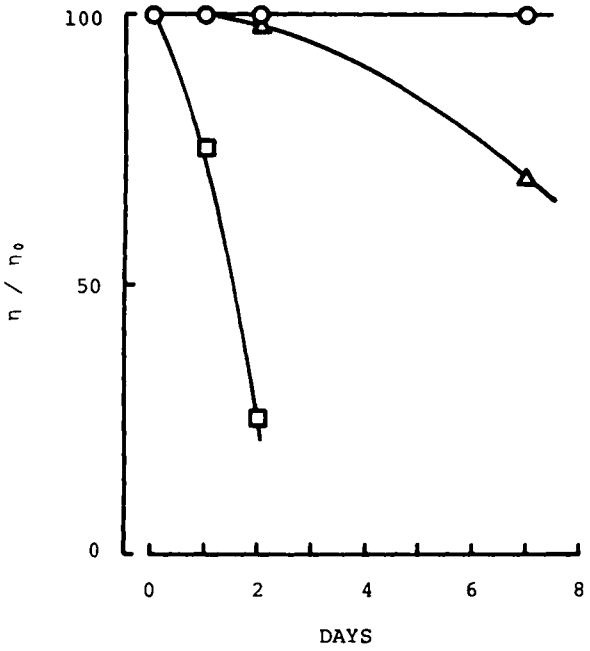

Figure 9—*Change of the viscosity of the ointment with storage time. Key: (○) o/w emulsion-type ointment; (△) hydrophilic ointment; (□) absorptive ointment. The ointments were stored at 40°C. The temperature in the measurement of viscosity was 20.0°C; the time of shear application was 30 s.*

measurements of the base over a 50-d experimental period at 40°C. Finally, the aforementioned properties suggest that these o/w-type emulsions prepared with sugar ester, I, sugar wax, and distilled water are suitable for the pharmaceutical preparation of ointments for clinical use. Future investigations will evaluate the effect of physicochemical properties of the o/w emulsion-type ointment on the indices of bioavailability through *in vivo* percutaneous absorption.

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NOTES

Determination of Calcium Gluconate by Selective Oxidation with Periodate

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Received July 19, 1982, from the *Second Pharmaceutical Factory of Wenchow, Chekiang, People's Republic of China.* Accepted for publication March 7, 1983.

Abstract □ A modified analytical method was developed which can accurately quantitate calcium gluconate and its pharmaceutical preparations in the presence of other calcium compounds or other cations able to complex with EDTA. The proposed method was based on the principle of the Malaprade reaction, according to which gluconic acid is selectively and quantitatively oxidized by sodium periodate. The content of calcium gluconate was calculated from the amount of gluconic acid found. The selective oxidation proceeded at 50°C for 10 min, yielding ~100% recovery of calcium gluconate. The proposed method was accurate, precise, and superior to the compendia EDTA-complexometric method in terms of specificity.

Keyphrases □ Calcium gluconate—determination by selective oxidation with periodate, quantitation, Malaprade reaction □ Periodate—selective oxidation, determination of calcium gluconate, quantitation, Malaprade reaction □ Malaprade reaction—determination of calcium gluconate with selective oxidation by periodate, quantitation

The EDTA-complexometric titration for the determination of calcium gluconate (I) and its pharmaceutical formulations is most commonly adopted by many current national pharmacopoeias (1–6). However, it is known that the official compendia method is only suitable for determining a sample of I which does not contain other calcium compounds or other

0022-3549/84/0500-0681$01.00/0

Table I—Molar Ratio of I to Periodate in the Selective Oxidation

I Reference Standard		Amounts of 0.05 M I_2 Subtracted by the blank, mL	Moles of $NaIO_4$ Reacted, $\times 10^{-3}$	Molar Ratio of I to $NaIO_4$	Equivalents of I for 1 mL of 0.05 M I_2, g
Added, mg	mol $\times 10^{-3}$				
55.64	0.1241	24.81	1.241	1:10.0	0.002242
50.23	0.1120	22.42	1.121	1:10.01	0.002241
51.32	0.1145	22.89	1.145	1:10.0	0.002241
48.32	0.1074	21.56	1.078	1:10.0	0.002243
53.53	0.1194	23.85	1.193	1:9.992	0.002242
Mean				1:10	0.002242

Table II—Recovery of I

I Added[a], mg	I Found[b], mg	Recovery of I, %
51.32	51.30	99.96
55.64	55.62	99.96
50.23	50.24	100.0
48.32	48.35	100.1
53.53	53.50	99.94
Mean		99.99
SD		0.0161

[a] According to Table I. [b] Calculated as 1 mL of 0.05 M I_2 equivalent to 2.242 mg of I.

cations capable of complexing with EDTA. However, I as prepared by some domestic manufacturers usually contains a certain amount of calcium oxalate, which is a byproduct produced in the fermentation of glucose with *Aspergillus niger*. Commercially available injections of I usually contain a small amount of solubilizing agent, such as calcium lactate (7). Therefore, it is impossible to accurately estimate I with the compendia methods. Because gluconic acid is considered to be a vicinal polyhydroxy alcohol and since some earlier investigators have applied the Malaprade reaction to determine vicinal polyhydroxy alcohols and aldoses (8–11), we were interested in studying the reaction of I with periodate to develop an assay method for I. The proposed method is based on the selective oxidation of gluconic acid with periodate, calculating the amount of I from the amount of gluconic acid found.

EXPERIMENTAL

Drugs and Chemicals—ChP-grade powder[1], tablets[1], and injections[1] of I, starch, talc, magnesium stearate, lactic acid, and analytical- or base-reagent-grade iodine, potassium iodide, arsenic(III) oxide, sodium oxalate, magnesium chloride, sodium bicarbonate, disodium EDTA, and sodium periodate were used.

Volumetric Solutions—Solutions of 0.05 M iodine, 0.025 M sodium arsenite, 0.05 M disodium EDTA, and 0.05 M sodium periodate were prepared and standardized by compendia methods.

Preparation of I Reference Standard—Parenteral grade I (1) was dissolved, recrystallized from redistilled water three times, and dried at 70°C. The content of I was determined by the ChP method (1), containing 100 ± 0.01% of $C_{12}H_{22}O_{14}Ca{\cdot}H_2O$. The oxalate content was <15 ppm, determined by the ferric perchlorate method (12, 13).

Stoichiometric Relation in the Selective Oxidation of I with Sodium Periodate—About 50 mg of I reference standard, accurately weighed, was placed in a glass-stoppered 500-mL flask, 20 mL of water was added, and the mixture was stirred until the sample was completely dissolved. Forty milliliters of 0.05 M sodium periodate was added and mixed. The solution was heated on a 50°C water bath for 10 min. After cooling, 1 g of potassium iodide, 1.5 g of sodium bicarbonate, and 50 mL of 0.025 M sodium arsenite were added. The flask was stoppered, thoroughly mixed, and allowed to stand in the dark for 5 min. The inner walls of the flask and the stopper were rinsed, and the residual sodium arsenite was titrated with 0.05 M I_2, using the starch test system as the indicator. A blank determination was performed simultaneously. The titration reading of 0.05 M I_2 for I reference standard, corrected for the blank, was equivalent to the amount of 0.05 M sodium periodate that reacted with I.

Selection of Optimum Condition for the Selective Oxidation of I with Sodium Periodate—To 5 mL of I reference standard solution (1.00%), were added 20 mL of water and 40 mL of 0.05 M sodium periodate. The solution was mixed as described above, except when the selective oxidation was carried out at different temperatures and time periods.

Recovery of I in the Presence of Other Substances in Accordance with the Sodium Periodate Method—Twenty milliliters of water was added to three 5-mL portions of I reference standard solution (1.00%), respectively. To the first portion, 20 mg of starch, 12.5 mg of talc, and 2.5 mg of magnesium stearate were added. To the second portion, 25 mg of sodium oxalate and 2 mg of magnesium chloride were added. Then, to each portion, 40 mL of 0.05 M sodium periodate was added, and the solution was mixed and heated on a 50°C water bath for 10 min. The procedure was continued as described above.

Comparison of the Sodium Periodate Method with the ChP Method (1) in the Determination of I and its Pharmaceutical Preparations—An aliquot of the sample, equivalent to ~50 or 500 mg of I, was accurately weighed or transferred and analyzed as described above or under the ChP method, respectively.

RESULTS AND DISCUSSION

In 1928, Malaprade (14) described that vicinal hydroxy groups in a C—C bond might be selectively oxidized by a reagent such as periodic acid to produce two aldehyde fragments. Kolthoff and Belcher (15) have reviewed this oxidation, known as the Malaprade reaction, at length and assured that all of the polyatomic alcohols, including vicinal hydroxyl groups, could be oxidized by periodate according to the following general formula (Scheme I):

$$HOCH_2(CHOH)_nCH_2OH + (n+1)IO_4^- \rightarrow 2CH_2O + nHCOOH + (n+1)IO_3^- + H_2O$$

Scheme I

The hydroxyl group at the terminal carbon atom was oxidized to yield formaldehyde, but the hydroxyl group at the intermediate carbon atom was alternatively oxidized to yield formic acid. If a carbonyl and a hydroxyl group were vicinal, the periodate oxidation would yield a carboxyl and a carbonyl function, respectively. Because gluconic acid belongs to a vicinal polyhydroxy alcohol having a terminal carboxyl group, it is possible that gluconic acid may be oxidized by periodate as in Scheme II:

$$HOCH_2(CHOH)_4COOH + 5IO_4^- \rightarrow CH_2O + 3HCOOH + (CO_2H)_2 + 5IO_3^- + H_2O$$

Scheme II

In the bicarbonate alkaline condition, the excess periodate can oxidize the added iodide to iodine quantitatively, but the iodate does not. The liberated iodine subsequently oxidizes the added arsenite and the residual arsenite is then back-titrated by 0.05 M I_2 (Scheme III):

$$IO_4^- + 2I^- + H_2O \rightarrow IO_3^- + I_2 + 2OH^-$$

$$I_2 + AsO_3^{3-} + 2OH^- \rightarrow 2I^- + AsO_4^{3-} + H_2O$$

Scheme III

As a consequence, it is obvious that 1 mol of gluconic acid is equivalent to

Table III—Recovery of I as a Function of Time and Temperature During Selective Oxidation

Temperatures, °C	Recovery of I, %[a]		
	5 min	10 min	30 min
15	89.2	90.9	91.3
40	99.2	99.9	100.2
50	100.0	100.0	100.3
60	100.0	100.2	100.6
80	100.2	100.9	102.1
100	100.9	102.6	104.2

[a] Average of triplicate analyses.

[1] Prepared by the Second Pharmaceutical Factory of Wenchow, China.

Table IV—Recovery of I in the Presence of Other Substances

I Added, mg	Other Substances Added, mg	I Found, mg[a]	Recovery of I, %[a]
50.00	Starch, 20; talc, 12.5 Magnesium stearate, 2.5	50.45	100.9 (*SD* 0.02)
50.00	Lactic acid, 25; calcium hydroxide, 2.5	50.37	100.7 (*SD* 0.08)
50.00	Magnesium chloride, 2; sodium oxalate, 0.5	49.98	99.96 (*SD* 0.05)

[a] Average of triplicate analyses.

5 mol of periodate or to 10 g equivalents of iodine. Hence, 1 mol of I is equivalent to 10 mol of sodium periodate or 20 g equivalents of iodine, *i.e.*, the molar ratio of I to periodate in the selective oxidation is 1:10, and each milliliter of 0.05 M I_2 is equivalent to 0.002242 g of I ($C_{12}H_{22}O_{14}Ca{\cdot}H_2O$, Table I). The average recovery of I is 99.99% (*SD* 0.0161, Table II).

When I reacts with periodate at 100°C over 10 min or at 80°C over 30 min, the formaldehyde would be oxidized successively so that the recovery of I may exceed 100%. On the other hand, if the reaction temperature is low and the period of reaction is short, the selective oxidation of I may be incomplete, resulting in a lowering of recovery. As a rule, it is favorable to keep the selective oxidation at 50°C for as long as 10 min, resulting ~100% recovery (Table III).

Table IV gives the results of recovery of I by the sodium periodate method in the presence of other substances. Some excipients such as starch, talc, magnesium stearate, lactic acid, calcium hydroxide, magnesium ions, and oxalate, *etc.*, occurring in amounts as much as 10-fold of formulation weight in tablets or injections of I, or far more than the compendia prescribed limits, do not interfere with the determination of I using the periodate method. Although lactic acid is an α-hydroxycarboxylic acid, it is not oxidized by periodate under the conditions described previously (16).

Table V—Results of the Determination by use of the Sodium Periodate Method and the ChP Method (1)

Sample of I	Content of Oxalate, ppm[a]	Content of I, %[b] $NaIO_4$ Method	Content of I, %[b] ChP Method
Oral			
Lot 820131	585.4	99.87	100.7
Lot 820303	909.6	99.50	101.0
Lot 820207	1527.1	99.24	101.7
Parenteral			
Lot 820314	<15	99.98	99.96
Lot 820310	<15	99.99	99.99
Tablets			
Lot 820405		98.92[c]	101.7[c]
Lot 820406		97.45[c]	99.83[c]
Injection			
Lot 801222		101.4[c]	103.2[c]
Lot 801226		100.6[c]	102.4[c]

[a] From Refs. 12 and 13. [b] Average of triplicate analyses. [c] Percent of labeled amount.

Because the amounts of oxalate present in oral grade I (1) commonly reach 500 ppm (sometimes >1000 ppm) and additive amounts of calcium lactate in I injections are approximated to 1.5% (7), results of analysis in oral grade I or its preparations with the EDTA-complexometric method are usually higher than with the sodium periodate method. Alternatively, owing to the low oxalate content in parenteral grade I (in general, the amounts of oxalate are controlled to <150 ppm in parenteral grade I; if the injections are prepared by I containing oxalate >150 ppm, microcrystalline calcium oxalate would precipitate after standing for several weeks), results of determination obtained by the two methods are almost identical (Table V).

In conclusion, it is apparent that the sodium periodate method is superior to the official EDTA-complexometric method with high specificity, no interference by any excipients or oxalate, *etc.* The proposed method is accurate and precise, not requiring any special apparatus or costly reagents.

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ACKNOWLEDGMENTS

The author thanks Ms. Chen Mei-Yu for her assistance with these experiments.