Inclusion Compounds in Pharmaceutical Analysis II: Assay of a Cream following Chemical Dehydration

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Abstract □ A nitrofurazone cream presented a strong emulsion problem which prevented the drug being isolated from other constituents by simple extraction. Chemical dehydration of the cream by acetone dimethyl acetal made possible the subsequent precipitation of long-chain, emulsifying constituents as channel-type inclusion compounds with urea. Both steps take place in a single tube. Good precision and recoveries of nitrofurazone were obtained using spectrophotometric measurement. Combination of urea inclusion methods with chemical dehydration expands the scope of this analytical methodology for complex dosage forms.

Keyphrases □ Nitrofurazone cream—analysis □ Dehydration, chemical—cream analysis □ Urea inclusion compound—nitrofurazone cream analysis □ UV spectrophotometry—analysis □ Emulsion—analysis □ Acetone dimethyl acetal—dehydration agent

Chemical dehydration based on the known, acid-catalyzed reaction of water with acetals has found practical application in recent years (for example, aviation fuel additives). Application of acetal dehydration to analytical problems also is receiving attention. Acetone dimethyl acetal[1] was used by Erley (1) to remove water from nonvolatile samples for IR analysis. Critchfield and Bishop (2) used the reagent in determining water in organic samples by measuring the IR absorbance of the acetone formed. Other work was done on water determination with acetone dimethyl acetal, but combined with gas-liquid chromatographic measurement of products, by Hager and Baker (3), Badinand *et al.* (4), and Mary (5), whose work was with natural products of pharmaceutical interest. This approach allows water determination using flame-ionization detectors and simplifies sample handling, as was observed by Martin and Knevel (6). Some other applications of acetal dehydration to analytical methodology should be noted. Bousquet *et al.* (7) used acetone dimethyl acetal to dry extracts of biological materials and even reacted it directly with tissues. Incomplete extraction of thyroxine from sera was overcome by Kowal *et al.* (8) by dehydrating the sample with acetone dimethyl acetal prior to radiometric determination.

Acetal dehydration apparently has yet to be applied to the considerable assay problems presented by pharmaceutical emulsions. This article reports that application to a specific problem, nitrofurazone cream NF. The use of inclusion chemistry to facilitate assay schemes for topical dosage forms was developed previously (9) in this laboratory for dienestrol cream NF and, in combination with acetal dehydration, constitutes a new, general approach to the construction of analytical methods for pharmaceutical emulsions and topical dosage forms.

[1] Also called 2,2-dimethoxypropane; each molecule reacts with 1 molecule of water to produce 1 molecule of acetone and 2 molecules of methanol.

METHODS AND MATERIALS

All chemicals were reagent grade. Acetone dimethyl acetal was used.[2] All evaporations were performed at 20–30° at reduced pressure with mixing.[3] Spectrophotometric measurements were made with a recording, double-beam instrument.[4] A sample of commercial cream[5] and reference standard nitrofurazone NF were used.

Standard Preparation[6]—Transfer to a 100-ml. volumetric flask about 25 mg. of reference standard nitrofurazone NF, previously dried at 100° for 1 hr. and accurately weighed. Dilute to volume with methanol and mix (original solution of standard). Transfer a 4-ml. aliquot to a 50-ml. glass-stoppered centrifuge tube and evaporate to dryness under reduced pressure, keeping the temperature below 30°. Add 0.3 ml. of water and continue with the *Procedure*, beginning with "...add 3 ml. of acetone dimethyl acetal."

Procedure—Transfer to a 50-ml. glass-stoppered centrifuge tube an accurately weighed amount of nitrofurazone cream, equivalent to about 1 mg. of nitrofurazone; add 3 ml. of acetone dimethyl acetal; warm in a 30° water bath with occasional swirling until the cream is dispersed. Add 2 drops of 0.1 *N* hydrochloric acid and warm in the water bath for 10 min. with occasional swirling. Add 3 ml. of dehydrated alcohol and, with swirling, add 1.3 g. of urea followed by an additional 2 ml. of dehydrated alcohol. Mix for approximately 2 min. and allow to stand, mixing every 20 min., for at least 2 hr.

Centrifuge and transfer the supernate to a second, similar tube. Wash the residue with one 3-ml. portion and two 2-ml. portions of methyl ethyl ketone, centrifuging each time and adding the supernates to the second tube. Add 25 ml. of ether to this tube, shake well, and centrifuge. Transfer the supernate to a third, similar tube and evaporate to dryness under reduced pressure, keeping the temperature below 30°. Wash the residue in the second tube with one 3-ml. portion and two 2-ml. portions of methyl ethyl ketone, centrifuging each time and adding the supernates to the third tube to be evaporated to dryness.

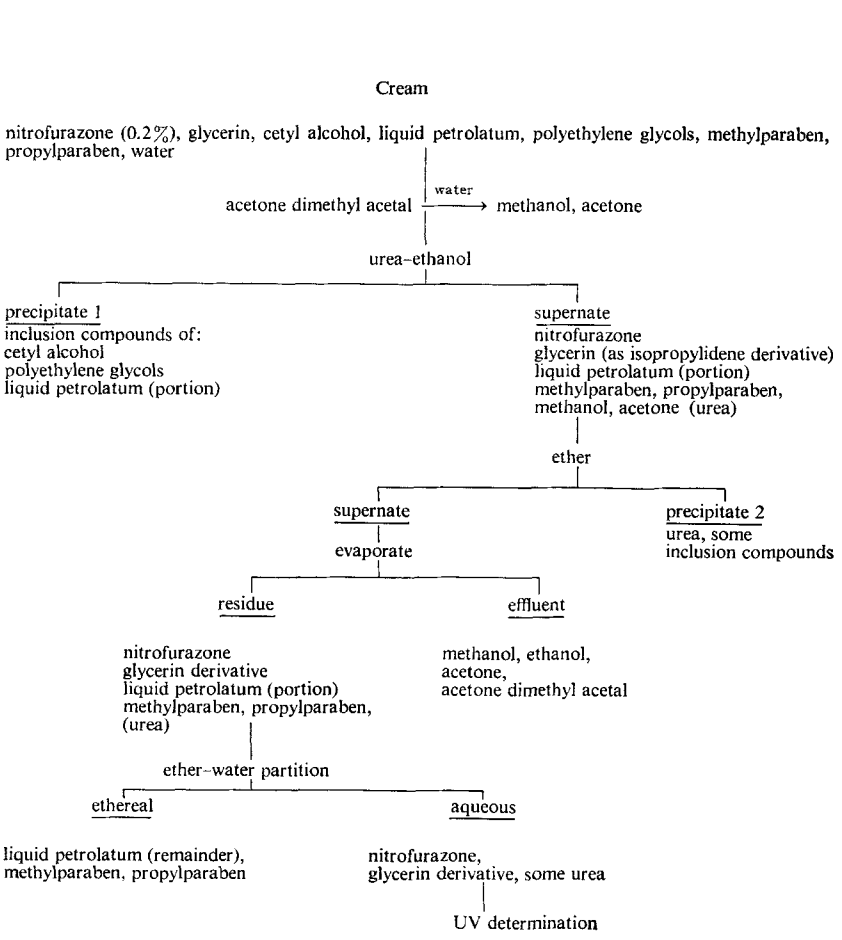
Transfer this residue to a 125-ml. separator with the aid of about 35 ml. of water. Add 20 ml. of ether to the separator and shake carefully. Transfer the aqueous layer to a 100-ml. volumetric flask. Wash the ether with three 15-ml. portions of water, adding these to the volumetric flask. Add water to volume and mix. Filter, if necessary, to obtain a clear solution.

Concomitantly determine the absorbance of the two solutions (sample and standard) in 1-cm. cells with a suitable spectrophotometer at the maximum at about 372 nm. using water as the blank. Calculate the quantity, in milligrams of $C_6H_6N_4O_4$ in the portion of cream taken, by the formula $0.004C \times (A_U/A_S)$, in which C is the exact concentration, in micrograms per milliliter, of the original solution of standard, A_U is the absorbance of the sample solution, and A_S is the absorbance of the standard solution.

RESULTS AND DISCUSSION

The assay in the NF XIII monograph covering nitrofurazone cream provides no isolating procedures. Where possible, compendial procedures require separation of the active ingredient from the other ingredients of the dosage form. A simple, workable assay sequence (Scheme I) was therefore needed which would separate the nitrofurazone from the other ingredients of a typical emulsion formulation. A placebo cream was prepared containing all constituents in common with the labeled ingredients of the commercial

[2] Matheson Coleman & Bell.
[3] Rotary Evapo-Mix, Buchler Instruments, Fort Lee, N. J.
[4] Cary model 14, Monrovia, Calif.
[5] Furacin Vaginal Cream; supplied by Eaton Laboratories, Norwich, N. Y.
[6] Perform assay with minimum exposure to light.



Scheme I—Flow diagram for the assay of nitrofurazone cream

item, although the actual proportions in that item were not known.

The standard (1 mg.) in the presence of this arbitrary placebo mixture yielded a reproducible recovery of 98.1% when compared to the standard put through the procedure (Table I). This 1.9% loss of drug when placebo is present is due in part to the retention of some drug by the large amount of precipitate formed when includable material is present. More rigorous washings of the precipitate are not desirable due to the chance of drawing the included material back into solution. Some drug is also lost during the ether–water partitioning, where sharper interfaces are obtained without the presence of placebo. The arbitrary placebo mixture used consisted of: glycerin, 5%; cetyl alcohol, 20%; liquid petrolatum, 10%; polyethylene glycols (equal amounts of 400, 1600, and 4000), 5%; methyl- and propylparabens, 0.25%; and water, 60%; 0.5-g. portions of the mixture were used.

The coefficients of variation of the standard through the procedure and of the standard plus arbitrary placebo through the procedure were 0.12 and 0.38%, respectively. When four 0.5-g. samples of the commercial cream were assayed, the recovery, based on the standard put through the procedure, was 95.9% of the labeled amount of nitrofurazone; the coefficient of variation was 3.6%. This lesser precision is most likely due to the small amount of sample used. No difficulties were apparent in applying the procedure to the commercial cream formulation. Commercial cream, 2-g. portions, assayed 98.5 and 99.0% by the present NF XIII procedure, in which the cream is dissolved in chloroform and dimethylformamide and the UV absorbance of that solution after a suitable dilution is determined.

As the 98.3% average recovery of standard (1 mg.) through the procedure shows, it is necessary either for standard drug to be carried through the procedure or for a suitable recovery factor to be inserted in the calculation to account for small amounts of drug lost during the washing of precipitates and partitioning. The former, use of a procedural standard, was chosen for this procedure.

Comments—Removal of long-chain compounds, particularly those capable of establishing an emulsion, is a recurrent problem. Dienestrol cream NF (9) presented a several thousandfold ratio of monostearin to dienestrol, which made polarographic assay impossible. An initial ether extraction separated the monostearin and dienestrol from the water and its solutes in the cream. At that point, monostearin was separated from the dienestrol by formation of a crystalline, channel-type inclusion compound with urea.

Nitrofurazone cream formulations present different problems. Nitrofurazone is a water-soluble compound, so an extractive separation of nitrofurazone from these typical water-insoluble cream constituents would appear to be the simplest isolation scheme. However, simple extraction fails, due to the formation of very stable emulsions. Direct partition-column chromatography also offers little hope of reliability in such cases.

Two goals were evident. The first was to destroy the emulsion composition without destroying the labile nitrofurazone. The second was to separate nitrofurazone from the emulsion-forming constituents of the cream. The second goal appeared amenable to the urea inclusion technique, provided the solubility of nitrofurazone in any system was not exceeded. Initial destruction of the emulsion system thus was the primary problem.

The chosen procedure was to dehydrate the emulsion system chemically with acetone dimethyl acetal. This technique does not appear to have been used previously to remove water from pharmaceuticals to facilitate assay. It is especially promising for cream preparations to remove the aqueous phase and thus destroy the emulsion. The resulting system can consist of a single phase, as in the case of this cream. The reaction is endothermic and acid catalyzed and proceeds at room temperature. The methanol and acetone formed and any excess acetone dimethyl acetal are evaporated off easily before further handling. The volume ratio of acetone dimethyl acetal used in this procedure should remove water in amounts up to 90% of the cream formulation.[7]

[7] One milliliter of acetone dimethyl acetal reacts with 0.147 g. of water.

A word of caution should be added. Acetals react with compounds other than water, so the possible effect of the acetal on the analyte should be anticipated. For example, acetals (ketals) react with organic acids to give esters (10) and with alcohols to give a mixture of ketals (11).

Following dehydration, and in the same tube, cetyl alcohol, polyethylene glycol, and a portion of the liquid petrolatum were separated by precipitating them as the urea inclusion compounds. The amount of urea used in this nitrofurazone cream assay is sufficient to react with includable materials of up to 40% of the cream formulation, to saturate the ethanol added as well as methanol formed in the dehydration, and to have some in excess. Methanol is generally used as the solvent from which urea inclusion compounds are formed (9). However, in the case of nitrofurazone cream, ethanol was chosen because at saturation it contains only about one-fourth the amount[8] of urea (12) contained by methanol and thus causes less urea to be carried on to the rest of the procedure by the supernate. Most urea in the supernate is precipitated in the next step of the procedure by addition of ether. This second precipitation also removes includable material not wholly precipitated at first. Of the various solvents tried for washing the precipitates [dioxane, cold ethanol–ether (1:1), ether, methyl ethyl ketone], methyl ethyl ketone seemed most satisfactory. It dissolves[9] the nitrofurazone, dissolves little urea, does not decompose the inclusion compound significantly, and is removed easily by evaporation.

Ether–water partitioning, impossible with this cream in its unmodified condition, is now quickly done after the removal of the emulsifying agents by inclusion. The discarded ether fraction contains the nonincludable branched-chain portion of the liquid petrolatum along with the parabens.[10] The absorbance of the nitrofurazone in the aqueous fraction is then measured. No interference is caused by the glycerin, probably present as its isopropylidene derivative (13) after reaction with the acetal, or by any added urea still present.

Solutions of nitrofurazone are sensitive to light, particularly direct sunlight and strong fluorescent lighting. A 6% loss in UV absorbance value was noted when a 1 in 100,000 solution in ethanol–water was allowed to stand in a fluorescent-lighted room for 6 hr. Heat speeds the decomposition and should be avoided. The 30° temperatures specified in this procedure do not cause observable loss of drug. Solutions of the drug, even at low concentrations, are stable for long periods when kept in complete darkness at room temperature.

Isolation Alternatives—Column chromatography was investigated as an alternative to this ether–water partition as a final separation. Silica gel[11] provided no separation. A diatomite[12] column that worked satisfactorily consisted of a lower bed of diatomite and water topped by a bed of diatomite, water, acid, and nitrofurazone. The drug was retained in the top layer while the column was washed with 50 ml. of ether–hexane (1:1) and was then eluted with 35 ml. of water. Work was done with various types of neutral and basic aluminas and various solvents. If one is willing to specify a definite batch of alumina (not just a definite pH), it is possible to have a procedure with a final column step, based on the NF XIII nitrofurazone ointment assay, which will give a complete separation of the nitrofurazone from every other ingredient of the cream. The column of alumina need be only 4 cm. high and 1.5 cm. in diameter and should be washed with dehydrated alcohol. To the dry residue, after chemical dehydration and urea inclusion, add 5 ml. of dehydrated alcohol and transfer the solution to the prepared column which is then eluted with additional dehydrated alcohol to remove the glycerin derivative, the urea, and the parabens. The drug is eluted with water, leaving any remaining liquid petrolatum behind on the column. Of the five aluminas tried, only one worked at all satisfactorily.[13] If the type of alumina is so critical, the use of an alumina column is not advisable. Chromatographic difficulties with nitrofurans, using other systems, were noted elsewhere (14).

[8] Urea solubilities at 20°/100 g. of solvent are: methanol, 22 g.; ethanol, 5 g.; and ether, 0.6 mg.

[9] The authors determined the solubility of urea as 219 mg./100 g. methyl ethyl ketone at 20° and nitrofurazone as 115 mg./100 g. at 24°.

[10] In the case of methylparaben, 89% transfers to the ether. This percentage would be expected to be higher in the case of propylparaben.

[11] Matheson Coleman & Bell, 60–80 mesh, chromatographic grade.

[12] Celite 545, Supelco, Inc., Bellefonte, Pa.

[13] Merck & Co. reagent aluminum oxide for chromatographic adsorption, 71707.

Table I—Assay Results

	Recovery, %
Standard, 1 mg., and arbitrary placebo, 0.5 g., through procedure. Results based on standard through procedure.	97.7 97.9 97.7 98.5 98.4 98.4
Average	98.1
Coefficient of variation	0.38
Standard alone through procedure, 1 mg.	98.2 98.2 98.2 98.4
Average	98.3
Coefficient of variation	0.12
	Labeled Amount
Cream, commercial, 0.5 g. Results based on standard through procedure.	92.0 95.3 100.4 95.7
Average	95.9
Coefficient of variation	3.6

SUMMARY

Isolation of drugs for assay from complex dosage forms containing large quantities of surfactants and other long-chain compounds in the past has required troublesome steps such as column chromatography or multiple extractions. In the case of the stable emulsions, especially pharmaceutical creams, this problem may be compounded by extreme difficulty in obtaining reliable destruction of the emulsion interface prior to initial isolation of drug. Chemical dehydration was applied to this problem of pharmaceutical emulsion destruction and was combined with subsequent removal of most of the cream-formulation surfactants and long-chain compounds by inclusion chemistry. With these techniques, which facilitate the construction of reliable, workable assay procedures, the authors were able to construct a separation-assay procedure for nitrofurazone cream NF XIII, giving good precision and recovery.

Cream of arbitrary formulation is dehydrated chemically by reacting the water present with acetone dimethyl acetal to yield methanol and acetone. Cetyl alcohol, propylene glycols, and some liquid petrolatum are precipitated as the urea inclusion compounds. These two steps are carried out in a single centrifuge tube. Excess urea is precipitated by adding ether to the supernate in a second tube. The nitrofurazone is isolated from this supernate by means of an ether–water partition and is quantitated spectrophotometrically.

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Rapid Identification and Estimation of Gitoxin in Digitoxin and Digoxin Tablets by TLC

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Abstract □ A rapid TLC procedure was developed for the identification and estimation of gitoxin in digitoxin and digoxin tablets. A solution of tablet material is spotted on a TLC plate directly, and the gitoxin is estimated by visual comparison of fluorescence with that of standards after spraying with acid–ferric chloride T.S. USP XVII. The method can detect 0.5% of gitoxin in the presence of 1 mcg. of cardiac glycosides.

Keyphrases □ Gitoxin—identification, estimation □ Digitoxin, digoxin tablets—gitoxin identification □ TLC—identification, estimation □ Fluorescence, estimation—UV light

The USP XVII monograph for digitoxin tablets (1) includes separate procedures for the identification of digitoxin and the determination of other digitoxosides. Identification Test A is a color reaction using a version of the Keller–Kiliani reagent (2). The test is neither sensitive nor convenient to use for large numbers of samples. The "Other Digitoxosides" determination is also a colorimetric method based on the Keller–Kiliani reagent. Gitoxin is the principal digitoxoside other than digitoxin occurring in digitoxin tablets (3), and separation on a diatomaceous earth[1] column used in the USP XVII method is based on this premise (4). Gitoxin is also the principal fluorescing substance mentioned in the monograph for digoxin tablets in USP XVII (5). Lanatoside C may also contain gitoxin as an impurity (3), although the NF XII monograph (6) does not include a test for it.

The aglycone of gitoxin with hydroxyl groups in the 14- and 16-positions is readily dehydrated (7, 8) at room temperature to dianhydrogitoxin, which exhibits visible fluorescence when activated by UV light. The anhydro derivative of digitoxin is not visible under the same conditions. The usual dehydrating reagent for this reaction has been either glycerin–HCl or propylene glycol–HCl. Acid–ferric chloride T.S. USP XVII was found to give the same reaction and has the advantage of being volatile. The nonvolatile residues from the acidic glycerin or glycol reagents result in diffusion of spots and lowered sensitivity.

A TLC system using methylene chloride–methanol–formamide on a silica gel plate (9) was found to separate the compounds sufficiently to carry out the tests. The gitoxin content of the sample on the sprayed plate is estimated by visual comparison of its fluorescence with that of standard spots under longwave UV light (3650 Å). The digitoxin chromatogram becomes visible after the plate is heated in a 100° oven for 10 min.

The same technique can be used for the identification of digitoxin, digoxin, acetyldigitoxin, and lanatoside C and for the determination of any gitoxin present in their drug preparations. Digoxin and gitoxin are not completely separated by this chromatographic procedure. Nevertheless, digoxin is not activated to visible fluorescence at room temperature by the acid–ferric chloride. Consequently, any fluorescence present immediately after spraying is due to gitoxin alone. Heating the plate at 100° destroys the gitoxin fluorescence and converts the digoxin to a fluorescent anhydro derivative which may be seen under both UV and visible light.

EXPERIMENTAL

Chromatographic Chamber—A Mitchell tank was used (10).

TLC Plates—Coat a 200–250-μ layer on a clean glass plate, using a 2:1 slurry of water–silica gel G.[2] Air dry; then activate 30 min. at 100°. Store in a desiccator. When several samples are to be run simultaneously, 0.5–1.0-cm. channels may be scored on the plate for convenience. Precoated silica gel GF plates[3] enhance the fluorescence of the spots obtained. A source for long wavelength (3650 Å) UV light was used.

Reagents—*Mobile Solvent*—Methylene chloride, distilled in glass, plus anhydrous acetone-free methanol (Mallinckrodt or

[1] Celite 545, Johns-Manville Products Corp., New York, NY 10016

[2] Catalog No. 7731, Brinkmann Instruments Co., Westbury, NY 11590

[3] Analtech, Inc., Wilmington, DE 19801