aggregates or clusters occurs in the plastic which hinders the over-all migration of individual sorbic acid molecules through the plastic. With more dilute solutions, the mobility of individual molecules increases, and diffusion proceeds at a more rapid rate.

SUMMARY

Studies on the interaction of sorbic acid by nylon 66 were continued for the purpose of (*a*) postulating the mechanism of interaction, (*b*) verifying the rate-determining step in the sorption process, and (*c*) comparing diffusion coefficients by the time-lag method to a sorption method. Results of the various experiments may be summarized as follows.

Dye–sorption studies indicated that sorbic acid was most likely interacting at the amide linkages in the polymer rather than at the end amino groups.

Differential infrared analysis on samples of untreated and treated (with sorbic acid) nylon suggested a double hydrogen bond formation between the acid and the nylon, confirming that the previously found ΔH° of -9.77 was correct.

X-ray diffraction studies on untreated and treated (with sorbic acid) nylon indicated that a greater degree of order results with the sorbic acid molecules acting as the ordering agent.

Application of the Rotinyan-Drozdov equation to kinetic sorption data confirmed that the rate-determining step is the diffusion step in the plastic.

Diffusion coefficients obtained from the time-lag method approximated within experimental errors those determined by a sorption method (Berthier method).

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Drug Standards

Calcium Assays for Official Use

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Comments recommending consideration of direct complexometric titration for the assay of the official calcium salts were investigated and the method used was the method chosen for the official compendia ("National Formulary" and "United States Pharmacopeia"). Selection of an indicator that conformed to requirements of these compendia introduced unexpected problems, and the selection of naphthol green screened murexide for the N.F. XI and U.S.P. XVI monographs represented a compromise between analytical suitability and compendial requirements. Further study of the indicator problem resulted in the selection of hydroxy naphthol blue as the indicator of choice; this indicator will be specified in the N.F. XII and U.S.P. XVII monographs.

DURING THE circulation of proof for N.F. X and U.S.P. XV, several reviewers recommended that direct complexometric (EDTA) titration be considered for the assay of the official calcium salts. It was not possible to complete the studies necessary to document the suitability of the method for compendial use before N.F. X and U.S.P. XV were published, so an investigation of the recommended procedure was postponed until the N.F. XI and U.S.P. XVI were in preparation.

Previous studies performed in the American Pharmaceutical Association Drug Standards Laboratory (1, 2) had shown the suitability of an EDTA back titration procedure for the assay of some calcium salts, but no attempt appears to have been made to include the procedure in the compendia. The number of comments received in favor of the direct EDTA titration method and the advantages claimed for this method over the oxalate-permanganate procedure suggested

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TABLE I.—COMPARISON OF THE OXALATE-PERMANGANATE AND EDTA TITRATION METHODS

Compd.	Analyst	U.S.P. XV Procedure: Assays, No.	U.S.P. XV Procedure: Av., %	U.S.P. XV Procedure: S.D., %	EDTA Procedure: Assays, No.	EDTA Procedure: Av., %	EDTA Procedure: S.D., %
Calcium carbonate	*A*	26	99.93	0.31	9	100.02	0.23
Calcium carbonate	*B*	10	99.95	0.14	8	100.03	0.03
Calcium gluconate ("as is" basis)	*A*	24	96.62	0.26	10	96.90	0.26

that the direct titration method should be evaluated for possible use in the N.F. XI and U.S.P. XVI monographs.

The following criteria are considered essential for adoption of a new method of assay for compendial use: (*a*) it must show distinct advantages over the current method; (*b*) it must conform to the specific requirements of the compendia insofar as equipment, reagents, and solutions are concerned; (*c*) it must yield results of equivalent or improved precision and accuracy.

Preliminary comparisons of the official oxalate-permanganate and direct EDTA titration procedures showed that eight oxalate-permanganate determinations required 6 hours of analytical time, whereas the same number of direct EDTA determinations required only 2 hours. On the basis of this average 30-minute saving in time per analysis, it was concluded that the recommended direct titration procedure conformed to the first criterion, at least in respect to time. Since the procedure could conform easily to the second criterion if a suitable indicator could be found, further work was indicated to demonstrate equivalence, precision, and accuracy.

EQUIVALENCE AND PRECISION

The decision was made that suitability of the direct EDTA titration method for compendial use could be demonstrated by a series of determinations whereby the results obtained from assaying samples by the official procedures were compared to those obtained by the proposed EDTA procedures to demonstrate equivalence. Since the authors were unaware of any published precision data on the official procedures, it was decided to obtain this as a part of the study.

PROCEDURES

The official oxalate-permanganate procedures given in U.S.P. XV and N.F. X were followed, with one modification. To standardize conditions and yield more precise results, the oxalate precipitate was washed with 10 ml. of 1% ammonium oxalate solution, then with 100 ml. of water cooled in an ice bath.

The EDTA procedure was as follows: weigh accurately a quantity of salt that will yield about 80 mg. of calcium and dissolve in a mixture of 100 ml. of water and sufficient diluted hydrochloric acid to yield a clear solution. Add 15 ml. of sodium hydroxide T.S. and dilute with water to 165 ml. Add 40 mg. of murexide indicator preparation (3) and titrate with 0.05 *M* disodium ethylenediaminetetraacetate to a clear blue color.

[*Note:* In these preliminary studies, most of which were performed by analyst *A*, the murexide indicator was used without screening with naphthol green T.S. Analyst *B*, whose results were obtained later in the study, used naphthol green screened murexide; the only difference in the procedure was that 3 ml. of naphthol green T.S. (4) was added to the sample solution before the titration was begun. In later studies, the same procedure was followed, but the other indicators were substituted for the murexide or screened murexide.]

The samples used for the first comparisons were Mallinckrodt calcium carbonate primary standard A.R. having an assay value of 100.03 ± 0.05% and calcium gluconate U.S.P. The calcium carbonate primary standard assay value was obtained by oxalate precipitation, followed by ignition to carbonate. The calcium gluconate U.S.P. assay value was not known at this time. A comparison of the results obtained by the two procedures is shown in Table I.

These data show that the precision of the direct EDTA titration procedure using either murexide or screened murexide indicator is equal to or better than the precision of the U.S.P. XV procedure. Insofar as the calcium carbonate assays are concerned, the data show that the direct EDTA titration procedure yields average assay results equivalent to the results obtained by the primary standard assay method. In the case of calcium gluconate, however, the 0.28% difference in the average results suggested that the gluconate ion may be interfering with one or the other of the two assay procedures. To determine which of the two procedures was affected by the gluconate ion and to insure that difficulty was actually caused by the organic portion of the molecule, samples of a different lot of calcium gluconate U.S.P. were treated with nitric and perchloric acids to destroy the organic matter; the solutions so prepared were then assayed by the U.S.P. XV and EDTA procedures. The results are summarized in Table II.

The data in Table II confirm that the presence of gluconate ion affects the U.S.P. XV oxalate-permanganate assay method but not the EDTA titration method.

Later, in preparing sample stock solutions for use in further studies, it was found that in freshly pre-

TABLE II.—GLUCONATE ION EFFECT ON CALCIUM ASSAY

Assay Procedure	Untreated Samples: Assays, No.	Untreated Samples: Av. Assay, %	HNO_3-$HClO_4$ Treated Samples: Assays, No.	HNO_3-$HClO_4$ Treated Samples: Av. Assay, %
U.S.P. XV	6	98.66	5	99.27
EDTA titration	6	99.36	6	99.29

TABLE III.--ASSAY OF AGED CALCIUM GLUCONATE STOCK SOLUTIONS

Assay Procedure	Samples, No.	Assays, No.	Av. Assay, %
U.S.P. XV	6	12	99.81
EDTA titration	6	12	99.85

pared solutions of calcium gluconate and in some solutions of calcium lactate precipitation of calcium was not complete when the official procedures were used. If the solutions were allowed to stand prior to oxalate addition, complete precipitation was attained. When aliquots of aged sample stock solutions were assayed by the oxalate-permanganate and direct EDTA titration procedures, the results were the same (Table III).

Since the EDTA method yields similar results with freshly prepared solutions, with aged solutions and with wet ashed samples, the conclusion was made that sample dissolution is complete in freshly prepared solutions, but that a fairly stable complex ion must be present. This complex ion apparently dissociates within about 2 days in aqueous solution.

To establish the suitability of the EDTA titration method for the assay of other compendial calcium salts, assays were performed by the official and the EDTA methods. The results of part of this study are shown in Table IV.

An additional 38 sets of analyses by both methods performed in duplicate or triplicate have substantiated the conclusion that the oxalate-permanganate and EDTA titration methods yield equivalent results. The precision of the EDTA assay is at least as good as the precision obtainable by the oxalate-permanganate, even when the latter is modified as described under *Procedures*. On the basis of these data, the decision was made to recommend inclusion of the direct EDTA titration method in the N.F. XI and U.S.P. XVI monographs for some calcium salts.

INDICATOR EVALUATION

Some of the early results reported here were obtained using murexide as the indicator. Since the color change of murexide is gradual, the analyst must remember the exact color chosen as the end point of the titration to obtain precise results. The inability of some analysts to obtain satisfactory results with this indicator and the color memory problem attendant when it is used on other than a routine basis was evidence that an improved indicator was needed. The screening of murexide with naphthol green dye was developed independently, but it was later found that other investigators had previously recommended such a screened indicator (5). The results reported in Tables III and IV were all obtained using the naphthol green screened murexide indicator; this indicator was specified in the N.F. XI and U.S.P. XVI monographs.

Despite the general acceptance of the naphthol green screened murexide indicator for compendial use and absence of adverse comments or criticism upon its use in the N.F. XI and U.S.P. XVI monographs, the unanimous opinion of the authors and of the analysts who participated in this study was that efforts should be continued to find a better indicator for the official monographs. It was felt that the ideal indicator should conform as closely as possible to the following criteria: (*a*) it should yield results showing accuracy and precision equivalent to or better than those obtained using the naphthol green screened murexide indicator; (*b*) it should have a single sharp color change at the end point; (*c*) it should be specific for calcium and be relatively unaffected by other ions likely to occur as impurities in compendial calcium salts under the specified conditions of analysis; (*d*) it should be simple to use and avoid the color memory problem encountered with murexide and some other indicators; (*e*) it should

TABLE IV.—COMPARISON OF ASSAYS ON OTHER COMPENDIAL CALCIUM SALTS

Sample	Assays, No.	Av. Result by N.F. X or U.S.P. XV Procedure, %	Assays, No.	Av. Result by Direct EDTA Procedure, %
Calcium carbonate U.S.P.	8	98.90	8	98.90
Calcium chloride U.S.P.	14	100.61	14	100.66
Calcium hydroxide U.S.P.	17	97.88	17	97.78
Calcium mandelate U.S.P.	10	100.30	10	100.42
Calcium bromide N.F.	19	84.50	19	84.56
Calcium glycerophosphate N.F.	10	88.11 ("as is" basis)[a]	10	87.70 ("as is" basis)
Calcium lactate N.F.	12	95.33 (dried basis)	12	95.59 (dried basis)
Calcium lactate N.F.	8	72.64 ("as is" basis)	8	72.54 ("as is" basis)

[a] For reasons unclear, the N.F. X assay procedure for this product specified that the calcium oxalate be dried and weighed rather than titrated with permanganate, as is the case with all of the other salts. Since we did not consider this to be a precise method, we also carried out some determinations wherein the calcium oxalate, obtained as directed in the N.F. X procedure, was dissolved in dilute sulfuric acid and titrated with permanganate. By this modified procedure, our results were 87.69% (average of 10 determinations), which checks well with the result obtained by the direct EDTA titration procedure.

TABLE V.—COMPARISON OF INDICATORS

Indicator	Pure $CaCO_3$ Soln. Assays, No.	Pure $CaCO_3$ Soln. Av.	Pure $CaCO_3$ Soln. S.D.	Soln. of $CaCO_3$ + Added mg. Assays, No.	Soln. of $CaCO_3$ + Added mg. Av.	Soln. of $CaCO_3$ + Added mg. S.D.
Calcon	5	100.00%	0.06%	5	100.20%	0.11%
Cal-Red	5	99.85	0.16	5	99.91	0.14
Calconcarbonsäure	5	99.76	0.03	5	99.98	0.03
Calcein	5	100.12	0.11	5	100.47	0.12
Eriochrome Blue SE	5	100.02	0.15	5	100.19	0.16
Hydroxy naphthol blue	6	99.98	0.10	6	100.04	0.08
Methyl thymol blue	8	100.26	0.06	5	100.94	0.58

TABLE VI.—COMPARISON OF INDICATORS FOR THE ASSAY OF CALCIUM GLUCONATE

Indicator	Assays, No.	Av., %	S.D., %
Naphthol green–murexide	7	99.24	0.10
Hydroxy naphthol blue	7	99.28	0.08
Cal-Red	8	98.65	0.20

On the basis of this screening, Calcon, Calconcarbonsäure, Calcein, Eriochrome Blue SE, and methyl thymol blue were dropped from further consideration since the results, and in some cases, the end points, were adversely affected by the added magnesium. The remaining two indicators, Cal-Red and hydroxy naphthol blue, then were used to assay an aged stock solution of calcium gluconate U.S.P. The results are shown in Table VI.

TABLE VII.—COMPARISON OF NAPHTHOL GREEN SCREENED MUREXIDE AND HYDROXY NAPHTHOL BLUE FOR THE ASSAY OF COMPENDIAL CALCIUM SALTS

Compd.	Naphthol Green Screened Murexide Assays, No.	Av. ("as is" Assay)	Hydroxy Naphthol Blue Assays, No.	Av. ("as is" Assay)
Calcium gluconate U.S.P.	5	98.53	7	98.59
Calcium carbonate	3	97.93	3	97.99
Calcium chloride U.S.P.	3	101.56	3	101.58
Calcium hydroxide U.S.P.	3	99.28	3	99.51
Calcium pantothenate U.S.P.	3	94.22	3	94.17
Calcium bromide N.F.	3	67.76	3	67.71
Calcium lactate N.F.	3	71.62	3	71.72
Calcium oxide N.F.	3	78.74	3	78.81

be suitable for use by such standards-setting organizations as the N.F., the U.S.P., and the Food Chemicals Codex, each of which has some specific criteria to be met before a reagent is accepted for use.

[*Note:* In general, the criteria of the compendia may be summarized as follows: (*a*) the reagent must be readily available; (*b*) it must be possible to describe the reagent by a common nonproprietary name; (*c*) it must be possible to prepare a reagent specification by which the suitability of the reagent for compendial use can be demonstrated readily.]

At the time this portion of the study was carried out, the following indicators were commercially available and were selected for evaluation: Calcon,[1] Cal-Red,[2] Calconcarbonsäure,[3] Calcein[4] (screened with thymolphthalein), Eriochrome Blue SE[5] (screened with naphthol green), hydroxy naphthol blue,[6] and methyl thymol blue.[4] Murexide and Eriochrome Black T, both of which have been used by numerous investigators, were also considered; but the earlier experience with murexide was not favorable, and it seemed inadvisable to study it further. Eriochrome Black T was rejected since, as reported by many workers, it also would determine any magnesium present in the calcium salts.

In carrying out the evaluation studies, stock solutions of primary standard calcium carbonate and of primary standard calcium carbonate containing 1% of magnesium (weight Mg/weight Ca) in hydrochloric acid were prepared. Each stock solution was then assayed by the direct EDTA titration procedure, using 0.05 *M* disodium ethylenediaminetetraacetate standardized against the same lot of primary standard calcium carbonate with naphthol green screened murexide as the indicator. The results are shown in Table V.

[1] Obtained from J. T. Baker Chemical Co., Phillipsburg, N. J.
[2] Obtained from Scientific Service Laboratories, Inc., Dallas, Tex.
[3] Obtained from E. Merck, Darmstadt, Germany.
[4] Obtained from Fisher Scientific Co., Pittsburgh, Pa.
[5] Obtained from Geigy Chemical Corp., Ardsley, N. Y.
[6] Obtained from Mallinckrodt Chemical Works, St. Louis, Mo.

On the basis of this comparison with calcium gluconate solution, only hydroxy naphthol blue remained as a possible substitute for naphthol green screened murexide. Since hydroxy naphthol blue most nearly conformed to the criteria considered necessary for a suitable indicator, the decision was made then to evaluate it for use with all other N.F. and U.S.P. calcium salts by comparison to the naphthol green screened murexide EDTA method. The results of this evaluation are shown in Table VII.

Hydroxy naphthol blue appears to conform best to criteria selected for an ideal compendial calcium assay indicator. It is equivalent to murexide in precision and accuracy and was preferred by the analysts because a sharper more simple color change was observed. It also conforms to the criteria of the standard-setting organizations. Hydroxy naphthol blue is the disodium salt of 1-(2-naphthol azo-3,6-disulfonic acid)-2-naphthol-4-sulfonic acid.

CONCLUSIONS

The comparative data reported here confirm the suitability of the direct EDTA titration method for the assay of the compendial calcium salts. Of the 10 indicators considered, only two—naphthol green screened murexide and hydroxy naphthol blue—are suitable in assaying all of the compendial calcium salts. Of the two, hydroxy naphthol blue is the indicator of choice since it has a sharper more easily remembered and simpler color change at the end point. For these reasons, hydroxy naphthol blue has been specified as the indicator to use in the assay of calcium salts in the forthcoming N.F. XII, U.S.P. XVII, and Food Chemicals Codex monographs.

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