

Spectrophotometric Determination of Clodronate with Thorium–Morin

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Clodronate, disodium(dichloromethylene)diphosphonate tetrahydrate, belongs to a group of diphosphonates of the type P–C–P.^{1–4} Diphosphonates are a class of ligands that are chemically related to, and mimic the physiological behaviour of, pyrophosphates. Clodronate is a synthetic analogue of pyrophosphate, which, together with conventional cancer therapy, has been used successfully for the treatment of hypercalcaemia related to osteolytic metastases and malignancies.⁵ Clodronate has also yielded good results in the treatment of Paget's disease and primary hyperparathyroidism.

In this paper a modified version of the procedure of Gallez *et al.*⁶ has been introduced for the determination of clodronate. The method is based on the interference of diphosphonate with the formation of cation–morin (3,5,7,2',4'-pentahydroxyflavone) complexes in slightly acidic solution. The change of the absorbance of a thorium–morin complex was used as a measure of diphosphonate concentration. Interference caused by the major components of human urine has also been examined.

Experimental

Apparatus. A Pye Unicam SP 8-100 UV spectrometer with quartz cells (optical path 20 mm) and a Philips PU 8700 UV spectrometer with quartz cells (optical path 20 mm) were used for the measurements.

Reagents. Morin (3,5,7,2',4'-pentahydroxyflavone, p.a.) was obtained from Merck. Methanol (HPLC reagent grade) was obtained from J. T. Baker Chemicals. Sodium acetate and glacial acetic acid were pro analysis grade and obtained from Merck. Clodronate, as the disodium(dichloromethylene)diphosphonate tetrahydrate (Na₂Cl₂MDP), was obtained from Huhtamäki Oy Leiras.⁷

Sodium acetate buffer was prepared by dissolving 20.507 g of sodium acetate in 250 ml of distilled water, and the pH was adjusted to 4.75 with glacial acetic acid.

Morin solution (0.5 mM) was prepared by dissolving 0.169 g of morin in 250 ml of methanol.

A buffered thorium solution was prepared by dissolving 0.276 g of Th(NO₃)₄ · 4H₂O in 100 ml of distilled water. An aliquot of the solution was mixed with the acetate buffer in the ratio 1/20. The Th concentration in the mixed solution was 25 μM.

Procedures.

Aqueous solutions: An aliquot of 1 ml of buffered Th solution was taken, 10 ml of clodronate sample were added and the solution was mixed. 2 ml of methanolic morin solution were added. The solution was thoroughly mixed and the absorbance was measured at 445 nm.

Interference in urine: 1 ml of buffered thorium solution was taken and an aliquot of 10 ml of solution containing clodronate (9.0 mg l⁻¹) and major substance (varying concentration) was added and mixed. 2 ml of methanolic morin were pipetted into the solution and the solution was mixed vigorously. Absorbance was measured at 445 nm. Blank solution (no major substances) was prepared and measured in every series.

Results and discussion

Morin has been used for the determination of traces of Al and Th in weakly acidic solutions. Thorium forms a

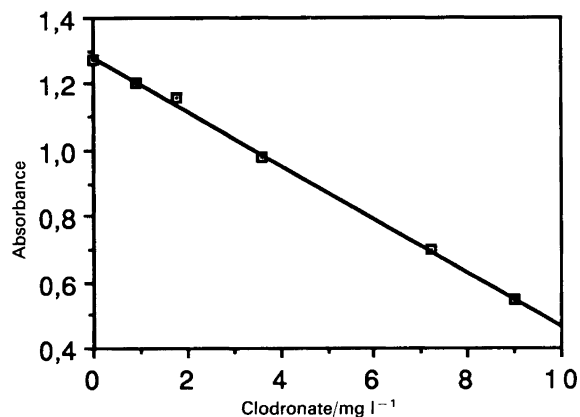


Fig. 1. Calibration graph with Th–morin.

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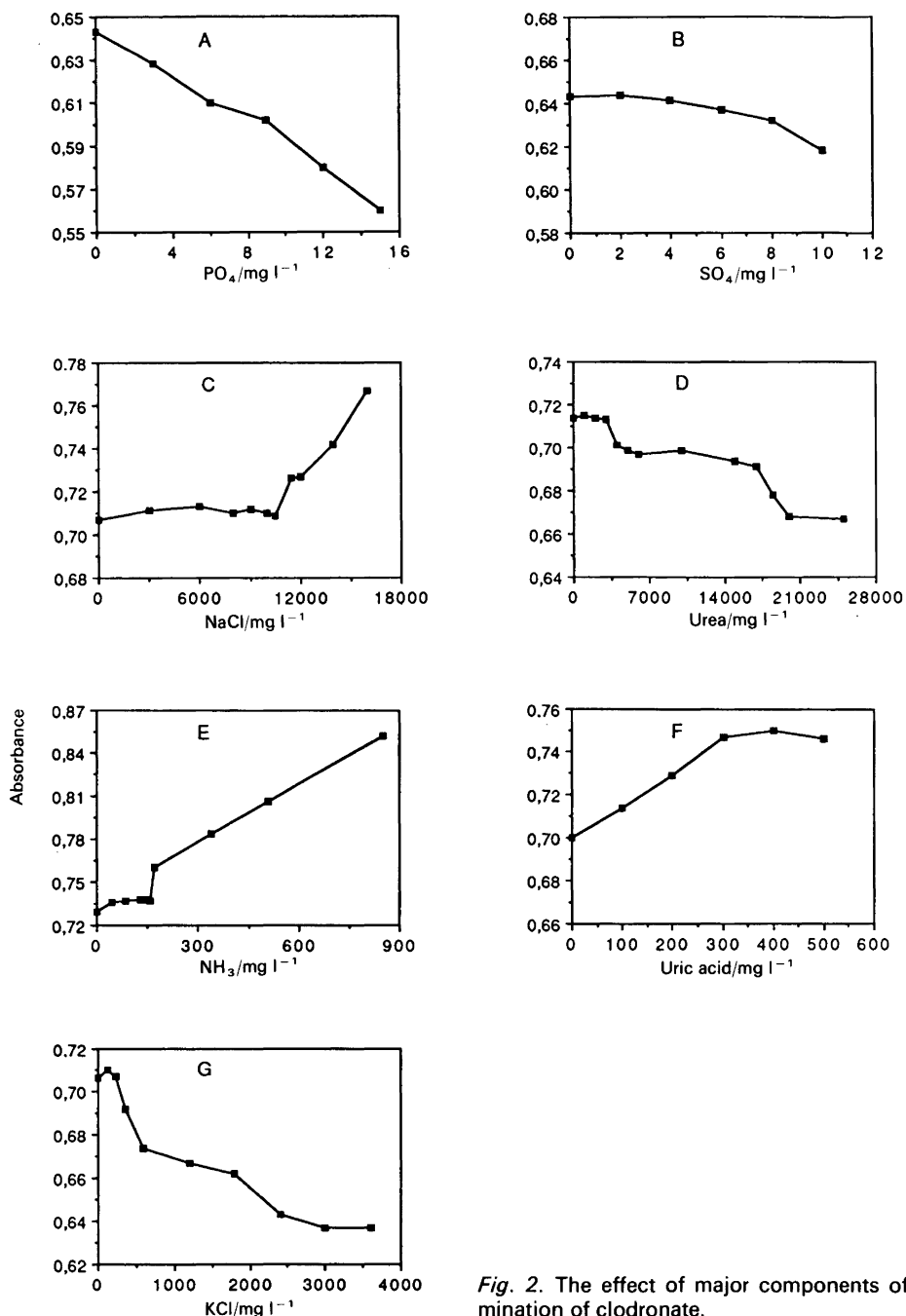
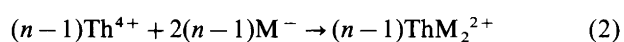
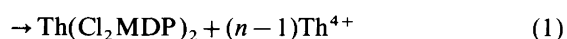
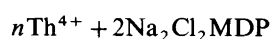


Fig. 2. The effect of major components of urine on determination of clodronate.

ThM_2^{2+} complex with morin which is weakly fluorescent but strongly coloured.⁸ The present method is based on reactions (1) and (2). Diphosphonate has a hypsochromic



effect on the UV-VIS spectrum of the Th-morin complex. In the differential UV-VIS spectra, the minimum at

Table 1. ΔA -values at different wavelengths.

λ/nm	Clodronate absorbances ^a			
	3.6-1.8	5.4-1.8	7.2-1.8	9.0-1.8
449.8	-0.063	-0.125	-0.176	-0.225
445.8	-0.064	-0.128	-0.181	-0.230
440.0	-0.064	-0.131	-0.183	-0.233
435.1	-0.062	-0.130	-0.181	-0.231

^a Absorbance value is the value of the clodronate sample (varying concentration) minus the value of the clodronate sample (1.8 mg l⁻¹).

Table 2. Determinations in aqueous solutions.

Clodronate/mg l ⁻¹	
Taken	Found
0.90	0.79
1.44	1.49
1.80	1.71 ± 0.12 ^b
2.70	2.51 ± 0.15 ^a
3.60	3.49 ± 0.10 ^b
3.96	3.85 ± 0.12 ^a
5.40	5.43 ± 0.13 ^b
7.20	7.17 ± 0.05 ^a
9.00	8.32 ± 0.06 ^a

^{a,b} Means of ^a double or ^b triple determinations with different samples.

445 nm is more reproducible than the maximum at 390 nm.⁶ According to our preliminary experiments the maximum in the UV-VIS spectra will change if the volume ratio of aqueous/methanolic solutions in the mixture is changed. The absorption maximum lies at 402, 407 and 446 nm with diphosphonate, and at 429, 427 and 455 nm without diphosphonate, when the ratio of aqueous and methanolic solution is 6:2, 11:2 and 4:5, respectively. Our measurements confirmed the earlier observations that in the differential spectra the minimum at 445 nm is more reproducible than the maximum.⁶

Measurements are also possible of the minimum peak of the differential spectra. This is exemplified in Table 1. The slopes of calibration graphs obtained at different wavelengths are practically the same, and the values of the correlation coefficient, *r*, for these graphs differ by less than 0.0015.

Better sensitivity is obtained if clodronate is allowed to react with thorium before addition of morin, instead of adding clodronate to the Th-morin complex solution.

A typical calibration graph is shown in Fig. 1. The correlation coefficient is 0.999, and the RSD value/3 replicants varies between 0.2 and 2% for the standard

solutions. The calculated limit of detection (LOD) for clodronate was 0.04 mg l⁻¹.⁹ Table 2 shows the results obtained with clodronate samples in the range 0.90–9.0 mg l⁻¹.

In order to use this method for the determination of clodronate directly in biological samples, possible interference caused by the major components of human urine on the method was examined (Fig. 2). However, organic polyoxo compounds are rarely specific for a single metal, and other polyoxo anions will cause interference by reacting with thorium. Thus morin and polyoxo anions compete to form a Th complex.

Clodronate can be determined by the present method in aqueous solution in the range 0.12–9.0 mg l⁻¹. However, if clodronate is determined in urine, it is necessary to separate it from the major matrix components before the determination. The separation could be made with HPLC using anion-exchange chromatography.¹⁰

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