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Laboratory Analysis of Toothpastes Containing Anticaries Agents

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Synopsis—In addition to the usual physical and chemical laboratory tests applied to cleansing toothpastes, analyses for the water-soluble anticaries species are required for evaluation of an anticaries toothpaste. Methods to determine stannous and fluoride ions in the toothpaste are described and discussed.

Toothpaste formulations containing anticaries agents should have all the characteristics, such as cleansing action, feel, and flavor, desired in a toothpaste which has good consumer acceptability. In addition, the abrasive and other agents present in the formulation and the tube must be compatible with the anticaries agent. In order to achieve these aims, knowledge of the physical and chemical characteristics of the anticaries agent is required. Specific analytical procedures for the agent and the products of its decomposition are necessary to define its stability in the presence of the other ingredients in the formulation. A thorough knowledge of the anticaries agent should be coupled with comparable information and analytical procedures for the other ingredients in the formulation, such as abrasives, humectants, binders, and foaming and flavoring agents. Complete information on available toothpaste ingredients and the anticaries agent should result in the selection of a combination of components to give the best over-all product.

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Some commercial toothpastes contain either stannous or sodium fluoride as anticaries agents. Stannous fluoride is described in the National Formulary, XII (1) and sodium fluoride in the U. S. Pharmacopoeia XVII (2). Qualitative and quantitative tests for stannous fluoride as well as other data on its reactions and stability have been described (3). Manly reported the soluble stannous and fluoride ion analysis of two stannous fluoride-containing toothpastes (4). The following procedures, which are based on an earlier publication (3), can be used with minor modifications on available toothpastes containing fluoride salts as anticaries agents.

Procedure

Preparation of sample: To a tapered 50 ml plastic centrifuge tube are added 10 g of paste, accurately weighed to the nearest 0.1 g, and 30 ml of water. The mass is hand-mixed with a stirring rod fitted with flared polyethylene or surgical rubber tubing for two minutes or until a smooth slurry is obtained. The tube is capped and centrifuged at 15,000 rpm for five minutes to obtain a clear supernatant. Total centrifuging time is about 15 minutes.

Fluoride Ion

The underside of the cover of a 54 mm Millipore®* plastic Petri dish is coated with an even film of potassium hydroxide by allowing 0.1 ml of 0.5 *N* alcoholic potassium hydroxide to evaporate on the cover. To one side of the bottom of the Petri dish is added 1.0 ml of perchloric acid and to the other side 0.3 ml of the suitably diluted supernatant equivalent to about 1–2 γ of fluoride ion. Two drops of a 0.1% solution of Tergitol®† are added, the cover is replaced, and the mixture is gently swirled. To diffuse the fluoride from the sample, the Petri dishes are placed into an oven at 65°C for 24 hours. Using small portions of water, the potassium hydroxide layer on the cover containing the diffused fluoride is quantitatively transferred to a 10 ml volumetric flask. Exactly 1 ml of SPADNS (see below) reagent is added. After diluting to the mark with water and mixing, the absorbance of the solution is read in a suitable colorimeter or spectrophotometer at 594 mμ against SPADNS reference solution. A standard curve is prepared similarly, as follows. Aliquots of a standard fluoride solution equivalent to 0, 1, 2, and 3γ of fluoride ion are diffused in the Petri dishes. The potassium

* Millipore Filter Corporation. Bedford, Mass.

* A nonionic NPX detergent, Union Carbide Corporation.

hydroxide phase is transferred to a 10 ml flask, 1 ml of SPADNS reagent is added, and the mixture is diluted to the mark with water. The degree of absorbance, which is inversely proportional to concentration of fluoride ion, is measured against the reference solution at 594 mμ.

Reagents

Solution A Exactly 3.16 g SPADNS (trisodium 4,5-dihydroxy-3(*p*-sulfophenylazo)-2,7-naphthalene disulfonate, Eastman No. 7309) is dissolved in 550 ml of water.

Solution B Precisely 0.133 g of zirconium oxychloride octahydrate, $ZrOCl_2 \cdot 8H_2O$, is transferred to a 500 ml volumetric flask and dissolved in 50 ml of water. To this are added 350 ml of hydrochloric acid and enough water to the mark.

Reference Solution To 50 ml of Solution A are added 500 ml of water and 35 ml of hydrochloric acid. This solution is stable.

SPADNS Reagent Equal volumes of Solutions A and B are mixed and stored in an amber plastic bottle.

Stannous Ion

A titration assembly, including a 250 ml beaker fitted with a tight rubber cover, stream of high purity nitrogen, platinum combination electrode, and suitable potentiometric equipment are prepared. An appropriate aliquot of the clear supernatant, equivalent to about 1500γ of stannous ion, is added. About 100 ml of 0.6 *N* hydrochloric acid, which has been previously degassed with high purity nitrogen, is added quickly, and the mixture is titrated with 0.005 *N* potassium iodate. Using the same apparatus, reagents, and timing, the potassium iodate is standardized with standard sodium thiosulfate. One ml of 0.005 *N* potassium iodate is equivalent to 297γ of stannous ion.

Discussion

Soluble ion analysis has been performed on a hand-stirred slurry of 1 part paste and 2 or 3 parts water. Although this 1:2 or 1:3 ratio is about that normally occurring during toothbrushing, many laboratories prefer to use a 1:10 dilution with water. This slurry may be somewhat more convenient to centrifuge and handle, but it is subject to the criticism of excessive dilution and thus the possibility of a higher soluble ion content.

It is important that soluble ion analysis be carried out on the clear supernatant. Most formulations will contain insoluble species which

could alter the results if not completely separated by centrifugation. The concentration and type of gum used in the formulation will have a pronounced effect on the speed and time required for centrifugation. Centrifuging at 1500 rpm for 30–60 minutes can achieve reasonable phase separation of slurries of most toothpaste formulations. Generally higher centrifugation speeds, of the order of 15,000 rpm, are necessary to achieve a clean separation in a reasonably short time.

The determination of fluoride in toothpaste formulations is a micro procedure, subject to many interferences from species normally present in the toothpaste but also from the chemical reagents, laboratory ware, and equipment used. Utmost care is required to insure consistently clean plastic and glassware, pure reagents, especially water, and reproducible dispensing of reagents.

Most toothpaste formulations contain sulfate and phosphate salts which will interfere with colorimetric fluoride determinations. Thus, it is necessary to separate the fluoride from the interfering soluble ions by diffusion or distillation with perchloric acid, or by pyrolysis (3). The diffusion procedures are usually preferred because a large number of samples can be run at the same time with less equipment and handling per sample. The alkali mixture containing the diffused fluoride can be easily transferred using an aspiration technique (5). The plastic micro Conway dishes, suggested by Wharton (6), are easy to use but require silicone grease to seal the cover to the base. After a determination, rather than wash the Conway dishes with a detergent it is preferable to rinse the dishes thoroughly with purified water and re-use them. From time to time a thorough soaking in alcoholic potassium hydroxide may be necessary to remove silicone contamination of the sample portion of the dishes and volumetric glassware. The Petri dishes, which were suggested by Rowley and Farrah (7), are normally discarded after one determination; however, they can be washed in a phosphate-free detergent, rinsed, and re-used.

The soluble stannous ion concentration in a toothpaste formulation tends to decrease as a function of time. This decrease is accelerated manifold when the toothpaste is diluted with water in the preparation of a slurry for centrifugation. Conditions necessary to hold this loss of soluble stannous ion to a minimum would include the use of a purified nitrogen atmosphere within the capped plastic centrifuge tubes, pre-cooling the centrifuge head at 0°C, and centrifugation at high speeds for a short time. A typical centrifugation of 400 g of toothpaste slurries at 15,000 rpm for five minutes results in a temperature rise of 10°C.

This procedure usually causes a loss of about 5–10% of the soluble stannous ion content present in the slurry.

The iodimetric titration of stannous ions involves oxidation to stannic ions with concomitant conversion of iodate to iodide. Although the usual starch-iodine color reaction can be used to detect the endpoint of this titration, it is desirable to follow the reaction potentiometrically because the detergents present in the formulation may interfere with the colorimetric endpoint.

Oxygen contamination of the reagents and the time required to carry out the titration have a pronounced effect on the number of milliequivalents of iodine required. All reagents should be degassed with high purity nitrogen. Water, degassed by boiling, can be used; however, polarographic measurements suggest that nitrogen degassing is more efficient (3).

Tin metal or arsenic oxide can be used for standardization of the iodine titrant (3). It is usually more convenient to use a recently standardized sodium thiosulfate solution. Since the over-all time of the procedure can have an effect on the titration, it is desirable to use semi- or automatic titration equipment which will tend to replicate the timing and other conditions of each determination. The standardization procedure should be done in a similar manner.

Total ion analysis is most useful for control of the manufacturing process since it can be used to indicate the amount of the particular species added. Assuming complete solubility at the concentration used, the difference between total and soluble ion analysis is a measure of the stability and compatibility of the agent and the other ingredients of the formulation.

One of the major problems in total ion analysis is trace quantities of fluoride present in other ingredients of the formulation. Rather than conduct a total ion analysis of each batch of each ingredient used in the formulation, analysis can be done on a sample of each batch of the complete formulation with no anticaries agent added.

For total fluoride analysis, the fluoride in the paste must be fixed with alkali so that it will not volatilize during subsequent treatment. Because of the variety of formulations it is difficult to recommend a procedure which is generally applicable. Ashing with calcium oxide is probably the most general procedure. If the sample contains a large excess of a calcium salt, making the paste alkaline with sodium hydroxide may be adequate. After the sample has been ashed at about 600°C

for one and one-half hours, the fluoride can be separated from the interfering ions and determined by the procedure described above.

For total tin concentration the toothpaste sample should be dissolved in dilute hydrochloric acid. Since some samples will not dissolve completely in dilute hydrochloric acid, it may be necessary to assume that all the tin salts will be soluble. They can then be reduced to stannous ion by boiling in an inert atmosphere with a mixture of nickel and iron powder in sulfuric acid (3). The total stannous ion concentration can be obtained by direct titration of the hydrochloric acid soluble portion of the paste. It is important to remember that stannous ion is not a stable species, even in strong hydrochloric acid.

Summary

Soluble ion analyses are probably the most commonly used tests applied to toothpastes containing anticaries agents. These analyses are done on the clear supernatant obtained from high speed centrifugation of a water slurry of the paste. Fluoride ion can be determined subsequent to separation by diffusion from interfering ions by the SPADNS colorimetric procedure. Stannous ion can be determined by a titrimetric iodine procedure. Both fluoride and stannous ion procedures are subject to a number of factors which can substantially alter the results obtained from analysis.

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