Precipitation of Calcium Gluceptate from Aqueous Solutions

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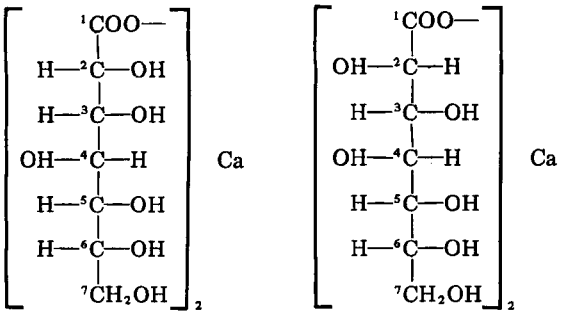
Abstract □ A precipitate encountered in solutions of calcium gluceptate was identified as hydrated calcium gluceptate. Precipitation was associated with a change from a very soluble amorphous anhydrous form to a sparingly soluble crystalline hydrate, the presence of seed crystals inducing crystallization, and unsuitable proportions of the α- and β-epimers of calcium gluceptate. Various commercial samples and the corresponding precipitates were examined by elemental analysis, thermal analysis, X-ray diffraction, IR spectroscopy, and GC–MS. The proportion of the α- and β-epimers in commercial samples was quantitated by GC. In this method, an aqueous solution of calcium gluceptate was converted into a mixture of glucoheptonic acids and their corresponding lactones by passage through a cation-exchange resin. The solution was freeze-dried, the acid–lactone mixture converted to the γ-lactones using concentrated hydrochloric acid, and the resulting material trimethylsilylated with trimethylsilylimidazole. Stability studies of solutions prepared from calcium gluceptate obtained from various commercial sources indicate that above ~50% α-epimer, stability decreased with an increase in the relative proportion of the α-epimer. Material complying with USP specifications (pure α-epimer) is the least stable in solution. It is suggested that calcium gluceptate containing approximately equal proportions of the α- and β-epimers be introduced in the USP monograph together with a method for estimating the proportions of the epimers.

Keyphrases □ Calcium gluceptate—precipitation in aqueous solutions, identification of α- and β-epimers, quantitation by GC □ Epimers—calcium gluceptate, quantitation by GC, role in precipitation in aqueous solutions □ Precipitation—calcium gluceptate in aqueous solutions, role of epimers, quantitation by GC

Calcium gluceptate used in the treatment of calcium deficiency (1), is described (2) as the calcium salt of D-*glycero*-D-*gulo*-heptonic acid (I), which is the α-epimer of glucoheptonic acid. However, the commercially available calcium gluceptate is often a mixture of the α- and β-[calcium D-*glycero*-D-*ido*-heptonate, (II)] epimers. These two forms differ only in their configuration at the second carbon atom (C-2).



I II

Calcium gluceptate is highly soluble in water, and solutions containing 85% solids have been prepared which have not crystallized on prolonged standing[1]. However, since 1976 solutions of calcium gluceptate have shown a tendency to precipitate on storage[2] (3). According to Muller *et al.* (3), the precipitate can exist in two forms (A and B). They believed that the presence of seed crystals of form A was responsible for the precipitation, and that heating calcium gluceptate powder to 115–120°C destroyed the form A seed crystals, thus preventing nucleation and growth. However, this method offered no absolute guarantee of stability, whereas heating solutions of calcium gluceptate to a minimum temperature of 80°C for 30 min destroyed the form A seed crystals and offered complete protection against later crystallization. Holstein[2] indicates that the preparation of calcium gluceptate in the amorphous form has been impossible in recent years. He attributes precipitation to the presence of seed crystals or some other factor which initiates crystallization and suggests that the product is stable if prepared hot or autoclaved after packaging.

Preliminary stability studies indicated that the time for precipitation to commence depended on the commercial source of calcium gluceptate (4). Various commercial samples and the precipitate from solutions of these samples are examined in greater detail in this paper.

EXPERIMENTAL

Materials—Four samples of calcium gluceptate (source A[3], B[4], C[5], and D[6]) were obtained. Potassium chloride[7] was ground and dried at 105°C for 2 h. α-D-Glucoheptonic acid γ-lactone (III)[8], sulfonic cation-exchange resin[9], hydrochloric acid[10], methanol[11], sucrose[12], trimethylsilylimidazole in pyridine[13], and Tris[14] were used as received.

Characterization of Calcium Gluceptate and the Precipitate—Calcium gluceptate samples from commercial sources A, B, C, and D were dried under vacuum at 60°C for 16 h. For differential scanning calorimetric studies, calcium gluceptate was used as received. The precipitate obtained from solutions prepared with calcium gluceptate from sources A, B, and C was dried under vacuum at room temperature to constant weight unless otherwise stated.

Elemental Analysis—The percentage of carbon and hydrogen in calcium gluceptate (source C) and the precipitate [from the solution of calcium gluceptate (source C)] was determined by a commercial testing laboratory[15]. Calcium was estimated complexometrically with EDTA (ethylenediaminetetraacetic acid) using a modification of the USP method (5). Theoretically, calcium gluceptate consists of 34.3% carbon, 5.34% hydrogen, and 8.17% calcium. The commercial sample from source C gave 33.4% carbon, 5.48% hydrogen, and 8.12% calcium; the precipitate gave values of 32.6, 5.67, and 7.88%, respectively.

Thermal Analysis—Thermal analyses were performed using a dif-

[1] Product information of calcium gluceptate, Pfanstiehl Laboratories, Waukegan, Ill.

[2] Dr. A. G. Holstein, Pfanstiehl Laboratories, Waukegan, Ill., personal communication.

[3] Lot R 3679 BA, Givaudan; supplied by Stanley Drug Products, North Vancouver, British Columbia, Canada.

[4] Lot R 1432 TJ, Italsintex; supplied by Stanley Drug Products, North Vancouver, British Columbia, Canada.

[5] Lot 12953-D, USP grade (pure α-epimer); Pfanstiehl Laboratories, Waukegan, Ill.

[6] Lot 7311, α- and β-mixture; Pfanstiehl Laboratories, Waukegan, Ill.

[7] Analytical reagent; BDH Chemicals, Vancouver, British Columbia, Canada.

[8] Aldrich Chemical Co., Milwaukee, Wis.

[9] Amberlite IR-120 H; Mallinckrodt, Paris, Ky.

[10] ACS grade; Allied Chemical, Pointe Claire, Quebec, Canada.

[11] ACS grade; Caledon Laboratories, Georgetown, Ontario, Canada.

[12] Analytical reagent; BDH Chemicals, Vancouver, British Columbia, Canada.

[13] TRI-SIL Z; Pierce Chemical Co., Rockford, Ill.

[14] Parr Instrument Co., Moline, Ill.

[15] Canadian Microanalytical Service, Vancouver, British Columbia, Canada.

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ferential scanning calorimeter[16] (DSC) equipped for effluent gas analysis. After grinding with a glass pestle and mortar, 1- to 5-mg samples were weighed on an electrobalance[17] directly into aluminum volatile sample pans. Scans were made at various rates using closed pans and pans with a 0.1- to 0.2-mm pinhole.

Heat of Solution—About 500 mg of the sample was accurately weighed into the sample cell of a solution calorimeter[18]. After equilibration, the cell was lowered into 100 g of water contained in a fully silvered double-walled glass vessel and rotated at a constant speed of ~450 rpm. The dissolution of calcium gluceptate samples was almost instantaneous. Throughout the reaction, the temperature was monitored with a thermistor bridge system and recorded on a strip-chart recorder.

The change in enthalpy ΔH, at the mean reaction temperature (expressed in J/g) is[19]:

$$\Delta H = -\frac{(T_f - T_i)e}{m}$$

where T_f is the final temperature, T_i is the initial temperature, m is the weight of the sample, and e is the energy equivalent of the calorimeter and its contents[19] determined using Tris (505.89 J per degree). The calorimeter was standardized using potassium chloride–water (1:400) which gave a mean value for the heat of solution at 25°C of 231.9 ± 0.8 J/g, compared with reported values (6, 7) of 234.8 and 231.0 J/g, respectively. The heat of solution of the precipitate could not be determined because of its low aqueous solubility.

Surface Area—About 200 mg of each sample was accurately weighed into the sample cell of a surface area analyzer[20]. Krypton was used as the adsorbate, and helium was the carrier gas. The specific surface area was determined by the multipoint BET method (Brunauer, Emmett, and Teller) (8) using three different concentrations of adsorbate (0.030, 0.072, and 0.104 mol % krypton in helium). The desorption signals were calibrated with pure nitrogen injected into the flow stream.

X-ray Diffraction—Approximately 300 mg of the ground sample was exposed to Ni-filtered CuKα radiation (40 kV, 15 mA) in a wide-angle X-ray diffractometer[21] over a range of 2θ from 10° to 60°.

Infrared Spectra—One milligram of the sample was dissolved in 40 mL of chloroform, and the solution was scanned[22] in the 3800- to 625-cm^{-1} range. IR spectra were also obtained using compressed disks containing 200 mg of potassium bromide and 5 mg of sample.

Equilibrium Solubility of the Precipitate—An excess of the sample was added to 50 mL of water, and the mixture was agitated in a water-bath[23] maintained at 30°C. Aliquots were taken periodically, filtered, and analyzed for calcium gluceptate by a modification of the USP assay method (5).

Stability of Calcium Gluceptate Solutions—Solutions of calcium gluceptate in water (26.7% w/v)[24] were prepared from each commercial sample. Aliquots of each solution were treated as follows: (*a*) heated in a water-bath[23] at 85°C for 30 min, (*b*) autoclaved[25] at 121°C for 20 min, (*c*) filtered through a 0.22-μm membrane filter[26], and (*d*) left untreated (control). Solutions were also prepared using calcium gluceptate which had been heated at 120°C for 12 h and cooled in a desiccator.

Estimation of the Proportions of α- and β-Epimers in Calcium Gluceptate and the Precipitate—A gas chromatograph[27] (GC) equipped with a flame-ionization detector and data terminal[28] was used. A 1.8-m × 4-mm glass column packed with 3% phenyl–cyanopropyl methyl silicone[29] on a diatomaceous earth support (OV-225 on Chromosorb W) was employed with a carrier gas (helium) flow rate of 30 mL/min. The injector and the detector temperatures were 250°C. The column was maintained at 200°C for 10 min and then was temperature programmed from 200°C to 215°C at 5°C/min. It was maintained at the upper temperature until elution of the last peak.

[16] DSC-1B; Perkin-Elmer, Norwalk, Conn.
[17] Gram; Cahn Instrument Co., Paramount, Calif.
[18] Model 1451; Parr Instrument Co., Moline, Ill.
[19] Instrument manual for solution calorimeter, Model 1451; Parr Instrument Co., Moline, Ill.
[20] Quantasorb; Quantachrome Corp., Syosset, N.Y.
[21] Philips Electronic Instruments, Mount Vernon, N.Y.
[22] Unicam SP 1000; Philips Electronics Instruments, Mount Vernon, N.Y.
[23] Magni Whirl; Blue M Electric Co., Blue Island, Ill.
[24] This corresponds to the concentration of calcium gluceptate in a commercial formulation marketed by Stanley Drug Products, North Vancouver, British Columbia, Canada.
[25] General Purpose; American Sterilizer Co., Erie, Pa.
[26] Type GS; Millipore Corp., Bedford, Mass.
[27] Model 5830 A; Hewlett-Packard, Avondale, Pa.
[28] Model 18850 A; Hewlett-Packard, Avondale, Pa.
[29] Western Chromatography Supplies, New Westminster, British Columbia, Canada.

Table I—Heats of Solution and Surface Energy of Calcium Gluceptate

Calcium Gluceptate Source	Heat of Solution[a], J/g	Specific Surface Area, m^2/g	Surface Energy, J/m^2
B	16.9	0.619	27.3
A	20.4	0.421	48.5
D	33.0	0.145	228
C	45.3	0.156	290

[a] At 22°C; average of two determinations.

Sample Preparation—About 1 g of calcium gluceptate (preliminary studies with source D material) was weighed and dissolved in 20 mL of water. This solution was passed through a column of formic acid-washed cation-exchange resin. The eluate was analyzed for calcium in an atomic absorption spectrophotometer[30]. The calcium levels were found to be <5 ppm, confirming the efficiency of the ion-exchange process. This solution was freeze-dried[31] to a constant weight. About 70 mg of this dried material was accurately weighed, dissolved in sufficient methanol to give 100 mL, and 150, 200, and 250 μL of the solution were transferred to 1-mL vials[32]. The methanol was evaporated under reduced pressure[33], 50 μL of concentrated hydrochloric acid was added to each vial, and the vials were vortex mixed[34]. The hydrochloric acid was evaporated under reduced pressure; the addition and evaporation of hydrochloric acid was repeated 3 additional times.

About 60 mg of sucrose (internal standard) was accurately weighed and dissolved in pyridine, and the volume made up to 100 mL. To each vial 100 μL of the sucrose solution and 200 μL of trimethylsilylimidazole (to form the trimethylsilyl derivative) was added, the solution was vortex mixed, and 5 μL was injected into the GC. The precipitate was subjected to the same treatment.

Gas Chromatography–Mass Spectrometry—A mass spectrometer[35] interfaced to a GC was used to characterize source A drug and the precipitate from a solution from source A. The method of sample preparation and the GC conditions were the same as above. A beam energy of 70 eV was employed, and the separator temperature was 250°C. The injection volume was 5 μL.

RESULTS AND DISCUSSION

DSC thermograms of the different samples of calcium gluceptate showed no exothermic or endothermic peaks between 25 and 180°C, indicating that the samples were anhydrous as received and did not undergo any phase transitions in this temperature range. However, the precipitate (from solutions of calcium gluceptate from sources A, B, and C) gave a single endothermic peak at ~110°C in a closed sample pan with a pinhole, which allows water to evaporate. A weight loss was found on weighing the closed pan with a pinhole before and after the endothermic peak, and a peak was obtained on the effluent gas analyzer following the endothermic peak. When the precipitate was dried under vacuum at 76°C for 2 h and then subjected to DSC, the endothermic peak was not present. This indicates that the precipitate is a hydrate, but the weight loss was found to be highly variable.

The heats of solution and the surface energies (Table I) differ for each sample, but the values do not correlate with the stabilities of the corresponding solutions (Table II).

X-ray diffraction patterns of the calcium gluceptate samples were typical of amorphous materials. There were no characteristic diffraction peaks, contrary to the information received from Holstein[2] that production of the amorphous form has been impossible in recent years. The dried precipitate diffracted X-rays, showing its crystalline nature, and the diffraction patterns of the precipitates from different sources were very similar. When the precipitate was dried under vacuum at 80°C for 46 h, there were minor changes in the diffraction pattern (Table III). The results indicate that the crystal lattice of the hydrate does not collapse to an amorphous form when the water of hydration is removed.

The IR spectra of chloroform solutions of the four samples of calcium gluceptate and the three samples of the dried precipitates were identical.

[30] Model AA-5; Varian Techtron, Melbourne, Victoria, Australia.
[31] Freeze-Drying Unit; Vitris Co., Gardiner, N.Y.
[32] Reacti-Vial; Pierce Chemical Co., Rockford, Ill.
[33] Vac Torr S 35; Precision Scientific Company, Chicago, Ill.
[34] Vortex-Genie; Scientific Industries, Springfield, Mass.
[35] MAT 111; Varian Mat GmBH, West Germany. The mass spectrometer was coupled to a computer, Model 620/L, Varian Data Machines, Palo Alto, Calif.

Table II—Relationship Between the Proportions of the α- and β-Epimers and the Stability of Calcium Gluceptate in Solution

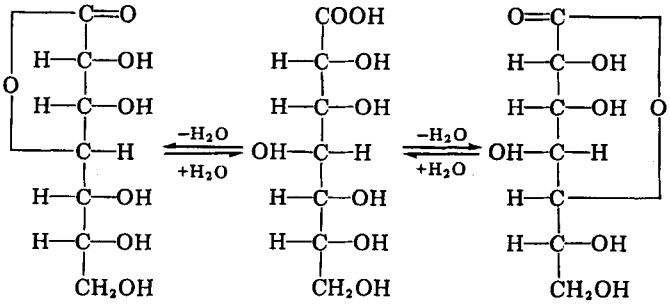
Calcium Gluceptate Source	Epimers in Calcium Gluceptate, % α	% β	Precipitation Begins, d	Epimers in Precipitate, % α	% β
C	100	0	<1	100	0
B	72.4	27.6	2	84.6	15.4
A	71.8	28.2	8	79.9	20.1
D	51.8	48.2	——Stable——		

The spectra of the potassium bromide disks of the samples were much less well defined than those of the chloroform solutions. The precipitate dried under vacuum at room temperature had a more clearly defined spectral pattern than the original sample, with an increase in the number of bands in the 1125- to 1000-cm^{-1} region, but after drying under vacuum at 80°C for 46 h the spectrum of the precipitate lost its sharpness and appeared like that of the original calcium gluceptate. This is in contrast with the X-ray results, where the more rigorous drying conditions caused only minor changes in the diffraction pattern rather than a return to the diffuse pattern of the original amorphous form.

Table III—X-ray Powder Diffraction Patterns of the Precipitate Obtained from the Solution of Calcium Gluceptate (Source C)

Dried Precipitate: Vacuum at Room Temperature[a] Interplanar Spacing, Å	Relative Intensity, %	Vacuum at 80°C[b] Interplanar Spacing, Å	Relative Intensity, %
		7.76	9
7.49	10	7.49	23
6.60	9	6.65	21
6.19	10	6.19	13
		5.86	12
5.50	13	5.50	22
5.34	27	5.37	36
5.27	6	5.24	14
4.87	7	4.87	34
4.74	22	4.72	18
		4.57	35
4.50	13	4.48	18
4.33	100	4.31	100
		4.23	22
		4.19	22
4.13	38	4.11	47
		4.02	7
3.90	31	3.88	23
3.85	44	3.83	56
		3.72	7
3.66	5	3.68	9
3.53	13	3.55	20
3.35	40	3.34	34
		3.29	18
3.25	14	3.25	22
3.23	7	3.22	10
3.16	5	3.17	8
3.10	22	3.09	22
3.05	8	3.05	16
2.98	27	2.96	23
2.92	6	2.92	8
		2.87	7
2.83	14	2.82	14
2.80	11		
2.70	19	2.70	22
2.64	5	2.64	8
2.49	11	2.49	14
2.43	31	2.43	28
2.38	14	2.38	12
2.33	15	2.32	16
2.26	8	2.25	12
		2.20	9
2.18	5		
		2.14	12
2.12	5		
2.07	8	2.07	9
2.03	10	2.03	7
1.97	6		
1.95	7		

[a] To constant weight. [b] For 46 h.



1,4-lactone of α-D-glucoheptonic acid (γ-lactone of α-D-glucoheptonic acid) | α-D-glucoheptonic acid | 1,5-lactone of α-D-glucoheptonic acid (δ-lactone of α-D-glucoheptonic acid)

Scheme I—Lactonization of α-D-glucoheptonic acid.

Attempts to determine the equilibrium solubility of each sample of calcium gluceptate were unsuccessful. Sources A, B, and D appeared to be infinitely soluble in water with >200% w/v going into solution. The accompanying increase in viscosity hindered dispersion and dissolution of additional solid. Concentrated solutions of source C were extremely unstable, becoming turbid and precipitating within minutes of dissolution. The precipitate (from the solution of source A) was found to have a solubility of 2.5% w/v in water.

Development of GC Method—Preliminary attempts to perform the GC analysis of calcium gluceptate were unsuccessful presumably due to the nonvolatile nature of this material. Hence, an aqueous solution of calcium gluceptate was first passed through a cation-exchange resin, freeze-dried, trimethylsilylated, and subjected to GC. This resulted in four peaks, presumably due to α-D-glucoheptonic acid, β-D-glucoheptonic acid, and the corresponding lactones, which result from lactonization of aldonic acids in solution (9). The formation of both the 1,4-lactone and 1,5-lactone is possible. The lactonization reactions of α-D-glucoheptonic acid are presented in Scheme I; β-D-glucoheptonic acid can undergo similar reactions.

In the presence of hydrochloric acid, aldonic acids are converted into their corresponding γ-lactones (1,4-lactones) (10). The number of substances eluted in the GC analysis was, therefore, reduced by treating the freeze-dried eluate from the ion-exchange column with hydrochloric acid. Treatment with hydrochloric acid also avoided possible complications due to the formation of 1,5-lactones (9, 10).

Identification of Calcium Gluceptate and the Precipitate—GC analysis of the treated calcium gluceptate (sources A, B, and D) indicated it to be a mixture of two compounds with retention times of 7.7 and 9.2 min (Fig. 1). GC–MS revealed that the compounds eluting as peaks 1 and 2 had similar mass spectral patterns as follows:

1. A molecular ion peak (M^+) recorded at m/z 568 in agreement with that calculated for the fully trimethylsilylated derivative of the lactone of glucoheptonic acid (11).
2. The ion M-15 (loss of one methyl group) was at m/z 553.
3. The ion M-43 was at m/z 525.
4. The ion M-105 was at m/z 463 due to the loss of methyl and trimethylsilanol groups.

The reference material III, when subjected to GC–MS analysis under the same conditions, had the same retention time and fragmentation pattern as the compound appearing as peak 2 in Fig. 1. Hence the identity of this compound as the trimethylsilyl derivative of III was confirmed.

The compounds eluting as peaks 1 and 2 (Fig. 1) have different retention times, but show mass spectral patterns with identical m/z values at the upper region of the spectrum. Hence, the chemical structures of the two compounds which gave rise to these peaks must be very similar. Since the identity of one of these compounds (peak 2) was established, peak 1 was ascribed to the trimethylsilyl derivative of β-D-glucoheptonic acid γ-lactone.

The precipitate, when subjected to the same treatment, was also found to be a mixture of two compounds with retention times of 7.7 and 9.2 min. Analysis by GC–MS showed that the mass fragmentation pattern of the compound eluting at 7.7 min was the same for both the treated calcium gluceptate and the treated precipitate. Similarly, the compounds appearing at 9.2 min also exhibited identical mass fragmentation patterns.

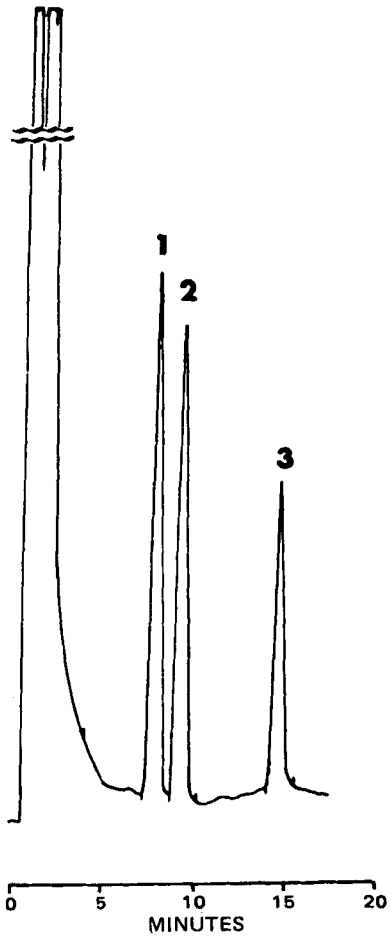


Figure 1—*Chromatogram of calcium gluceptate (source D) prepared for GC analysis. Key: (1) β-D-glucoheptonic acid γ-lactone; (2) α-D-glucoheptonic acid γ-lactone; (3) sucrose (internal standard).*

A linear relationship was established between the area ratio of peak 2 (trimethylsilyl derivative of III)–peak 3 (trimethylsilyl derivative of sucrose) and the weight ratio of III–sucrose (correlation coefficient 0.9989, slope 0.5174, intercept −0.0437). It was not possible to establish a similar relationship for β-D-glucoheptonic acid γ-lactone since this is not commercially available. Since β-D-glucoheptonic acid γ-lactone and III are epimers, the response factors were taken as being identical.

Elemental analysis, IR spectral (chloroform solutions), and GC–MS studies showed that calcium gluceptate and the precipitate were chemically similar. Since the DSC studies indicated that the precipitate was a hydrate, it was concluded that the precipitate was hydrated calcium gluceptate.

Stability of Solutions of Calcium Gluceptate—Calcium gluceptate consisting of the pure α-epimer (source C) has the lowest stability in solution, whereas the α–β mixture (source D) has the highest stability in solution (Table IV). Filtration increases the time before precipitation occurs, and autoclaved solutions showed no precipitation during 24 months of storage (Table IV). It is thought that precipitation could be due to one or more of the following factors:

1. A change from a very soluble amorphous anhydrous form to a sparingly soluble crystalline hydrate.
2. Unsuitable proportions of the α- and β-epimers. The problem of precipitation started only in 1976; prior to this time, solutions were apparently stable (3). It seems probable that in that year, a change occurred in the manufacturing procedure for calcium gluceptate which led to a change in the relative proportions of the α- and β-epimers.
3. The presence of seed crystals in the solution. Impurities or crystals of the required solute can act as nucleating agents or seed crystals (heterogeneous nucleation), inducing crystal growth at much lower degrees of supersaturation than for spontaneous (homogenous) nucleation.

Recrystallization of a Stable Form—X-ray studies showed that all the samples of calcium gluceptate were amorphous, whereas the precipitates were crystalline. Thermal analyses indicated that the initial materials were anhydrous, but the precipitates were hydrated. Hence, precipitation is due to the recrystallization of a stable hydrate from a solution made using an amorphous anhydrous form. This change produces a dramatic decrease in solubility from an infinitely soluble material to a precipitate with a water solubility of ~2.5% w/v.

Table IV—Stability of Calcium Gluceptate (26.7% w/v) in Aqueous Solution Stored at Room Temperature

Treatment	Time for Precipitation, d			
	Source A	Source B	Source C	Source D
Control[a]	2	8	<1	—[c]
Solution heated at 85°C × 30 min	13	7	<1	—
Solution autoclaved at 121°C × 20 min	—	—	—	—
Filtered	210	240	2	—
Solid heated[b] at 120°C × 12 h	3	3	<1	—

[a] Solution prepared by dissolving solid in water at room temperature; frequently contaminated with microbial growth. [b] Causes caramelization. [c] — no precipitation (during 24 months of storage).

Proportions of the α- and β-Epimers in Calcium Gluceptate and the Precipitate—There is a marked variation in the proportions of the α- and β-epimers in the different samples of calcium gluceptate, and there is a relationship between the stability in solution and the proportion of α- and β-epimers (Table II). Solutions prepared with source C (100% α-epimer) precipitated from solution within a day, whereas solutions prepared with source D (51.8% α-epimer) showed no precipitation during 24 months of storage.

The proportions of the α- and β-epimers in the precipitates obtained from solutions of calcium gluceptate were determined (Table II). There was an increase in the proportion of the α-epimer when compared with the original material. Since the precipitate is hydrated calcium gluceptate, it would appear that the hydrate of the β-epimer has a higher solubility in water than the hydrate of the α-epimer. Thus, the relative proportion of the α-epimer in the precipitate would increase.

Heating solutions of calcium gluceptate does not produce any change in the proportions of the α- and β-epimers. This became evident by determining the proportions of the α- and β-epimers before and after autoclaving solutions of calcium gluceptate.

Presence of Seed Crystals Inducing Crystallization—It has been shown that membrane filtration increases the time before precipitation occurs (Table IV), possibly by excluding the majority of seed crystals. The stability of the autoclaved solutions suggests that seed crystals are destroyed by heating at 121°C. This is above the DSC temperature of 110°C, at which the crystalline precipitate dehydrates, which suggests that the seed crystals are hydrated calcium gluceptate. Our results indicate that all three causes of instability postulated earlier are involved in precipitation, but the most important factor affecting the stability of nonautoclaved solutions is the relative proportion of the α- and β-epimers in the commercial material.

Some Comments About the USP Specifications of Calcium Gluceptate—Only the α-epimer of calcium gluceptate is official in the USP (2), but the stability results in Table IV indicate that the pure α-epimer (source C) is the least stable in aqueous solution, with precipitation occurring within a day. Moreover, the pharmacopeia offers no method of identification of the α-epimer. The USP identification test states that the IR spectrum of the sample under investigation must exhibit maxima only at the same wavelength as a similar preparation of USP Reference Standard (2). It has been found that the IR spectra of calcium gluceptate obtained from different sources (*i.e.*, containing different proportions of the α- and β-epimers) are superimposable. Thus, IR spectroscopy is incapable of distinguishing between the α- and β-epimers of calcium gluceptate.

The USP assay method consists of the complexometric estimation of calcium with EDTA and cannot distinguish between the α- and β-epimers. The rationale behind the choice of the pure α-epimer form of calcium gluceptate in the USP monograph is not known. It is apparent that in order to be stable in aqueous solution, calcium gluceptate must contain approximately equal proportions of the α- and β-epimers (as in the source D material). Hence, it is suggested that the USP should consider an α–β mixture, and the monograph should include a method for estimating the proportion of epimers in the mixture.

In our studies, based on the close structural similarity of the α- and β-epimers, the response factors of the two epimers were taken as being identical. For an absolute calculation of the proportions of the α- and β-epimers, it would be necessary to have pure reference standards for both

III and β-D-glucoheptonic acid γ-lactone. At present only III is commercially available.

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Quantitative Determination of the Stabilizers Octanoic Acid and *N*-Acetyl-DL-tryptophan in Human Albumin Products

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Abstract □ Methods were developed for the determination of octanoic acid and *N*-acetyl-DL-tryptophan, which are used as stabilizers in the human blood-derived therapeutic products normal serum albumin and plasma protein fraction. The method for octanoic acid uses GC; quantitation is achieved using heptanoic acid as the internal standard. The method for *N*-acetyl-DL-tryptophan is based on UV spectrophotometry of the acid-soluble fraction remaining after precipitation of the protein (ϵ_{280} for *N*-acetyl-DL-tryptophan, 5250). The coefficient of variation for replicate determinations of octanoic acid averaged 3.9% (range 2.1–5.5%); that of *N*-acetyl-DL-tryptophan averaged 1.9% (range 0.5–4.0%). Use of these methods for the analysis of 138 lots of commercial products for octanoic acid and 159 lots for *N*-acetyl-DL-tryptophan showed that the stabilizer contents of 132 and 158 of these lots, respectively, were within 20% of the value indicated on the product label.

Keyphrases □ *N*-Acetyl-DL-tryptophan—quantitative determination of stabilizers, octanoic acid, human albumin products □ Octanoic acid—quantitative determination of stabilizers, *N*-acetyl-DL-tryptophan, human albumin products □ Human albumin products—quantitation of stabilizers, octanoic acid and *N*-acetyl-DL-tryptophan

The major therapeutic human albumin products manufactured for intravenous use in the United States are normal serum albumin (prepared as a 5% or 25% protein solution) and plasma protein fraction (prepared as a 5% protein solution). These products are heated at 60°C for 10 h (1) to inactivate hepatitis viruses (2); to minimize changes in the protein during the heating procedure, stabilizers are added (3–5). The stabilizers permitted by federal regulations are the sodium salt of *N*-acetyl-DL-tryptophan (0.16 mmol) or a combination of sodium salts of *N*-acetyl-DL-tryptophan and octanoic acid (0.08 mmol of each) per gram of protein (1); in practice, only the combination is used. Both octanoic acid and *N*-acetyl-DL-tryptophan bind to human albumin (6). The strength of binding varies with the length of the carbon chain of the fatty acids (7, 8) and the steric configuration of tryptophan (9). Depending on the experimental conditions, lowering the concentration of either stabilizer can affect the thermal stability of albumin (5, 10, 11).

Numerous biological effects of octanoic acid have been reported, including inhibition of platelet aggregation (12, 13), hypoglycemia (14, 15), narcotic action in several animal species (16, 17), and suppression of liver clearance of long-chain fatty acids (18). The ability of the sera of certain individuals to agglutinate all human erythrocytes when octanoic acid is present has been described by several investigators (19). Although no untoward responses were elicited by administering octanoic acid-stabilized albumin to a recipient with such fatty acid-dependent ("caprylate-dependent") antibodies (20), some patients who had undergone anaphylactoid reactions to albumin products exhibited positive skin reactions to octanoic acid-treated albumin (21). In contrast, no adverse reactions have been reported for *N*-acetyl-DL-tryptophan. When given intravenously to human recipients, both the D- and the L-form are poorly utilized and rapidly excreted (22).

The present study was undertaken to develop precise and accurate methods for the quantitative determination of these stabilizers in concentrated protein solutions. The methods developed were then used to assess the stabilizer content of commercially manufactured normal serum albumin and plasma protein fraction[1].

[1] All lots of these products were submitted to the Bureau of Biologics before release for marketing; therefore, a sufficient number of samples could be analyzed to assure that the values obtained were representative of products in commercial distribution.

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