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Short Communication

Determination of calcium ion in the presence of phosphate anion and collagen by capillary-type isotachophoresis

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ABSTRACT

A new procedure for the determination of Ca^{2+} in the presence of phosphate anion and collagen was developed using capillary-type isotachophoresis. Ca^{2+} could be determined successfully using a leading electrolyte containing $1 \cdot 10^{-2}$ *M* potassium acetate–acetic acid (pH 5.4) and a terminating electrolyte containing $1 \cdot 10^{-2}$ *M* *n*-hexanoic acid in the presence of collagen. Phosphate anion influenced the determination of Ca^{2+} at pH greater than 3 but not at pH 2–3. The calcium in several calcium phosphates was determined successfully by adjusting the pH of sample solutions made from them to between 2 and 3 with hydrochloric acid.

INTRODUCTION

We are studying the synthesis of collagen–hydroxyapatite composites using the hydration [1–6] of α-tricalcium phosphate (α-TCP) in collagen solution. As the determination of Ca^{2+} in this process is influenced by phosphate anions, etc., it is necessary to pretreat samples before analysis [7]. Capillary-type isotachophoresis (ITP) has excellent selectivity [8] for each ion, but it has rarely been applied to the estimation of Ca^{2+} in calcium phosphates. Thus, we examined the determination of Ca^{2+} in the presence of phosphate anions and collagen by ITP in order to apply this method to the analysis of Ca^{2+} in the synthesis of collagen–hydroxyapatite composites and in these materials.

EXPERIMENTAL

Apparatus

A Model IP-2A Shimadzu (Kyoto, Japan) isotachophoretic analyzer equipped with a potential gradient detector was used. As the main column, a fluorinated ethylene propylene copolymer (FEP,

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TABLE I

OPERATING CONDITIONS

Parameter	Electrolyte a		Electrolyte b	
	Leading	Terminating	Leading	Terminating
Cation	H^+	Tris	K^+	H^+
Concentration	$1 \cdot 10^{-2}$ *M*	$1 \cdot 10^{-2}$ *M*	$1 \cdot 10^{-2}$ *M*	$1 \cdot 10^{-2}$ *M*
Counter ion	Cl^-	OH^-	CH_3COO^-	$CH_3(CH_2)_4COO^-$
Concentration	$1 \cdot 10^{-2}$ *M*	$1 \cdot 10^{-2}$ *M*	$1 \cdot 10^{-2}$	$1 \cdot 10^{-2}$ *M*
Solvent	Water	Water	Water (pH 5.4)	Water

Shimadzu) tube (10 cm × 0.5 mm I.D.) and as a precolumn a polytetrafluoroethylene (PTFE, Shimadzu) tube (10 cm × 1.0 mm I.D.) were used. A 2- to 10-μl aliquot of sample solution containing $5 \cdot 10^{-3}$–$1 \cdot 10^{-2}$ *M* Ca^{2+} was injected into the ITP apparatus.

To compare ITP with atomic adsorption spectrometry (AAS), we analyzed the same samples with a Model AA-180 atomic adsorption spectrometer (Nippon Jarrell-Ash, Kyoto, Japan).

Procedure

As operating conditions, the electrolytes a and b shown in Table I were examined. In electrolyte a, $1 \cdot 10^{-2}$ *M* hydrochloric acid was used as the leading electrolyte and $1 \cdot 10^{-2}$ *M* tris(hydroxymethyl)aminomethane (Tris) was used as the terminating electrolyte. The ITP of samples was carried out for 14 min at 150 μA, and then at 100 μA. In electrolyte b, $1 \cdot 10^{-2}$ *M* potassium acetate–acetic acid (pH 5.4) was used as the leading electrolyte and $1 \cdot 10^{-2}$ *M* *n*-hexanoic acid was used as the terminating electrolyte. The ITP of samples was carried out for 13 min at 200 μA, and then at 100 μA.

Materials

All solutions were prepared using guaranteed reagent-grade chemicals. The Ca^{2+} solution was obtained from calcium carbonate dried at 100°C and the phosphate anion solution was obtained from potassium dihydrogenphosphate dried at 100°C. The collagen solution was prepared by dissolving commercial pepsin-solubilized collagen (Koken, Tokyo, Japan) in $1 \cdot 10^{-3}$ *M* hydrochloric acid at 5–10°C. Sample solutions were obtained by mixing the phosphate anion solution or the collagen solution with the Ca^{2+} solution and by dissolving calcium phosphates in diluted hydrochloric acid (pH 2–3).

RESULTS AND DISCUSSION

Operating conditions

Fig. 1 shows the isotachopherograms of Ca^{2+} when 5 μl of a $5 \cdot 10^{-3}$ *M* Ca^{2+} solution were injected into the ITP apparatus. The potential unit (PU) value and the molar response in electrolyte a were larger than in electrolyte b. The calibration

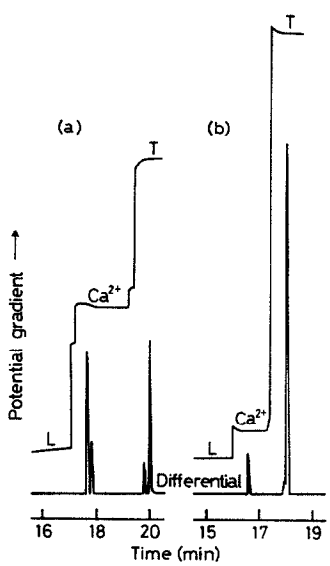

Fig. 1. Isotachopherograms of Ca^{2+}. Conditions (a) and (b) are shown in Table I. Injected sample: 5 μl of 5 m*M* Ca^{2+}. L = leading ion; T = terminating ion.

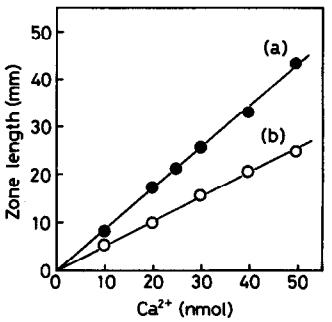

Fig. 2. Calibration curves of Ca^{2+}. Conditions (a) and (b) are shown in Table I.

curves in both conditions were linear from 10 to 50 nmol (Fig. 2). However, as the collagen could be precipitated in the analysis because of the basicity (over pH 7) of the terminating electrolyte in electrolyte a, we adopted the electrolyte system of electrolyte b in this experiment.

The reproducibility of the zone length obtained in analyses of 5 μl of a $1 \cdot 10^{-2}$ M Ca^{2+} standard solution was as follows: $\bar{x}$ (n = 26), 25.3 mm; S.D., 1.1; coefficient of variation (C.V.), 4.4%.

Influence of collagen

To examine the influence of native and denatured (heated for 30 min at 45°C) collagens on the determination of Ca^{2+}, their concentration in $5 \cdot 10^{-3}$ M Ca^{2+} solutions was varied, and the Ca^{2+} in these solutions was estimated when 5 μl of sample solution were injected. The zone length did not change with an increase in collagen concentration, whether native or denatured, when the collagen concentration was low (below about 0.1%).

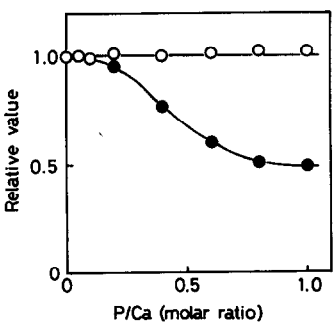

Fig. 3. Comparison of analytical methods for the determination of Ca^{2+} in the presence of phosphate anions. ○ = capillary-type isotachophoresis; ● = atomic adsorption spectrometry.

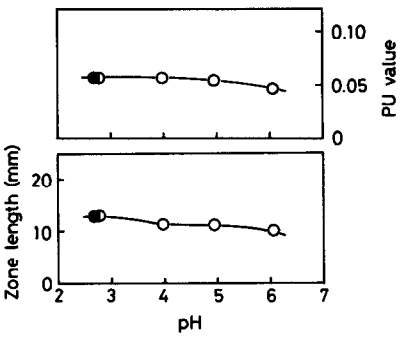

Fig. 4. Influence of pH on the zone lengths and the PU values of Ca^{2+} in the presence of phosphate anions. ○ = pH adjusted with acetic acid and ammonia, containing 0.1 M acetic acid; ● = containing 0.1 M acetic acid; ◐ = no pH adjustment.

Influence of phosphate anion

To examine the influence of the phosphate anion on the analysis of Ca^{2+}, its concentration in $5 \cdot 10^{-3}$ M solution was varied to produce an equimolar ratio (phosphorus/calcium = 1). The Ca^{2+} in these solutions was determined when 5 μl of sample solution were injected. The zone length and the PU value of the samples were the same when the phosphorus/calcium ratio was equimolar. These samples were analyzed by AAS, and the values of these samples relative to samples containing no phosphate anion were calculated for both methods.The relationship between phosphorus/calcium ratio and relative values is shown in Fig. 3. The relative values measured by ITP did not change, but those obtained with AAS decreased as the phosphorus/calcium ratio increased to 0.8, and then to about 0.5. The phosphate anion was found to influence the determination of Ca^{2+} by AAS, but not by ITP.

Influence of pH in the presence of phosphate anion

As the hydration of α-TCP occurs mainly at pH 5–6, we need to analyze the concentration of Ca^{2+} in these solutions. Thus, we examined whether the pH of solutions influences the determination of Ca^{2+} in the presence of equimolar phosphate anion. As shown in Fig. 4, the pH of solutions with no pH adjustment and containing 0.1 M acetic acid was between 2 and 3, and these zone lengths and PU values were the same. When the pH of these solutions was adjusted to 2–3 with dilute hydrochloric acid, the zone lengths and PU values recovered.

TABLE II

CALCIUM CONTENT OF SEVERAL CALCIUM PHOSPHATES

Calcium phosphate	Phosphorus/calcium ratio	Calcium content (%)	
		Measured[a]	Calculated
Calcium pyrophosphate[b]	1.00	31.6	31.5
α-Tricalcium phosphate[c]	0.67	38.3	38.7
Hydroxyapatite[d]	0.60	39.4	39.8

[a] Measured by capillary-type ITP.
[b] Synthesized by heating calcium hydrogenphosphate at 500°C for 1 h.
[c] Synthesized by heating an equimolar mixture of calcium carbonate and calcium pyrophosphate at 1300°C for 2 h.
[d] Commercial product (Isizu, Osaka, Japan).

Consequently, by adjusting the pH of solutions to 2–3, Ca^{2+} could be determined without the influence of the phosphate anion.

Determination of Ca^{2+} in calcium phosphates

We measured Ca^{2+} in sample prepared by dissolving several calcium phosphates with different phosphorus/calcium ratios in dilute hydrochloric acid, and determined their calcium content. As shown in Table II, these measured values were consistent with calculated ones.

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