Spectrophotometric Method for Quantitation of Peroxides in Sorbitan Monooleate and Monostearate

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Abstract □ A rapid and sensitive spectrophotometric method has been developed to quantitate the peroxides present in sorbitan monooleate and monostearate. The method relies on the peroxide conversion of iodide to iodine. The method has also been found to work for polysorbate 60.

Keyphrases □ Peroxides—sorbitan monooleate and monostearate by spectrophotometry □ Sorbitan monooleate—spectrophotometric quantitation of peroxides □ Sorbitan monostearate—spectrophotometric quantitation of peroxides

Nonionic surfactants, used extensively as emulsifying agents, are integral components of semisolid dosage forms. However, surfactants tend to form peroxides, which have caused stability problems in numerous oxidizable drugs (1-3). The formation of peroxides can also lead to chemical modifications in the surfactants themselves (*e.g.*, chain shortening) that may unfavorably modify their physical properties (1, 4, 5). Titrimetric assay methods have been developed to determine the peroxide content in surfactants, but most of these are limited to the water soluble surfactants, *e.g.*, polyoxyethylene surfactants (6, 7). One titrimetric method, that has been used to quantitate the peroxides present in fats and oils, relies on the partitioning of the desired fat or oil between an organic and aqueous layer. The fat or oil is then transferred with "constant and vigorous shaking" from the organic layer to the aqueous layer where the actual titration occurs (8). Another limitation of titrimetric quantitation of peroxides can arise when methylene blue is used as an indicator. With the yellow surfactant sorbitan monooleate, for example, the color change at the end point, from green to aqua, is extremely difficult to detect.

In the course of our studies of the stability of steroids in surfactant systems, an analytical method was needed to quantitate the peroxides that were initially present in the surfactants. This method also had to be sensitive enough to ensure that the peroxide content had been significantly reduced in each of the surfactants following a purification procedure designed to eliminate peroxides from the surfactants.

To overcome the limitations of water insolubility and a difficult titrimetric assay, we have developed a rapid and sensitive spectrophotometric method to quantitate the peroxides present in sorbitan monostearate and monooleate, two poorly water soluble surfactants. This method was also shown to work for polysorbate 60.

EXPERIMENTAL SECTION

Materials and Instrumentation—Peroxides were removed from polysorbate 60[1], sorbitan monostearate[1], and sorbitan monooleate[1] according to the method of Segal *et al.* (9), with modifications. Hexane was substituted for methylene chloride to solubilize sorbitan monooleate, and hexane with ~5% methylene chloride was used to solubilize sorbitan monostearate.

The following reagents were used: resublimed iodine[2], potassium iodide[2], sodium thiosulfate[2], sodium metabisulfite[2], reagent grade isopropyl alcohol[2], methylene blue[3], and double-distilled water. UV spectral scans and absorbances were obtained on a rapid-scanning UV-VIS spectrophotometer[4].

Titrimetric Peroxide Number Determination of Polysorbate 60 and Sorbitan Monooleate—To 2.0 g of surfactant was added 3 mL of glacial acetic acid-methylene chloride (3:2). The mixture was stirred until a clear solution was obtained. After 50 μL of a KI-saturated aqueous solution was added, the mixture was stirred for exactly 2.0 min and 5 mL of isopropyl alcohol and 100 μL of an aqueous 0.025% methylene blue solution were added. The solution was titrated with standardized sodium thiosulfate to an aqua end point.

Iodine Standards for Spectrophotometry—A 0.010 M iodine standard was prepared by dissolving iodine in an aqueous 5% KI solution. This standard solution was further diluted with the 5% KI solution to give an iodine concentration of 10^{-4}-10^{-7} M. Iodine standards containing 2% purified surfactant were also prepared as described above at an iodine concentration range of 10^{-4} to 5×10^{-6} M.

Spectrophotometric Analyses of Polysorbate 60 and Sorbitan Monooleate—Aqueous saturated KI solution (50 μL) was added to 2 g of surfactant dissolved in 3 mL acetic acid-methylene chloride (3:2) and the solution was stirred under a blanket of nitrogen for 2 min. A 5.0-mL portion of isopropyl alcohol was added and immediately 200 μL of this solution was diluted to 2.0 mL with isopropyl alcohol. The absorbance at 360 nm was then measured within 2-3 min after mixing. Two blanks were determined under the same conditions, one without surfactant, and the other without KI.

Spectrophotometric Analysis of Sorbitan Monostearate—The procedure followed for analysis of sorbitan monostearate was exactly the same as for sorbitan monooleate with one exception: prior to the final addition of 2.0 mL of isopropyl alcohol, 100 μL of methylene chloride was added to maintain the solubilization of the monooleate.

RESULTS AND DISCUSSION

This spectrophotometric method takes advantage of the iodine generated from the reaction of peroxides on iodide (6). From the stoichiometry of the reaction, 1 mol of iodine is produced for each mole of hydroperoxide present. The quantitation of iodine formed is thus a direct measure of the peroxides present in the sample[5].

In the absence of surfactant, the absorbamce at 360 nm was obtained as a function of the concentration of iodine. Beer's law was obeyed over the 10^{-4}-10^{-7} M iodine concentration range. Beer's law was also followed in the presence of purified sorbitan monostearate at an iodine concentration range of 10^{-4}-10^{-6} M at 360 nm. The molar absorptivities in the absence of surfactant and in the presence of sorbitan monostearate were 24,000 M^{-1} cm^{-1} and 27,000 M^{-1} cm^{-1}, respectively, indicating that purified surfactant had little effect on the value of the molar absorptivity at 360 nm.

Two solutions prepared for peroxide quantitation by the spectrophotometric method, one without KI and the other without surfactant, showed no absorbance at 360 nm. Therefore, the absorbance determined in samples assayed by this method is due entirely to the generation of iodine by the peroxides present in the surfactant.

To eliminate iodine fading[6], one of the complications of iodimetric assays, part of the experimental procedure was conducted under a blanket of nitrogen. This nitrogen blanket provided several minutes to obtain the final absorbance reading after the dissolution of the surfactant. When the nitrogen blanket was not used, iodine fading was detected immediately after dilution with the final portion of isopropyl alcohol.

The quantitation of peroxides is frequently expressed in terms of the peroxide number (PN). By definition, the PN is the number of equivalents of peroxide contained in 1000 g of surfactant.

[1] Emery Industries, Cincinnati, Ohio.
[2] Mallinckrodt Chemical Works, St. Louis, Mo.
[3] Matheson Coleman and Bell, East Rutherford, N.J.
[4] Model 8450A; Hewlett-Packard, Palo Alto, Calif.
[5] Tri-iodide, in equilibrium with iodine and iodide in the presence of water, is actually the species that is quantitated (6).
[6] Azaz *et al.* (6) have developed a kinetic spectrophotometric assay for peroxide quantitation in water soluble surfactants which corrects for iodine fading.

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Table I—Peroxide Number (PN) of Sorbitan Monooleate, Sorbitan Monostearate, and Polysorbate 60 Using the Spectrophotometric Method

Surfactant	Peroxide Number Before Peroxide Removal	Peroxide Number After Peroxide Removal
Sorbitan Monooleate	0.24[a]	<0.10
Sorbitan Monostearate	1.3	<0.10
Polysorbate 60	9.8[b]	<0.10

[a] By titrimetric assay the PN was determined to be 0.39. [b] By titrimetric assay the PN was determined to be 5.8.

The PN of the three surfactants in this study were determined by the spectrophotometric method before and after peroxide removal (Table I). For comparison, the PN of polysorbate 60 and sorbitan monooleate were determined before peroxide removal by the titrimetric assay.

The lower limit of <0.10 for the PN by the spectrophotometric method (Table I) was chosen because it corresponded to the lowest iodine concentration in the presence of purified surfactant used in establishing the linearity of the method. This lower limit was sensitive enough to assure that the peroxide level had been significantly reduced after extraction with sodium metabisulfite[7].

From the results shown in Table I, the titrimetric and the spectrophotometric method for polysorbate 60 and sorbitan monooleate agreed fairly well. However, the color transition at the titrimetric end point for sorbitan monooleate, from green to aqua, was difficult to observe. No such problems were encountered using the spectrophotometric method; hence, this method may provide a more accurate measure of the peroxide content of sorbitan monooleate than the titrimetric assay.

While the titrimetric assay worked well for polysorbate 60 and fairly well for sorbitan monooleate, it did not work at all for sorbitan monostearate. It was not possible to keep the sorbitan monostearate solubilized during the titration with aqueous thiosulfate using the titrimetric method. Therefore, the PN of sorbitan monostearate could only be determined using the spectrophotometric method detailed in this report.

In summary, a sensitive spectrophotometric method has been developed to quantitate the peroxides present in sorbitan monostearate and monooleate, and polysorbate 60. This method should be easily extended to include other poorly water soluble as well as water soluble surfactants.

[7] These authors feel that the lower limit of peroxide quantitation by this spectrophotometric method can be extended by 10-fold, to PN values at levels <0.01. This is based on our findings that Beer's law is valid at a 50-fold lower iodine concentration in the absence of surfactant.

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Gas Chromatographic Method for Solvent Residues in Drug Raw Materials

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Abstract □ A gas chromatographic (GC) method for screening drug raw materials, soluble in aqueous media, for volatile solvent residues has been developed. After dissolution, separate portions of the drug are each separately extracted with *n*-octane, toluene, and ether and injected into a chromatograph equipped with a porous polymer column and a flame ionization detector. The range of extractant polarities provides chromatograms which, taken together, are free of interfering peaks from 0 to ~20 min. Peaks due to solvent residues in the drug are identified by retention time with confirmation of identity by GC-MS.

Keyphrases □ GC—solvent residues in drug raw materials, comparison with MS □ Solvent residues—drug raw materials, GC, comparison with MS

Drug raw materials are manufactured in an increasing number of countries. Whether, for a given drug, the same synthetic route is used universally or whether different routes are used, the necessarily diverse sources of raw materials, technical experience, and manufacturing conditions can lead to differences in the amount and kind of drug-related impurities (1-4). Solvent residues may also be present (1). These may be revealed by modern techniques such as liquid chromatography but, due to their volatility, not by thin-layer chromatography (a method often used to assess drug impurities). Besides being undesirable contaminants, solvent residues may interfere with the determination of drug-related impurities. The problem of solvent residues is recognized by the USP (5) which includes monographs involving, for example, tests for chloroform and ethyl acetate in colchicine, pyridine in diethylstilbestrol diphosphate, and isopropyl alcohol in dihydroxyaluminum aminoacetate. Work in this laboratory, reported herein, has shown for example, that some sulfinpyrazone and flurazepam hydrochloride raw materials are contaminated with toluene and acetone, respectively. To maintain surveillance over this situation, a method for the detection and quantitation of solvent residues in drug raw materials has been developed.

Each of three portions of an aqueous solution of the drug to be examined is individually extracted with ether, toluene, and *n*-octane, and the extracts are tested for volatile organic solvents by gas chromatography (GC). The three solvents were chosen to provide a range of polarities. This enhances the probability that any solvent residue in the drug will be substantially extracted into at least one of them. In addition, each of the three provides a different retention time range free of

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