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ON THE CALCIUM DETERMINATION IN HUMAN SERUM

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A complexometric method for calcium determination in human serum is described which uses 1,2-diaminocyclopentane-NN′-tetraacetic acid as titrant and calcein as indicator. It was observed that serum iron can be masked with cupferron which is more convenient for this purpose than KCN which causes the results to be too high. The method is applicable in the concentration range from 5 to 200 mgCa/100 ml.

Complexometric methods for calcium determination in human serum are widely used for they have numerous advantages, *e. g.* short time required for the analysis as well as the simplicity of the procedure. In this communication we describe an extremely simplified method for calcium determination which uses calcein as indicator and either EDTA (ethylenediamine-tetraacetic acid) or CPDTA (1,2-diaminocyclopentane-NN′-tetraacetic acid) as titrant.

Acid dissociation constants of CPDTA as well as the stability constant of its calcium complex were recently determined in this laboratory (1) and they are quoted together with respective values for EDTA (2) and DCTA (1,2-diaminocyclohexane-NN′-tetraacetic acid) (3) in Table 1.

CPDTA shows an increased affinity for calcium relative to EDTA but lower than DCTA. The basicity of CPDTA (expressed as pK_4 value), however, is remarkably lower than that of either DCTA or EDTA, so that the effective complex formation constants, defined by

$$K'_{MY} = K_{MY}/\alpha_H \qquad (1)$$

may – for moderate *p*H values – be more favourable for CPDTA than for the other two compounds. In Eq. (1) K'_{MY} stands for the effective stability constant of calcium complex, K_{MY} is the »true« stability constant while α_H is defined as

$$\alpha_H = 1 + h/K_4 + h^2/K_3K_4 + h^3/K_2K_3K_4 + h^4/K_1K_2K_3K_4 \qquad (2)$$

where K values denote the conventional acid dissociation constants and h the hydrogen ion concentration. We have studied the possibilities of use of CPDTA for calcium determination in human serum and in inorganic material. Besides this, we have studied two masking agents, KCN and cupferron which can be used for the masking of serum iron.

MATERIALS AND METHODS

All the reagents used were of analytical reagent grade and were not further purified. The water was deionized and distilled in an all-glass (»Pyrex«) apparatus.

Calcium standard solution

24.9725 g of pure $CaCO_3$ (»Merck«) was dissolved in conc. HCl and diluted to 1 liter. This solution contained 10.085 mg Ca/ml.

EDTA and CPDTA solutions

0.37210 g of disodium salt of EDTA ($Na_2EDTA.2H_2O$, BDH) was dissolved in water and made up to 1 liter to give a 1 m*M solution.* CPDTA solution, also 1 m*M*, was prepared by dissolving 0.33231 g of CPDTA in sufficient KOH to give a solution of dipotassium salt and then made up with water to 1 liter.

Calcein solution

0.0263 g of solid calcein (»Riedel de Haën«) was dissolved in 0.7 N KOH and brought up to 100 ml.

Masking solutions

For masking of serum iron 1% solutions of KCN (»Schering-Kahlbaum«) or cupferron (»Chemapol«) were used.

Procedure

0.05 or 0.10 ml of non-haemolyzed serum is pipetted into an Erlemmeyer flask which contains 0.1 ml 0.7 N KOH and *ca.* 3 ml water. After addition of 2 drops (*ca.* 0.1 ml) of calcein the titration is started with. At the end-point the greenish-yellow colour of solution changes to a slightly pink colour (or, sometimes, the solution becomes colourless) which should remain stable for more than 1 minute. 1 ml 1 m*M* CPDTA corresponds to 40.08 μg Ca. For Fe^{++} masking, if necessary, 0.1 ml of 1% Cupferron solution should be added prior to the titration.

RESULTS AND DISCUSSION

The reliability of the method was checked by adding varying amounts of calcium to the mixture of sera of 12 healthy adult humans (this mixture had a normal content of 130 μg Ca/ml). For EDTA titrations minimum 100 μl serum had to be taken for analysis while with CPDTA 50 μl could still easily be titrated. The results are shown in Table 2. By regression analysis of these data statistical parameters were calculated which are shown in Fig. 1. Although the standard error of the estimate of calcium concentration is somewhat higher for CPDTA than

Table 1

Acid dissociation constants and calcium complexes stability constants of EDTA (2), CPDTA (1) and DCTA (3), t = 20°C, medium 0.1 M KCl

Constant	EDTA	CPDTA	DCTA
pK_1	1.99	1.87	2.43
pK_2	2.67	2.44	3.52
pK_3	6.16	7.48	6.12
pK_4	10.23	10.09	11.70
log K_{CaY}	10.59	11.26	12.50

Table 2

Results of calcium determination using EDTA and CPDTA. Results are expressed as μg Ca/ml

EDTA*		CPDTA**	
Ca present	Ca found	Ca present	Ca found
17.01	16.83	10.51	10.18
20.02	20.01	13.52	13.78
23.02	23.65	16.52	16.86
33.04	32.81	26.54	26.75
53.08	52.51	46.58	46.10
93.00	93.40	86.50	86.40
113.20	112.50	106.70	100.20
133.00	132.20	126.50	126.70
173.00	173.50	166.50	165.80

* 0.1 ml of serum sample (Ca content 13 mg/100 ml).

** 0.05 ml of serum sample (Ca content 13 mg/100 ml).

for EDTA method it is to be pointed out that the former is a more sensitive reagent, which could be ascribed to its higher affinity for calcium.

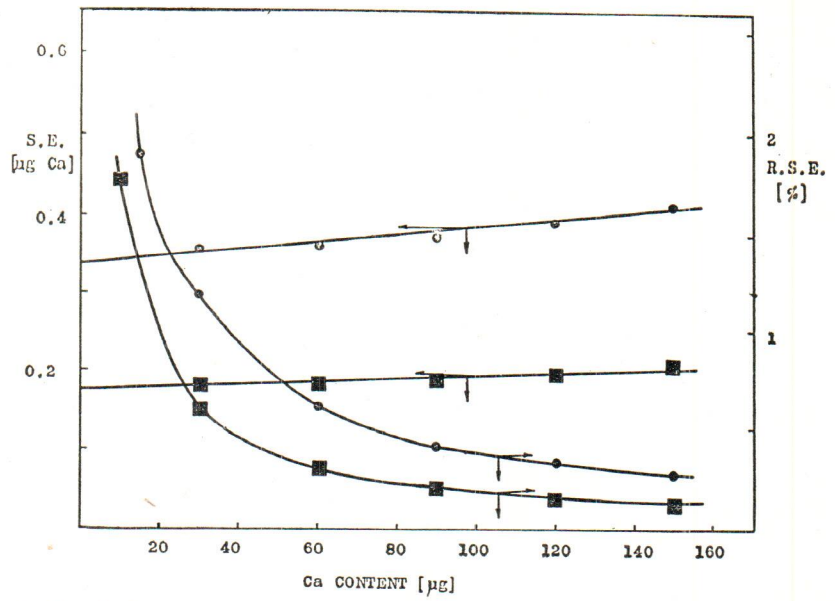

Fig. 1. *Standard error (S. E.) and relative standard error (R. S. E.) of the estimate of calcium content using EDTA (■) and CPDTA (●) as titrants*

Possible interferences with such determination were also examined, especially those arising from the presence of Fe and Mg. The latter was almost quantitatively precipitated since the working *p*H was about 12. It is frequently recommended to mask iron with KCN (5–7). Although the normal serum iron content is more than 100 times lower than the calcium level this precaution seems to be fully justified because the serum can be markedly haemolyzed.

The use of KCN in order to mask eventually present iron seems, however, to be a source of error. As can be seen from Tables 3. and 4. the presence of KCN causes the results to be too high. This applies to the results of the analyses of pure $CaCl_2$ samples as well as to the results of titration of serum samples. If instead of KCN cupferron is used the results appear to be correct. Since the error caused by KCN tends to increase with increasing KCN concentration it might be believed that there are some impurities but with the data at hand a decisive explanation cannot be given.

The efficiency of cupferron masking can be illustrated by the data from Table 5. To the analyzed samples, both those containing mineral

Table 3

Effect of addition of masking agents (MA). Analyses of pure $CaCl_2$ solutions. Results in μg Ca/ml

Calcium present	Calcium found		
	1% MA. soln. added. ml	MA. = Cupferron	MA. = KCN
10.02	0	10.02	10.02
	0.1	9.62	10.42
	0.5	10.02	10.82
	1.5	10.42	10.82
	2.0	10.42	11.62
40.08	0	40.08	40.08
	0.1	39.68	40.48
	0.5	39.28	41.28
	1.5	40.08	41.28
	2.0	40.08	42.08
80.00	0	79.76	79.76
	0.1	80.16	83.78
	0.5	79.36	83.78
	1.5	79.76	84.57
	2.0	79.76	85.37

Table 4

Effect of masking agents (MA) in the titrations with serum present. Results in μg Ca/ml

Total Ca present*	Ca found		
	1% MA. soln. added, ml.	MA. = Cupferron	MA. = KCN
26.02	0	26.05	26.05
	0.1	26.05	36.87
	0.5	26.05	37.27
	1.5	25.65	40.08
	2.0	26.45	42.08
56.08	0	55.71	55.71
	0.1	56.11	55.71
	0.5	55.31	56.51
	1.5	55.71	60.12
	2.0	56.51	62.92
96.00	0	95.97	95.79
	0.1	96.19	96.19
	0.5	95.79	97.79
	1.5	95.79	102.60
	2.0	96.59	106.21

* In each titration 0.1 ml serum was taken (Ca content 16.00 mg/100 ml).

calcium only and those with serum added, a considerable amount of $FeCl_2$ was added but this resulted in no change in result (*cf.* also Tables 3 and 4).

From the practical point of view the use of cupferron is to be recommended since it is an efficient masking agent which does not influence the results and it is more comfortable to use than highly toxic potassium cyanide. It is to be pointed out that, if serum is handled carefully to prevent haemolysis, the masking of iron is not essential. Finally, it can be concluded that the suggested method is sufficiently reliable and sensitive for clinical purposes, especially because of the use of colorimetric rather than fluorescence end-point.

Table 5

Efficiency of iron masking with cupferron. To all samples 12.28 μg Fe^{+2} (as $FeCl_2$) was added. Results in μg Ca/ml

Calcium present	1% Cupferron soln. added, ml.	Calcium found
40.08*	0.1	39.68
	0.5	40.08
	1.5	40.08
	2.0	40.48
56.08**	0.1	55.71
	0.5	56.51
	1.5	55.71
	2.0	56.11

* Samples with inorganic Ca only.

** Samples containing 0.1 ml serum (Ca content 16 mg/100 ml).

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Sadržaj

ODREĐIVANJE KALCIJA U LJUDSKOM SERUMU

Opisan je kompleksometrijski postupak za određivanje kalcija u ljudskom serumu. Kao titrant upotrijebljena je 1,2-diaminociklopentan-NN′-tetraoctena kiselina (CPDTA), koja je nešto osjetljiviji reagens nego EDTA, a kao indikator upotrebljava se kalcein. Opaženo je da je u većini slučajeva potrebno maskirati željezo, i za tu se svrhu predlaže upotreba kupferona, jer se upotrebom KCN dobivaju nešto previsoki rezultati. Metoda je primjenljiva u koncentracijskom području od 5 do 200 mg Ca/100 ml.

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ALDOMET

SREDSTVO ZA LIJEČENJE SVIH OBLIKA HIPERTENZIJE!

SASTAV

Jedna tableta sadržava 250 mg metildope.

KLINIČKE PREDNOSTI

Signifikantno smanjuje krvni pritisak i kod ležanja i kod stajanja; na taj način kontrolira krvni pritisak u toku sva 24 sata na dan.

Simptomi ortostatske hipotenzije mnogo su rjeđi nego kod drugih djelatnih antihipertenzivnih sredstava.

Hipotenzija kod napora je rijetka.

Od naročite je koristi u slučajevima kad je renalna funkcija oštećena ili postoji mogućnost da je oštećena, jer u efikasnoj dozi ne smanjuje veličinu glomerularne filtracije i renalnu cirkulaciju.

Na srčani minutni volumen obično ne utječe.

Doziranje je jednostavno, elastično i brzo se regulira.

OPREMA

Bočice sa 25 tableta.

PROIZVODI

TOVARNA FARMACEVTSKIH IN KEMIČNIH IZDELKOV

LEK

LJUBLJANA

Preparat se proizvodi u suradnji s tvornicom Merck& Co. Inc., Rahway, N. J., SAD