The Determination of Polysorbate 60 in Foods[1]

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ABSTRACT

Analytical methods have been developed for the determination of polysorbate 60 (Tween 60, Atlas Chemical Industries, Inc.) in several food products including baked bread and cake, cake mix, yeast-raised doughnuts, shortening, foam-mat dried tomato paste and citrus fruit juices, dressings for salad, and potato flakes. Most of the methods involve extraction of the polysorbate 60 from the sample with a suitable solvent. In all cases the polysorbate polyol moiety is recovered, desalted by ion exchange and measured gravimetrically as the barium phosphomolybdate complex. The polysorbate 60 content of the sample is calculated using a gravimetric factor obtained by subjecting known amounts of polysorbate 60 to the same analytical procedure. Each method is designed to eliminate interferences from other constituents of the food so that blank samples are unnecessary.

INTRODUCTION

Polysorbate 60, hereafter denoted by PSB-60, is a food additive approved by the FDA for a number of applications. It is often employed in conjunction with other emulsifiers to impart certain desirable qualities to the food product. Used in conjunction with monoglycerides, it is an excellent dough conditioner and enhances the softness retention properties of mono- and diglycerides in yeast-leavened bakery goods such as breads and doughnuts. In cake and cake mixes, PSB-60 may be combined with one or more emulsifiers to give a close uniform grain, improved texture, larger cake volume, and increased moistness. In products such as dressings for salad, the function of the PSB-60 is simply that of an efficient surfactant which maintains the stability of the emulsion. In an experimental study for the production of foam-mat dried products such as dried tomato paste and dried citrus fruit juices, PSB-60 acts as a foaming agent which increases the efficiency of the drying process. For reasons not fully understood, but probably related to the complexing reaction with starch, the presence of PSB-60 in potato flakes reduces unwanted stickiness. It should be noted that for the last two categories, foam-mat dried products and potato flakes, no request for approval of the use of PSB-60 has been filed with the FDA.

Analytical procedures of suitable accuracy and precision were required for the quantitative determination of this versatile surfactant at low concentration levels in the diverse food products cited. Generally, levels of use range in concentration from 0.1% to 0.5%, depending upon the particular application.

The determination of PSB-60 in a food product is a two phase problem: (a) it must be isolated from other components of the particular food, e.g., starches, fats, sugars, etc., which might otherwise interfere with the analysis, and (b) a suitable quantitative measurement of the PSB-60 in the isolate must be conducted.

The problem of isolating the PSB-60 from the food product was solved in most cases by careful selection, based on experiment, of optimum extracting solvents. Likewise it was necessary to develop different techniques of sample preparation and extraction for particular foods.

PSB-60 is a complex, but reproducible mixture of polyoxyethylated fatty acid esters of sorbitol and its anhydrides. In choosing a method for quantitative measurement, attention was directed to the polyoxyethylated portion, i.e., polyol moiety of the molecule, since it would be impossible to distinguish the fatty acid moiety from that of natural fats and added shortenings found in many foods.

The polyoxyethylated polyol moiety is detectable by a wide variety of methods including a resorcinol-glucose precipitation, a colorimetric method using ammonium cobaltothiocyanate reagent, a method based on reaction with hydriodic acid, a turbidimetric procedure and methods based on complex formation and precipitation with an alkaline earth metal and silicotungstic or phosphomolybdic acid. Shaffer and Critchfield (1) originally developed a colorimetric procedure for polyethylene glycols based on the barium phosphomolybdic complex. Other workers including Oliver and Preston (2) used the same reaction in a gravimetric procedure. The latter procedure has been found to offer the simplicity, specificity and sensitivity required for the analysis of the food products investigated.

EXPERIMENTAL PROCEDURES

PSB-60 is separated from the food product by a suitable extraction procedure. In a few instances, e.g., shortening and dressing for salad, no separation is necessary. The sample or extract is saponified directly with alcoholic potassium hydroxide. After acidification, the fatty acids are removed by extraction with hexane. The aqueous polyol solution is passed through a mixed-bed ion exchange column to remove ionic impurities. The PSB-60 polyol in the column effluent is precipitated as the barium phosphomolybdate complex. The precipitate is filtered into a preweighed Gooch crucible, dried and weighed. Finally, the PSB-60 content of the sample is calculated, using a gravimetric factor which correlates weight of complex per unit weight of PSB-60. The factor is obtained by subjecting known amounts of PSB-60 to the analytical procedure.

Apparatus

Extraction apparatus, Soxhlet (two sizes) extraction tubes, i.d. 40 and 50 mm. Extraction thimbles, Whatman, 80 x 33 mm and 123 x 43 mm. Extraction flasks, capacity 250 and 1000 ml. Fritted glass filter funnel, capacity ca. 350 ml; porosity, coarse. Erlenmeyer flasks, alkali resistant, capacity 300 ml. Ion exchange column, 28 mm i.d., 300 mm length, Teflon stopcock, coarse fritted disc resin bed support. Gooch crucibles, size 4.

Reagents

Solvents include chloroform and *n*-propanol, both of reagent grade, absolute ethanol, U.S.P., and redistilled commercial grade hexane. Mixtures of solvents used for particular extraction applications include *n*-propanol 72%–water 28%, and chloroform 93%–absolute ethanol 7%. Alcoholic potassium hydroxide (1 N in absolute ethanol). Hydrochloric acid, concentrated, and 3 N Barium chloride dihydrate, 10% solution. Phosphomolybdic acid, 10% solution. Asbestos, medium fibre, Gooch grade. Mixed-bed ion exchange resin – Illco Exchange Resin Research Grade TMD-8 (anion dyed) available from Illinois Water Treatment Co., Rockford, Illinois was used. Hyflo Super-Cel (Johns-Manville Products Corp.) was used; any nonadsorbing filter aid may be equally suitable.

[1]Presented at the AOCS Meeting, San Francisco, April 1969.

TABLE I

Recovery of PSB-60 in Foods

Food	Number of samples	Level(s) of PSB studied (% d.b.)	Recovery, % Range	Recovery, % Mean
Baked bread	10	0.270, 0.450	87.0-90.2	89.0
Raised doughnuts	10	0.282	88.3-92.6	91.3
Baked cake	10	0.375	89-96	92.0
Chocolate cake mix	10	0.292	96-101	99.0
Foam-mat dried tomato paste	5	0.43, 0.094	96-106	100.0
Foam-mat dried lemon juice	7	0.102, 0.500, 1.000	82-113	94.0
Dressing for salad	4	0.27	104-108	106.0
Shortening	7	0.60, 1.00, 2.00	95-108	101.0
Potato flakes	10	0.09, 0.36	78-122	96.0

Sample Size

The sample taken for analysis should contain from 10 to 40 mg of PSB-60 for optimum results.

Baked Bread, Baked Cake, and Raised Dougnuts

Take a representative sample of the baked bread or cake (at least 100 g) and dry in a vacuum oven at 45-50 C to constant weight. Pulverize the dried material in a Waring Blendor and mix well. In the case of doughnuts cut away and discard the outside shell of the doughnuts which contains absorbed frying oil. Dry the central portion of the doughnuts to constant weight in a vacuum oven, as described for bread. Pulverize the dried doughnuts in a Waring Blendor and mix well. Weigh a sample of the appropriate size (not more than 5 g for bread and not more than 3 g for cake or doughnuts) into a 150 ml beaker and mix well with 5 g of Super-Cel. Transfer the mixture to an 80 x 33 mm Soxhlet thimble and extract for 48 hr with the azeotropic binary distillate, *n*-propanol-water, in a 40 mm i.d. Soxhlet apparatus fitted with a 250 ml flask. Adjust the heat such that siphoning occurs at intervals of not longer than 20 min. The extraction directions given here must be followed strictly to obtain satisfactory recoveries. Transfer the extract to a 300 ml alkali resistant flask, insert a boiling rod and evaporate to dryness by supporting the flask in an opening of a steam bath. Add 50 ml of 1 N alcoholic potassium hydroxide and reflux for 45 min on a hot plate. Add 30 ml of water and continue refluxing for an additional 45 min. Transfer the saponified mixture to a 250 ml separatory funnel with 50 ml of hot water. Add 4.5 ml concentrated HCl and mix contents by swirling. Cool somewhat so that the funnel may be held comfortably in the hand. Add 50 ml of hexane, shake vigorously and allow the layers to separate. Withdraw the lower layer into a second separatory funnel and extract with another 50 ml portion of hexane. Withdraw the lower layer into a 600 ml beaker. Combine the hexane extracts and wash with two 20 ml portions of 1:1 alcohol-water. Combine the aqueous alcoholic extracts in the 600 ml beaker and evaporate in the steam bath (lip of beaker supported on edge of bath opening) to a volume of about 50 ml. Cool to room temperature. Prepare a mixed-bed ion exchange column by pouring an aqueous suspension of the mixed-bed resins into a glass column of approximately 28 mm i.d. to a height of 12 in. Add the polyol solution to the column and adjust the flow rate to approximately 2 ml/min. Collect the effluent in a 600 ml beaker. Rinse the residual solution in the beaker into the column, rinse the sides of the column with water and finally wash the resin bed with 200 ml of water. The rate may be increased to 3 to 4 ml/min during the washing of the column. Adjust the combined column effluent and washings to a volume of 300 ml and heat for 15 min or more in the steam bath in the manner previously indicated. Remove from the steam bath and add 2.0 ml of 3 N hydrochloric acid, 4.0 ml of 10% barium chloride solution and 4.0 ml of 10% phosphomolybdic acid solution to precipitate the barium phosphomolybdate-polyol complex. Stir the solution and allow it to stand overnight. Filter the contents of the beaker through a preweighed Gooch crucible provided with an asbestos mat, previously dried at 110 C. Use a rubber policeman and, with the aid of a water wash bottle, complete the transfer of the precipitate. Wash the precipitate in the Gooch crucible with 50 ml of distilled water. Dry the crucible and precipitate in an oven at 110 C for 1 hr. Cool in a desiccator and weigh. A gravimetric factor correlating the weight of complex per unit weight of PSB-60 must be obtained by carrying known quantities of PSB-60 through the analytical procedure starting with the saponification step.

Calculation. Per cent of PSB-60 in baked food product (dry basis)

$$= \frac{C \times 100}{F \times W}$$

where: C = grams of dry precipitate, W = gram sample of dried product, F = gravimetric factor representing the weight of complex per unit weight of PSB-60.

Cake Mix

Weigh a sample of the appropriate size (not more than 5 g) into a 150 ml beaker and mix thoroughly with 7 g of Super-Cel. Transfer the mixture to a Soxhlet thimble. Extract in a 40 mm i.d. Soxhlet apparatus for 48 hr using the azeotropic binary distillate, chloroform-ethanol, as the extraction solvent. Transfer the extract to a 300 ml alkali resistant flask and evaporate to dryness in a steam bath using a boiling rod. To ensure complete removal of chloroform from the residue, add 50 ml of absolute ethanol and evaporate to dryness. Repeat the evaporation with an additional 50 ml of ethanol. Complete the analysis as directed under the procedure for baked bread, starting with the saponification step.

Shortening

No sample preparation of extraction is necessary for shortening. Weigh a sample of an appropriate size (not more than 9 g) into a 250 ml alkali resistant flask. Follow the procedure given under the analysis of baked bread, starting with the saponification step.

Foam-Mat Dried Tomato Paste and Citrus Fruit Juices

Weigh a sample of appropriate size (not more than 20 g in the case of dried tomato paste and not more than 10 g in the case of dried citrus fruit juices) into a 1000 ml round bottom flask. Add 50 to 100 ml of distilled water depending on sample size and swirl with warming on a steam bath until the sample is completely dispersed. Add 10 g of Super-Cel with stirring to make a smooth paste.

Add chloroform to the flask until approximately half full, attach a reflux condenser via a trap for the continuous removal of water. Reflux the mixture on a steam bath until all water has been azeotropically distilled out of the sample. Periodically discard the chloroform-water distillate in the trap. Filter the chloroform solution in the flask through a coarse sintered glass funnel into a 1000 ml suction flask. Wash the solids on the funnel with several portions of chloroform. Discard the insoluble matter. Evaporate the chloroform filtrate to dryness in a 300 ml alkali resistant flask on the steam bath. To ensure complete removal of chloroform from the residue, add 50 ml of absolute ethanol and evaporate to dryness. Complete the analysis as directed in the procedure for baked bread, starting with the saponification step.

Dressing for Salad

No sample preparation or extraction is necessary for dressings. Weigh a sample of the appropriate size (not more than 5 g, dry basis) into a 250 ml alkali resistant flask. Follow the procedure given under the analysis of baked bread, starting with the saponification step.

Potato Flakes

Weigh a sample of an appropriate size (not more than 12 g) into a 250 ml beaker and add 5 g of Super-Cel. Add a few milliliters of the solvent mixture consisting of 72% *n*-propanol-28% water and mix well. Transfer the mixture to a 123 x 43 mm Soxhlet thimble, cover with a wad of cotton and extract for 24 hr with the azeotropic distillate, *n*-propanol-water, in a 50 mm i.d. Soxhlet apparatus fitted with a 1000 ml extraction flask. Transfer the extract to a 300 ml alkali resistant flask and evaporate to dryness in the steam bath, adding absolute alcohol near the end to help remove remaining water. Add 50 ml of 1 N alcoholic potassium hydroxide solution and reflux for 45 min. Add 30 ml of water and continue refluxing for an additional 45 min. Transfer the saponified mixture to a 250 ml separatory funnel with 20 ml of water followed with about 50 ml of 1:1 alcohol-water dispensed from a wash bottle. Add 4.5 ml of concentrated HCl and mix the contents by swirling. Add 50 ml of hexane, shake vigorously and allow the layers to separate. Withdraw the lower layer into a second separatory funnel and extract with another 50 ml portion of hexane. Withdraw the lower layer into a 600 ml beaker. Combine the hexane extracts and wash with two 100 ml portions of 1:1 alcohol-water. Combine the aqueous alcoholic extracts in the 600 ml beaker and evaporate in the steam bath to a volume of about 50 ml. Complete the analysis as directed in the procedure for baked bread, beginning with the ion exchange of the polyol solution.

RESULTS AND DISCUSSION

Regular production batches of PSB-60 were subjected to the analytical procedure beginning with the saponification step to obtain a gravimetric factor relating weight of complex per unit weight of PSB-60. Gravimetric factors determined on ten batches were found to have a mean value of 2.74 (range 2.71-2.78; standard deviation, 0.026). The close agreement of data indicates that PSB-60 is a consistently reproducible product from batch to batch.

Table I lists the results obtained for the analysis of baked bread, raised doughnuts, baked cake, chocolate cake mix, dried tomato paste, dried lemon juice, dressing for salad, shortening, and dried potato flakes.

The absence of any precipitation upon the addition of the barium phosphomolybdate reagent in the analysis of blank samples shows that there is no interference by contribution caused by the other constituents of the various food products, when the recommended extraction procedures are employed.

It is noted that the mean recoveries of PSB-60 in cooked foods are about 90%, while those in uncooked foods are in general more nearly complete. The somewhat low, but nevertheless acceptable recovery of PSB-60 from the cooked foods, all of which contain starch, is attributed to a small degree of irreversible adsorption or complexation, or both, which does not yield to the most expedient of solvents studied.

The apparent wide ranges of recoveries in the dried lemon juice and dried potato flakes products are explained by the difficulties attending the preparation of reasonably accurate and precise samples of these products for analysis.

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REFERENCES

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