ratio should be 1 : 20.

4.1.9 Calculate on oven-dry basis, the percentage loss in weight of the pieces treated as in **4.1.8** by the following formula:

$$b = \frac{W_2 - W_4}{d} \times 100$$

where

b = loss in weight, percent, of starch;

W_2 = oven-dry weight, in g, of the untreated starched piece;

W_4 = oven-dry weight, in g, of the treated starched piece; and

d = amount of starch present on the piece (*see* **4.1.3**).

4.1.10 Calculate the desizing efficiency at 50°C and 70°C separately by the formula given below:

$$E = a - b$$

where

E = desizing efficiency of the enzyme;

a = loss in weight, percent, due to enzymic action (*see* **4.1.7**); and

b = loss in weight, percent, due to blank (*see* **4.1.9**).

4.2 Repeat the test by substituting 0·05 and 0·1 percent of enzyme or a lower concentration of enzyme as required in place of 0·02 percent of enzyme.

NOTE — The amount of sodium chloride taken for the test should be equal to the amount of the enzyme taken for the test.

4.3 Repeat the test as given in **4.1.1** to **4.2** for the remaining enzymes under test.

NOTE — It is not necessary to repeat the blank for tests carried out on the same day for the same setting of squeezing rollers.

4.4 Compare the results (*see* **4.1.10, 4.2** and **4.3**) to determine the relative efficiency of the enzymes under test bearing in mind that higher the desizing efficiency, more efficient is the corresponding enzyme.

5. SECOND METHOD

5.1 Apparatus

5.1.1 *Fluidity Tube* — with a short length of rubber tubing attached to the wider end of the tube (*see* Fig. 1) which is fitted vertically on a stand with a spring clamp.

5.1.2 *Stop-Watch* — to read correct to one-tenth of a second.

5.1.3 *Water-Bath*

5.2 Procedure

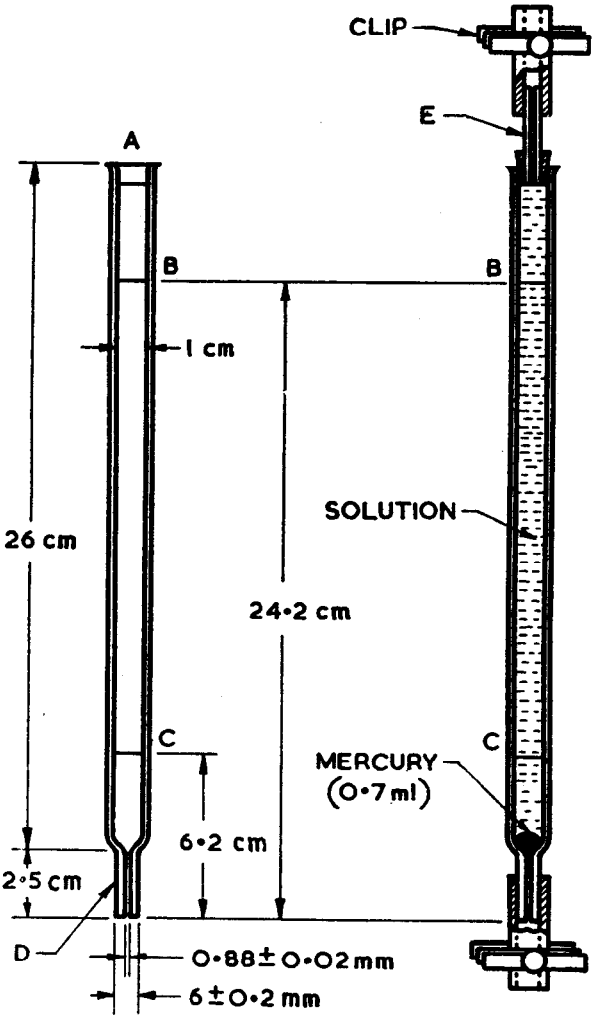
5.2.1 To about 800 ml of vigorously boiling distilled water, add in a thin stream of a dispersion of 40 g of maize starch made in 150 ml of cold distilled water. Stir well, boil for 5 minutes, transfer to 1-litre measuring cylinder and make up the volume with hot distilled water. Mix well and strain through a fine gauze (or mull cloth). Adjust the viscosity of the starch paste with small amount of distilled water so that 50 ml of starch paste when diluted to 100 ml with distilled water gives a flow time of about 80 ± 5 seconds at 50°C.

5.2.2 Weigh out accurately 1 g of the enzyme and 1 g of sodium chloride, dissolve them in distilled water and make up the volume to 1 litre.

5.2.3 Measure out 50 ml of the prepared starch paste (4 percent) in a 100 ml measuring cylinder, dilute to about 90 ml with distilled water, mix well and ascertain the temperature to be below 50°C (in case of testing pancreatic enzymes) or 70°C (in case of bacterial enzymes). Adjust *p*H to an optimum value recommended for the enzyme under test by adding small amount of sodium bicarbonate solution. Add 0·25 ml of the enzyme solution and make up to 100 ml. Mix well and transfer to a 250-ml beaker, and keep in a water-bath maintained at $50° \pm 2°C$ (or $70° \pm 2°C$ as the case may be). Note the time when enzyme is added. At the end of 15 minutes during which time the temperature is carefully maintained, suck into the fluidity tube sufficient amount of liquefied starch-enzyme paste and determine the outflow time of the solution from the top mark to the bottom mark of the fluidity tube.

5.2.4 Repeat the procedure given as in **5.2.3** with 0·5, 0·75, 1·0, 2, 3, 4, 5 and 10 ml of the enzyme solutions and record the corresponding outflow times.

5.2.5 Plot a graph showing the flow time against the corresponding concentration of enzyme used for test.



A = Fluidity tube
B = Top mark
C = Bottom mark
D = Capillary of the fluidity tube
E = Capillary tube

FIG. 1 FLUIDITY TUBE ASSEMBLY

5.3 Repeat the test given in **5.2.1** to **5.2.4** with the other enzymes and similarly draw the graphs (as in **5.2.5**) for each of the other enzymes under test on the same graph paper.

5.4 From the graphs obtained in **5.2.5** and **5.3**, determine the amount of each enzyme under test required for liquefying the starch to the specific arbitrary flow time, say, between 30 to 40 seconds for the particular fluidity tube. Take four readings on the graph near the arbitrary flow time and determine the mean of these readings.

5.5 Compare the results (*see* **5.4**) to determine the relative desizing efficiency of the enzyme under test bearing in mind that higher the amount of enzyme required, lesser is the desizing efficiency.

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