Designation: D 3598 – 89 (Reapproved 1995)ε1

Standard Test Method for Citrate in Synthetic Detergents[1]

This standard is issued under the fixed designation D 3598; 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.

ε[1] NOTE—Keywords were added editorially in February 1995.

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 *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:*

D 501 Test Methods of Sampling and Chemical Analysis of Alkaline Detergents[3]

D 1193 Specification for Reagent Water[4]

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.[5] Other grades may be used, provided it is first ascertained that the reagent is of 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 D 1193.

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

[1] This test method is under the jurisdiction of ASTM Committee D-12 on Soaps and Other Detergents, and is the direct responsibility of Subcommittee D12.12 on Analysis of Soaps and Synthetic Detergents.

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

[3] *Annual Book of ASTM Standards*, Vol 15.04.

[4] *Annual Book of ASTM Standards*, Vol 11.01.

[5] *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. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.