Designation: D3598 – 89 (Reapproved 2009)

Standard Test Method for Citrate in Synthetic Detergents[1]

This standard is issued under the fixed designation D3598; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the enzymatic determination of citrate in both liquid and solid synthetic detergents. The test method is applicable to most detergents containing citrate at a minimum concentration of approximately 5 % (**1-8**).[2]

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Material Safety Data Sheets are available for reagents and materials. Review them for hazards prior to usage.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D501 Test Methods of Sampling and Chemical Analysis of Alkaline Detergents

D1193 Specification for Reagent Water

3. Summary of Test Method

3.1 This test method employs an enzyme system that is based upon the selective cleavage of citrate by citrate lyase (citrate oxaloacetate-lyase; EC 4.1.3.6) (**1**). One of the products, oxaloacetate, is reduced to malate by malic dehydrogenase (L-malate: NAD oxidoreductase; EC 1.1.1.37) with the simultaneous oxidation of reduced β-nicotinamide adenine dinucleotide to β-nicotinamide adenine dinucleotide, oxidized form. The course of the reaction is measured spectrophotometrically. The decrease in absorbance at 340 nm caused by the formation of β-nicotinamide adenine dinucleotide, oxidized form, is directly proportional to the concentration of citrate.

4. Interferences

4.1 The test method is highly specific for citrate. Other organic acids, for example, *cis*and *trans*-aconitic, *d,l*-isocitric, α-ketoglutaric, oxalic, succinic, or tartaric acids, do not interfere.

4.2 Although low levels of zinc or magnesium, or both, are required as an activator for the enzyme citrate lyase, excessively high levels of divalent metallic ions including zinc and magnesium will cause inactivation of the enzyme and potentially interfere with the test method (**7**).

4.3 The test method is not applicable to those detergents containing components with excessive absorptivity at 340 nm such that ultraviolet measurements are inappropriate at 340 nm under test conditions.

5. Apparatus

5.1 *Interval Timer*.

5.2 *Micropipet*, suitable Eppendorf pipets for dispensing 10 and 100-µL volumes and with disposable tips.

5.3 *Spectrophotometer*, suitable for measuring ultraviolet absorbance at 340 nm and equipped with 1-cm matched quartz cells with tapered TFE-fluorocarbon stoppers and a minimum volume of 4 mL.

6. Reagents

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[4] Other grades may be used, provided it is first ascertained that the reagent is of

---

[1] This test method is under the jurisdiction of ASTM Committee D12 on Soaps and Other Detergents and is the direct responsibility of Subcommittee D12.12 on Analysis and Specifications of Soaps, Synthetics, Detergents and their Components.

Current edition approved Oct. 1, 2009. Published December 2009. Originally approved in 1977 as D3598 – 77 T. Last previous edition approved in 2003 as D3598 – 89 (2003). DOI: 10.1520/D3598-89R09.

[2] The boldface numbers in parentheses refer to the references at the end of this test method.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] *Reagent Chemicals, American Chemical Society Specifications* , American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean Type II reagent water conforming to Specification D1193.

6.3 *Citrate Lyase Solution (40 units/mL)*—Add sufficient cold water to a vial of citrate lyase containing a premeasured weight of enzyme protein such that the resulting solution will contain 40 units/mL; for example, 2.0 mL of water is added to a vial containing 5 mg of enzyme protein with an activity of 16 units/mg of enzyme protein. One unit of activity will convert 1.0 µmol of citrate to oxaloacetate per minute at pH 7.6 at 25°C. The citrate lyase solution should be maintained in an ice bath for the duration of the analyses and can be used for 5 days if refrigerated. Citrate lyase (EC 4.1.3.6) from *Aerobacter aerogenes* is commercially available as a lyophilized powder containing approximately 24 % citrate lyase, 24 % albumin, 48 % saccharose and 4 % magnesium sulfate ($MgSO_4 \cdot 7\ H_2O$). The citrate lyase powder should be stored as specified by the supplier.

6.4 *Disodium β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (0.0032 M)*—Dissolve 10 mg of disodiumβ -nicotinamide adenine dinucleotide, reduced form, (β-NADH) in 4.0 mL of water. The β-NADH should be approximately 98 % pure and essentially free of the alpha isomer. The β-NADH solution should be protected from light and maintained in an ice bath for the duration of the analyses. The solution should be prepared fresh daily.

6.5 *Hydrochloric Acid (sp gr 1.19)*—Concentrated hydrochloric acid (HCl).

6.6 *Hydrochloric Acid (1 N)*—Slowly add 85 mL of HCl (sp gr 1.19) to 700 mL of water and with mixing dilute to 1 L with water.

6.7 *Malic Dehydrogenase Solution (2500 units/mL)*— Add sufficient cold water to a vial of malic dehydrogenase (MDH) suspension containing a premeasured volume such that the resulting solution will contain 2500 units/mL; for example, 1.5 mL of water is added to a vial containing 5 mg of enzyme protein in 0.5 mL of suspension with an activity of 1000 µM units/mg of enzyme protein. One micromolar unit of activity will convert 1.0 µmol of oxaloacetate and β-NADH to *l*-malate and β-NAD per minute at pH 7.5 at 25°C. The MDH solution should be maintained in an ice bath for the duration of the analyses and can be used for 5 days if refrigerated. MDH (EC 1.1.1.37) from Porcine heart is commercially available as a suspension in 2.8 *M* ammonium sulfate solution, pH 6. The MDH suspension should contain <0.01 % transaminase activity and should be stored as specified by the supplier.

6.8 *Triethanolamine Buffer Solution (0.1 M, pH 7.6)*—Dilute 6.65 mL of triethanolamine in approximately 250 mL of water. Adjust to pH 7.6 with 1 *N* HCl.

6.9 *Trisodium Citrate Dihydrate Standard Solutions I and II*—Dissolve approximately 150 mg of trisodium citrate dihydrate, accurately weighed, in water and dilute to 100 mL. Dilute 2.0 and 4.0-mL aliquots of this solution each to 100 mL with water. These are the standard Solutions I and II, respectively. Calculate the actual concentration of trisodium citrate dihydrate in each standard solution as follows:

$$C_{I,II} = \frac{S \times V}{10} \quad (1)$$

where:

$C_{I,II}$ = concentration of trisodium citrate dihydrate in the standard Solutions I or II,µ g/mL,
S = standard weight of TSC, mg, and
V = volume taken for the final dilution, mL.

Prepare all solutions fresh daily.

6.10 *Zinc Chloride Solution (0.003 M)*—Dissolve 41 mg of zinc chloride in 100 mL of water.

7. Sampling

7.1 Collect the sample in accordance with Test Methods D501.

8. Procedure

8.1 Dissolve an accurately weighed detergent sample equivalent to approximately 300 mg of trisodium citrate dihydrate in distilled water and dilute to 200 mL. Dilute a 3.0 mL aliquot of this solution to 100 mL with water. This is the sample test solution.

8.2 During the following steps, use the appropriate micropipet for the 10 and 100-µL volumes, replacing the tip after each addition. Standard volumetric pipets can be used for the 1.0 and 2.0-mL additions.

8.3 Into a 1-cm quartz cell, pipet 1.0 mL of either a water blank, standard Solutions I or II, or a sample test solution.

8.4 Pipet 2.0 mL of the triethanolamine buffer solution, 100 µL of the β-NADH solution, and 100 µL of the zinc chloride solution into the cell.

8.5 Pipet 10 µL of the MDH solution below the liquid surface in the cell and start the interval timer. Stopper the cell and mix by inverting several times. Do not shake the cell so as to cause foaming.

8.6 After 2.0 min, measure the absorbance (A_1) at 340 nm versus water.

8.7 After an additional 1.0 min, pipet 10 µL of the citrate lyase solution below the liquid surface in the cell. Stopper the cell and mix by inverting several times. Again do not shake the cell too vigorously.

8.8 After an additional 3.0 min, measure the absorbance (A_2) at 340 nm versus water.

9. Calculation

9.1 Calculate the trisodium citrate dihydrate standard factor ($F_{I,II}$) for each of the standard solutions as follows:

$$F_{I,II} = \frac{\Delta A_{STD} - \Delta A_B}{C} \quad (2)$$

where:

$\Delta A_{STD} = (A_1 - A_2)$ = decrease in absorbance due to the trisodium citrate dihydrate content of the standard solution,
$\Delta A_B = (A_1 - A_2)$ = decrease in absorbance for the water blank, and
C = concentration of trisodium citrate dihydrate in the standard solution, µg/mL.

9.2 Calculate the trisodium citrate dihydrate content of the sample as follows:

$$\text{Trisodium citrate dihydrate, \%} = \frac{\Delta A_{\text{SAMPLE}} - \Delta A_B}{W \times F_{\text{AVG}} \times 1.5} \quad (3)$$

where:

$\Delta A_{\text{SAMPLE}} = (A_1 - A_2)$ = decrease in absorbance due to the trisodium citrate dihydrate content of the sample,

$\Delta A_B = (A_1 - A_2)$ = decrease in absorbance for the water blank,

W = sample weight, g, and

$F_{\text{AVG}} = F_1 + F_{II}/2$ = average of the factors calculated for each of the standard solutions.

10. Precision and Bias

10.1 Under the most favorable conditions the precision may be expressed as follows:

$$S_o = 0.02X \quad (4)$$

where:

S_o = single-operator precision, % w/w, and

X = trisodium citrate dihydrate content, % w/w.

11. Keywords

11.1 citrate; enzyme cleavage; synthetic detergents

REFERENCES

(1) Taraborelli, J. A., and Upton, R. P.," Enzymatic Determination of Citrates in Detergents," *Journal of the American Oil Chemists' Society,* JAOCA, Vol 52, p. 248.

(2) Berka, A., and Hilgard, S., "Bestimmung organischer Stoffe durch Oxidation mit Permanganat. IV. die Oxidation von Milch, Apfel, Zitron und Salicylsaure," *Mickrochim Acta,* Vol 174, 1966.

(3) Sucha, I., and Volka, K., "Anwendung von Zitratkomplexen mit Ionen des Systems Fe^{+3}/Fe^{+2} bei der Massanalytischen Bestimmung von Zitronensauren." *Collection of Czechoslovak Chemical Communications,* CCCA, Vol 29, 1964, p. 1361.

(4) Hartford, C. G., "Rapid Spectrophotometric Method for the Determination of Itaconic, Citric, Aconitic, and Fumaric Acids," *Analyt-ical Chemistry,* ANCHA, Vol 34, 1962, p. 426.

(5) Pucker, G. W., Sherman, C. C., and Vickery, H. B., "A Method to Determine Small Amounts of Citric Acid in Biological Material," *Journal of Biological Chemistry,* JBCHA, Vol 113, 1936, p. 235.

(6) Dagley, S., in *Methods of Enzymatic Analysis,* edited by Bergmeyer, H. U., Verlag Chemie Weinheim and Academic Press, New York, NY, 1965, p. 313.

(7) Moellering, J., and Gruber, W., "Determination of Citrate with Citrate Lyase," *Analytical Biochemistry,* ANBCA, Vol 17, 1966, p. 369.

(8) Bergmeyer, H. U., *Methods of Enzymatic Analysis,* Verlag Chemie Weinheim and Academic Press, New York, NY, p. 38, 1965.