Potentiometric Titration of Allantoin in Cream Formulations

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Abstract □ An analytical procedure is described which uses a rapid partition chromatographic separation of intact allantoin from cream formulations. The allantoin content is measured by potentiometric titration in a mixed solvent system. Accuracy and precision data are presented, and good results are obtained with commercial preparations.

Keyphrases □ Allantoin cream formulation—analysis □ Column chromatography—separation □ Potentiometric titration—analysis

The current methods of analysis of allantoin involve hydrolysis and quantification of the resulting glyoxylic acid (1–3) or urea (3–5). These procedures are deficient for the analysis of residual allantoin, since they measure urea or glyoxylic acid which are the products of normal allantoin degradation (6). Using these methods of analysis after preliminary isolation of allantoin from its degradation products requires a difficult separation step. Other procedures use paper chromatography (7) or paper electrophoresis (5, 7), which are rather slow and have been applied only to urine samples. A TLC separation and UV assay of allantoin at 220 mμ was reported for creams and lotions (8), but data on samples were not included and the molar absorptivity is only about 1200.

This report describes an assay for allantoin in cream formulations based on a partition chromatographic separation of allantoin from interfering substances and potentiometric titration of the allantoin with alkali. Those substances, which are eluted with the allantoin, are either nontitratable or have acid-dissociation constants sufficiently different from allantoin to permit its analysis.

EXPERIMENTAL

Apparatus—The following were used: recording pH meter[1] equipped with a glass-reference combination electrode[2] and a magnetic stirrer;[3] a motorized, constant-speed syringe buret[4] to deliver the titrant; and a glass chromatographic column, 30 × 2 cm.

Reagents—The following were used: sodium hydroxide, 0.1 *N* solution standardized potentiometrically against primary standard potassium biphthalate; acetone, A.R.; acid-washed diatomaceous earth;[5] ethyl acetate, A.R.; ethyl ether, A.R.; glass wool; and *p*-dioxane.[6] Allantoin[7] was used as received.

Preparation of Solvent Mixtures—Ethyl acetate (200 ml.) and water (20 ml.) were combined in a separator and shaken to equilibrate. The layers were allowed to separate. The water phase (lower) serves as the stationary phase in the column and for sample preparation. The ethyl acetate layer serves as the organic mobile phase for column elution.

Ether saturated with water was prepared by adding 25 ml. of water to 100 ml. of ethyl ether and separating after equilibration. The aqueous phase was discarded.

Column Preparation—To 4 g. of acid-washed diatomaceous earth, 3.5 ml. of stationary phase is added and mixed thoroughly. This material is added in portions to a 2-cm. i.d. chromatography column containing a pledget of glass wool at the bottom. The column is tapped on a wooden block after each addition of support until it is compact. The column is then tamped semifirmly with a column-packing rod to a length of 5.6 cm. The column is washed with 50 ml. of water-saturated ethyl acetate.

Preparation of Artificial Sample—A placebo batch of Cream E, containing all ingredients except allantoin, was prepared. Allantoin was added to samples of this placebo cream, lightly heated, mixed to give a homogeneous mix, and then rapidly cooled. The samples were put into beakers, sealed with Parafilm to avoid evaporation, and stored at room temperature for 4 days before being analyzed.

Procedure—Accurately weigh a quantity of sample equivalent to about 25 mg. of allantoin into a 50-ml. beaker, and add 1.5 g. of acid-washed diatomaceous earth. Mix thoroughly with a spatula and then add 0.5 ml. of stationary phase and mix thoroughly again. Add the sample preparation to the prepared column and tamp semifirmly. Place a pledget of cotton on top of the sample, and elute the column with 100 ml. of mobile phase. Allow the ethyl acetate mobile phase to run just below the surface of the diatomaceous earth, add 5 ml. of ethyl ether (water saturated), and again allow this to run just below the surface. Add an additional 25 ml. of ethyl ether (water saturated) and allow the column to run dry. Discard all solvent eluted. Strip the allantoin from the column by eluting with 40 ml. of deionized water. Only the water is collected into a 150-ml. beaker. The boundary between the residual ethyl ether on the column and the water eluate containing the allantoin is easily determined by the initially cloudy nature of the water eluate.

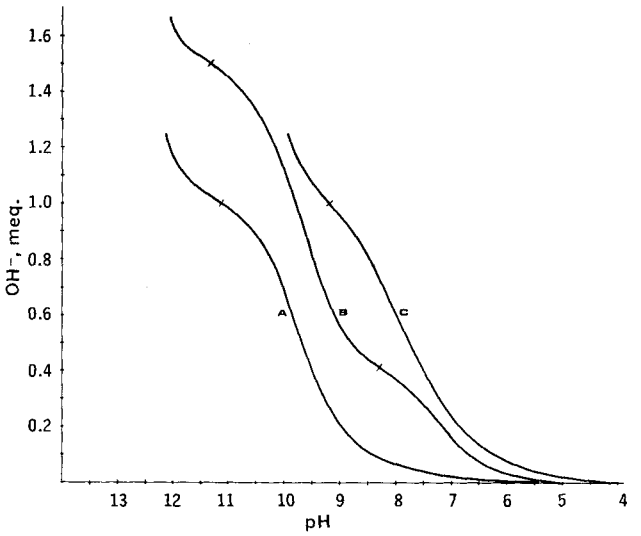

Figure 1—*Potentiometric titration curves of allantoin in 66% acetone–water (A); allantoin + triethanolammonium ion in 66% acetone–water (B); and allantoin in water (C).*

[1] Heath Recording.
[2] Sargent-Welsh.
[3] Sargent-Welsh.
[4] Micro-Metric Instrument Co., Model MIP-2.
[5] Celite 545.
[6] Matheson Coleman and Bell.
[7] Aceto Chemical Co.

Table I—Solvent Effect of the pKa′ of Allantoin, Triethanolamine, and Glyoxylic Acid

Substance	pKa′ H_2O	Dioxane–H_2O 1:2	Dioxane–H_2O 2:1	Acetone–H_2O 1:2	Acetone–H_2O 2:1
Allantoin	8.01	9.13	10.38	8.75	9.91
Triethanolamine	7.66	7.52	7.33	7.25	7.10
Glyoxylic acid	3.50	Reaction	Reaction		4.98

Collect all eluent until the column runs dry. Add 70 ml. of acetone to the 30–35 ml. of eluate collected and potentiometrically titrate the sample with standard 0.1 *N* NaOH to an apparent pH of 11.5–12.0. Usually, two inflections are observed, one near pH 8 and the other near pH 11. Calculate the milligrams of allantoin per gram of sample by substituting the observed values into the following equation:

$$\frac{V \times N \times 158.12}{\text{(sample wt., g.)}} = \text{mg. allantoin/g. sample} \qquad \text{(Eq. 1)}$$

where V is the milliliters of titrant consumed between the two inflections, N is the normality of the titrant, and 158.12 is the equivalent weight of allantoin.

DISCUSSION

Potentiometric Titration of Allantoin—The potentiometric titration curves of allantoin in water and 66% acetone–water are shown in Fig. 1. The equivalence points, although short, are distinct and analytically useful. Many cream samples were found to contain a basic emulsifying agent such as triethanolamine, which was eluted from the column with the allantoin and interfered with its titration in a purely aqueous solvent system. The addition of acetone or dioxane to the aqueous solution caused a large increase in the pKa′ of allantoin and a smaller decrease in the pKa′ of the amine. These shifts are due to the combined effects of a lower solvent dielectric constant and changes in the solvation sphere of the molecules (9). The effects of different solvents on the pKa′ of allantoin, triethanolamine, and glyoxylic acid are given in Table I. Dioxane has a lower dielectric constant than acetone and, therefore, produced a greater separation in pKa′ of allantoin and triethanolamine than was found for acetone. The titration curve of allantoin plus triethanolammonium ion in 66% acetone–water is given in Fig. 1. Glyoxylic acid, a natural degradation product of allantoin, reacted very rapidly with a substance in the *p*-dioxane to give a product which interfered with the titration of allantoin. This problem was avoided by using acetone as the solvent. Dioxane, besides containing material reactive to glyoxylic acid, also contains small amounts (9.6×10^{-4} meq./ml.) of an acid substance which also interferes with the titration of allantoin.

Partition Column Separation of Allantoin—The partition coefficient of allantoin estimated from the ratio of its solubility in ethyl acetate to its solubility in water is 3×10^{-3}. Therefore, in an aqueous stationary phase the allantoin is essentially immobile and is easily separated from nearly all excipients after sufficient ethyl acetate mobile phase has been passed through the column. Those excipients that are not separated are either not titratable or have a sufficiently different pKa′ to allow differentiation using a mixed solvent system.

Table II—Elution of Allantoin[a] from Partition Column with Water

Fraction	Total Milliliters Eluted	Allantoin Observed in Fraction, meq.
1	4	0.0400
2	8	0.0995
3	12	0.0529
4	16	0.0231
5	20	0.0074
6	24	Negligible
		Sum 0.2229[b]

[a] The sample analyzed was Product E. [b] The sum represents 101.18% of theory.

Table III—Accuracy Data for the Assay of Allantoin in Creams[a]

Sample	Placebo Weight, g.	Allantoin Added, mg.	Theoretical Allantoin, meq.	Observed Allantoin, meq.	Theory, %[b]
1	1.5253	28.20	0.1783	0.1792	100.50
2	1.4829	30.25	0.1912	0.1897	99.21
3	1.5842	29.82	0.1885	0.1885	100.00
4	1.4417	32.50	0.2055	0.2001	97.37
5	1.4045	29.46	0.1850	0.1862	100.64
6	1.3810	36.20	0.2289	0.2280	99.60
7	1.6279	31.14	0.1969	0.1941	98.57
8	1.6254	30.13	0.1905	0.1908	100.15

[a] Samples were prepared by the addition of allantoin to a placebo cream. [b] The mean percent recovery is 99.51%, and the 95% confidence interval is ±1.07%.

The apparent partition coefficient on a chromatographic column has been shown (10) to be a function of the stationary phase volume. The present assay uses a column with a ratio of milliliters of stationary phase to grams of support of 0.875. To maintain equilibrium conditions and produce a satisfactory elution pattern, it was found useful to add water to the sample preparation sufficient to give a ratio of aqueous phase to grams of support close to 0.875. This produced a satisfactory elution pattern (Table II) Excellent analytical results were obtained with flow rates as high as 5 ml./min.

The function of the ethyl ether wash is to remove residual ethyl acetate from the column. Any ethyl acetate present during the titration of allantoin interferes due to its rapid hydrolysis. This rapid hydrolysis was indicated by the fact that when the titration was stopped at high pH values (greater than 8), the pH began to drift toward lower values.

Accuracy and Precision—Accuracy data for the assay of allantoin added to placebo samples of Cream E are given in Table III. The data indicate that both the accuracy and precision of the analysis are excellent. The results of repeated analysis of a commercial sample of Cream E are given in Table IV. The 95% confidence interval is nearly the same as was calculated for the data of Table III. This suggests that the samples prepared for the accuracy study are truly representative of commercial creams and that the data in Table III are valid estimates of the accuracy of the assay.

The results of the analysis of a variety of commercial creams are given in Table V. The assay gave acceptable results in every case, and repeat assays of Products A and D showed good precision.

It has been shown (6) that under conditions of temperature and pH that would be expected in cream formulations, the products of allantoin hydrolysis are allantoic acid and then urea and glyoxylic acid. Potentiometric titration of mixtures of allantoin, glyoxylic acid, and triethanolammonium chloride and allantoin plus allantoic acid in 66% acetone–water gave allantoin recoveries of 100.3 and 100.0%, respectively. These results demonstrate that no interference is caused by these compounds.

Alkaline hydrolysis of allantoin (1.5×10^{-3} *M* NaOH at 80° for 30 min.), acidification, and then analysis showed a loss of allantoin of 10.5%. If ammonium ion had been produced, the recovery of allantoin would have been greater than 100% since urea, an initial hydrolysis product of allantoin, gives two molecules of ammonium ion when it hydrolyzes. The pKa′ of the allantoin from the hydrolyzed sample was 9.95, in excellent agreement with the value 9.91 previously determined.

Table IV—Precision Data for Assay of Allantoin

Sample[a]	Sample Weight, g.	Observed Concentration, mg./g.	Formula Strength, %[b]
1	1.2765	20.28	96.57
2	1.4072	20.53	98.10
3	1.4026	20.42	97.24
4	1.3113	19.97	95.10
5	1.5625	20.28	96.57
6	1.2270	20.54	98.10

[a] All samples are from a single tube of Cream E. [b] The mean percent formula strength is 96.9% and the 95% confidence interval is ±1.18%.

Table V—Assay Results for Allantoin in Commercial Creams

Product	Sample Weight, g.	Weight Allantoin/g. Cream, mg.	Label Strength, %
A	1.1495	18.6	93.0
A	1.0523	18.4	92.0
B	1.0670	20.9	104.5
C	0.9141	20.2	101.0
D	3.2041	2.44	97.6
D	3.3492	2.47	98.8
E	1.2765	20.28	96.57

Chromatography and potentiometric titration of a mixture of allantoin and ammonium chloride showed that the ammonium ion is eluted with the allantoin and interferes with the quantification of allantoin in the titration. However, since no significant production of ammonia from allantoin in creams occurs, this causes no problem in the assay. Precipitation of ammonium ion from equimolar allantoin–ammonium-ion mixtures using sodium tetraphenyl borate was 96% effective in removing the ammonium-ion interference and resulted in 103% apparent recovery of allantoin. Smaller amounts of ammonium ion gave correspondingly smaller interferences.

REFERENCES

(1) G. Young and C. Conway, *J. Biol. Chem.*, **142,** 839(1942).

(2) S. A. Katz, R. T. Turse, and S. B. Mecca, *J. Soc. Cos. Chem.*, **15,** 303(1964).

(3) R. Crokaert, *Bull. Soc. Chim. Bio.*, **41,** 1001(1959).

(4) G. Siest, *Bull. Soc. Pharm. Nancy*, **64,** 55(1965).

(5) R. Zimmermann, *Naturwissenschaften*, **43,** 399(1956).

(6) G. D. Vogels, F. E. De Windt, and W. Bassie, *Recueil*, **88,** 940(1969).

(7) J. Wagner and E. Franzen, *Arch. Tierenahrung*, **9,** 11(1959).

(8) I. Bonadeo and G. Bottezzi, *Ital. Essenzy Profumi*, **50,** 78 (1968).

(9) H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold, New York, N. Y., 1958, p. 683.

(10) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35,** 1358 (1941).

(11) "The Merck Index," 8th ed., Merck and Co., Rahway, N. J., 1968, p. 33.

(12) "Handbook of Chemistry and Physics," 45th ed., The Chemical Rubber Co., Cleveland, Ohio, 1964, p. D77.

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NMR Analysis of Mestranol Bulk Drug

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Abstract □ A double assay procedure for mestranol is described. The method is based upon measurement of the NMR spectrum of mestranol in pyridine, using diphenylacetic acid as an internal standard. The signals chosen are those from the methoxyl and ethinyl groups. Three commercial lots of the steroid were studied, and a TLC study of each lot is described. The impurities are tentatively identified.

Keyphrases □ Mestranol bulk drug—analysis □ TLC—separation □ Potentiometric titration—analysis □ NMR spectroscopy—analysis

The synthetic oral estrogen mestranol (17α-ethinyl-3-methoxyestra-1,3,5(10)-trien-17β-ol) is now in widespread use, chiefly as a component of oral contraceptive preparations. At present, the only official assay (1) for the raw material is a potentiometric titration. Other methods reported in the literature are colorimetric (2–4), UV (5–7), GLC (4, 6, 8, 9), TLC (7), and fluorometric (4, 10–12).

Mestranol lends itself very well to quantitative analysis by means of NMR spectroscopy, since the signals from the protons of both the ethinyl and methoxyl groups are single sharp peaks. With a suitable choice of solvent, these peaks appear in a region of the spectrum that is unaffected by signals from other protons.

The effect of solvents on the chemical shift of acetylenic protons is well documented (13). In particular, addition of pyridine to a dilute solution of a monosubstituted acetylene in carbon tetrachloride can result in deshielding of up to 1 p.p.m. of the acetylenic proton (14). Such significant deshielding is due to the fact that acetylenic compounds can form weak hydrogen bonds with molecules containing electronegative centers, such as acetone, acetonitrile, and pyridine (14–16).

Since the impurities present in mestranol bulk drug might interfere with either the signal from the ethinyl group or that from the methoxyl group, a study of the assayed samples was made by means of TLC.

EXPERIMENTAL

Spectra were obtained at 60 Mc.p.s., using a Varian A-60A analytical NMR spectrometer. A sweep time of 50 sec. for a chart width of 500 c.p.s. was used for all integrals. A r.f. power of 0.25 mG. (nominal dial setting) gave the maximum integral amplitude (17) and was used for the integrations. Tetramethylsilane in chloroform was used as an external reference to measure chemical shifts.

Assay Procedure—*NMR*—Approximately 200 mg. of mestranol and 150 mg. of pure diphenylacetic acid (DPAA) were accurately weighed and dissolved in the minimum amount of pure pyridine (approximately 0.5 ml.). The NMR spectrum was obtained in the usual manner and integrated five times in each direction through the region of interest.

Potentiometric Titration—Approximately 200 mg. of mestranol,