ON THE QUANTITATIVE DETERMINATION OF CONCREMENTS

I. Communication

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The chemical composition of concrements has been of clinical interest for long. For that reason methods for its determination have already been devised. A method for the qualitative examination of concrements is proposed by Halman. These methods are old in many respects and do not meet the present capacities of chemical analysis. A method for quantitative determination has been proposed also by Schpet and Keiser ([1]) but this method is neither contemporary.

The interest shown in internal and urological practice toward the quantitative composition of concrements stimulated us to elaborate a method for quantitative analysis, primarily of renal and vesical concrements. We directed our attention mainly toward modern methods. Our definite experimental work consisted of the following: 1) comparative study of known methods on model solutions prepared by us; 2) application of selected methods for definite analysis of concrements. We chose mainly complexometric methods which imposed a necessity to specify the conditions for determination of Ca^+, Mg^{2+}, $C_2O_4^{2-}$, PO_4^{3-} in model solutions, resembling concrement solutions. The results are reported in the present work.

I. Determination of calcium cathions — Ca^{2+}

The composition of the model solution was the following: calcium oxalate, calcium phosphate and magnesium chloride dissolved in hydrochloric acid and further diluted in distilled water. The concentration of calcium cathions amounted to 0,4008 mg/ml.

1. Permanganometric determination of Ca^2+.

M a t e r i a l s: 0,01 n potassium permanganate, 2 n sulphuric acid, model solution.

Mode of work: Determination was performed after the classic permanganometric method (2, 3, 4). Ten determination were done.

Calculations: 1 ml 0,01 n solution of $KMnO_4$ corresponds to 2004 mg Ca^2+.

2. Complexometric determination of Ca^{2+}. In concentrations of Mg^2+ lower than half the concentration of Ca^{2+}, Mg^{2+} does not hamber the correct determination of Ca^2+. In greater concentrations better results are obtained. The infavourable effect of Mg^2+ is avoided by additional dilution.

As far as the influence of PO_4^{3-} is concerned we established that in concentrations of PO_4^{3-} above 1 mg/ml low results for Ca^{2+} occur.

This necessitates the removal of PO_4^{3-} from the solution. For this purpose the method of Woy was used (5).

M a t e r i a l s: Model solution (indicated above): 0,02 or 0,002 M solution of complexon III, murexide, diluted with NaCl 1 : 50, eryochromschwarz T, diluted with NaCl 1:100, fluorexon-thymolphtalein in a ratio of 1 :1, diluted with KNO_3 1 : 20, ammonium buffer $NH_4OH.NH_4Cl$ in a ratio of 1 : 5, 10% sodium base, 3% ammonium. The method of Schwarzenbach is used (6).

a) D e t e r m i n a t i o n   w i t h   m u r e x i d e   a s   i n d i c a t o r. Two ml model solution is alkalinized with 10% sodium base up to pH 12 and is titrated with 0,02 M solution of complexon III to the point of overstaining — the transition of the stain fro, pink to purple.

Our studies indicate that in concentrations of Mg^{2+} lower than the half of that of Ca^{2+} and in concentrations of PO_4^{3-} less than 1 mg/ml coordinated results are obtained between permanganometric and complexometric determinations.

b) D e t e r m i n a t i o n s   w i t h   e r y o c h r o m s c h w a r z   T   a s i n d i c a t o r   (method of Schwarzenbach) (6). Two ml of the solution are alkalinized immediately before titration with 3% ammonia and buffered with 5 ml buffer $NH_4OH.NH_4Cl$ solution. It is titrated in cold with 0,02 M complexon III solution until the purple color becomes pale grayish blue, persisting for one minute.

Lower results are obtained for the concentration of Ca^{2+} after this method as compared with the abovementioned one.

c) D e t e r m i n a t i o n   w i t h   a   m i x e d   i n d i c a t o r — f l u o rexon-thymolphthalein. Two ml of the examined solution are alkalinized with 10% sodium base until the colour of the indicator changes from yellow into bluish-green (pH-12). It is titrated in cold with 0,02 M complexon III solution until a clearly purple colour without greenish fluorescence is achieved.

The determinations performed indicate that with the abovementioned mixed indicator results are obtained which coincide with the abovementioned methods even increased concentration of Mg^2 up to 2/3 of this of Ca^{2+}, also in PO_4^{3-} concentration up to 2 mg/ml. This fact established by us broadens the capacity of the method without the need of any additional operations.

Calculations: In all complexometric determination of Ca^{2+}: to 1 ml 0,02 M complexon III solution corresponds 0,80 mg Ca^{2+}.

Results

The results of the determinations are presented on Table 1. These data are the mean arythmetic of 35 determinations.

Discussion and conclusions

If our experimental results related to the level of Ca^{2+} are compared with those of permanganometric determinations it should be pointed out that from the standpoint of punctuality, coordinated results

are obtained in working with murexide and the mixed indicator — fluorexon-thymolphthalein. Having in mind that work with the latter may

Table 1

Method	Taken	Found	The mean deviation
Permanganometric	0,4008 mg/ml	0,3931 mg/ml	0,001
complexometric :			
with murexide	0,4008 "	0,3881 "	0,005
with eryochromschwarz	0,4008 "	0,3743 "	0,0187
with fluorexon—thymolphtalein	0,4008 "	0,3866 "	0,0065

be done within wider limits of concentrations for Mg^{2+} and PO_4^{3-} with almost identical exactness the complexometric method with mixed indicator of thymophthalein and fluorexon should be the method of choice for determination of Ca^{2+} in the model solution and in the solution of concrements.

II. Determination of Magnesium cathions (Mg^{2+})

To determine Mg^{2+} in model solutions we tried a series of methods from which only the following seemed adequate to our purposes. The model solution was prepared by dissolving pure $MgCO_3$ in hydrochloric acod. mixed with a solution of $CaCl_2$ and $Ca_3(PO_4)_2$. The concentration of Mg^{2+} amounted to 0,3450 mg/ml.

1. Weight determination of Mg^{2+} as $Mg_2P_2O_7$.

M a t e r i a l s. Model solution, $NH_4Cl,(NH_4)_2HPO_4$ saturated solution, 10% and 2,5% ammonia.

Mode of work: Determination is made after the me thod of Schmitz. Five determinations were performed.

Calculations: 48,64 mg Mg^{2+} correspond to 222,60 mg $Mg_2P_2O_7$.

2. Complexometric determination.

a) S i m u l t a n e o u s   d e t e r m i n a t i o n   of   C a $^{2+}$ and   M g $^{2+}$ a t   e r y o c h r o m s c h w a r z   T   a s   i n d i c a t o r.

M a t e r i a l s. Model solution (as in weight determination), 2,5% ammonia buffer $NH_4Cl.NH_4OH$ 1:5 0,02 M solution of complexon III, eryocthromschwarz T, diluted with NaCl 1:100, fluorexon-thymolphtalein at a ratio of 1:1, diluted with KNO_3 1:20.

Mode of work: 2 ml of the model solution are heated up to 60—70°C. Exactly before titration it is alcalified with 2,5% ammonia to pH 10,5 ml of butter solution being also added. Titration is performed with 0,002 M solution of complexon III until a pale grayish blue colour without a purple tint occurs. The colour should persist for 1 minute.

In another sample of the model solution Ca^{2+} is determined complexomertically with a mixed fluorexon-thymolphthalein indicator.

Calculation. The amount of Mg^{2+} is calculated by the difference between the first determination, the simultaneous determination of Ca^{2+} and Mg^{2+} and the second determination

$$[Ca^{2+}+Mg^{2+}]-[Ca^{2+}]=[Mg^{2+}]$$

To 1 ml 0,002 M solution of complexon III corresponds 0,4864 mg Mg^{2+}.

Our experimental data for Mg^2 indicate a correlation with the data of the weight determination of Mg^{2+}. The possible error amounts to 0,01 mg/ml.

b. D e t e r m i n a t i o n   o f Mg^{2+} i n   a   t e s t   i n   w h i c h Ca^{2+} h a s   b e e n   d e t e r m i n e d.

M a t e r i a l s. As abovementioned plus murexide as indicator.

Mode of work. The concentration of Ca^{2+} is initially determined in a sample of 2 ml at pH 12 with indicator murexide. The solution is then acidified with diluted hydrochloric acid (1 : 4) in order to destroy murexide (until decoloration is achieved). The colorless solution is heated to 60—70⁰C, alkalinized with 3% ammonia to pH 10,5, 5 ml of ammonia buffer is added and it is titrated on hot with 0,002 M solution of complexon III with eryochromschwarz T as indicator.

Calculations. 1 ml complexon III 0,0002 M corresponds to 0,4864 mg Mg^{2+}.

The results coincide with those in weight determination with an error of 0,05 mg/ml.

c. D e t e r m i n a t i o n   o f Mg^{2+} a f t e r   a   p r e l i m i n a r y   p r e-c i p i t a t i o n   o f Ca^{2+} a s   c a l c i u m   o x a l a t e.

M a t e r i a l s : 2% and 5% ammonia, 0,2% solution of methylred, 0,5% $(NH_4)_2C_2O_4$, 1 n hydrochloric acid. 10% sodium base 0,002 M solution of complexon III, eryochromschwarz T.

Mode of work: The method of Banewicz and Kenner is applied (8). A sample of 2 ml is measured in a centrifugal test tube, the solution is neutralized with 5% ammonia with methylred as indicator and about 3 ml of a 5% solution of $(NH_4)_2C_2O_4$ is added. The mixture is carefully stirred up with a glass rod with a simultaneous rubbing against the walls of the container; then it is allowed to stay for 6 hours. The rod is washed and the sample centrifugated for 15 minutes at 3000 rotations per minute. The supernatant fluid is decanted and 2 ml of 1 n hydrochloric acid is added; it is then heated up to 60—70⁰C, alkalinized with 10 ml 2% ammonia and titrated on hot with 0,002 M solution of complexon III with eryochromschwarz as indicator until a bluish green colour appears which results from the pale grayish blue colour of the eryochrom and the yellow of methylred. The transition is not very clear and for its more precise detection several determinations are needed. The amount of Mg^{2+} is calculated after the formula

$$Mg^{2+} = ml\ complexon \times 2,43$$

The concentration of Ca^{2+} may be determined also after this method in the following way: after decantation in a centrifugal test tube 5 ml of 1 n hydrochloric acid is added. A complete dissolving of the crystal oxalate sediment is achieved by bending and revolving the test tube. 0,2 ml equivalent quantity of $MgCl_2$ and complexon III are added to the solution. The latter is alkalinized with 10% soduim base up to pH — 7—8, it is then heated to 60—70⁰C; 5 ml of 3% ammonia is added to pH — 10.5

just before titration; it is then titrated to pale grayish blue with 0,002 M solution of complexon III with eryochromschwarz T as indicator. Calculations: 1 ml 0,002 M complexon III corresponds to 0,08 mg Ca^{2+}.

Experimental data concerning the concentration of Ca^{2+} and Mg^{2+} are presented on Table 2. They are the mean arytlimetic values of 35 calculations.

Table 2

Method	Taken mg/ml	Ca^{2+} mg/ml	Mg^{2+} mg/ml	Error
Permanganometric	0,888	0,8871	—	0,001
By weight	0,3450	—	0,3456	0,005
Complexometric				
a) simultaneous determination	the samo	0,8879	0,3355	0,01
b) in one and the same sample	„	0,8840	0,3105	0,05
c) separately by means of preliminary sedimentation of Ca^{2+}	„	0,8772	0,3752	0,08

Comparative data reveals that most appropriate and most convinient appears the method of simultaneous determination of Ca^{2+} and Mg^{2+} and calculation of Mg^{2+} concentration by the difference.

III. Determination of oxalate ions

1. Permanganometric determination.
M a t e r i a l s: 0,01 n $KMnO_4$, 2n sulfuric acid, a model solution, containing $C_2O_4^{2-}$ in a concentration of 6,4 mg/ml.

Mode of work: The classical method for the determination of oxalate ions.

Calculation: 1 ml of $KMnO_4$ corresponds to 0,4401 ml $C_2O_4^{2-}$.

The permanganometric determination of $C_2O_4^{2-}$ is not feasible, unless the examined solution is free of other reducing substances. This requirement obviously limits the application of this method for the analysis of concrements.

2. Plumbometric determination of $C_2O_4^2$ with 0,1 M solution of $Pb(NO_3)_2$ with ditisone as indicator (9).
M a t e r i a l s: Model solution, 0,01 M solution of $Pb(NO_3)_2$, 95% ethanol, a saturated solution of diphenyl thyocarbasone (ditisone) in 95% ethanol as internal indicator.

Mode of work: To 1 ml model solution 2 ml 95% ethanol are added plus 5—6 drops of the indicator. It is titrated with 0.1 M solution of $Pb(NO_3)_2$. The point of discoloration is characterized with a clear transition of the dark violet colour of the solution of the indicator into a milky rosy (milky from the PbC_2O_4 sediment and rosy from the resulting plumbum dithiosnate). Titration is performed with the aid of electromagnetic stirring-ladle.

Calculation: 1 ml of 0,01 M solution of $Pb(NO_3)_2$ corresponds to 0,8802 mg $C_2O_4^{2-}$ rel. 0,9002 mg $H_2C_2O_4$.

The results of the permanganometric and the plumbometric determination are presented on Table 3.

Table 3

Method	Taken			Difference	
permanganometric	plumbometric		mg/ml	permanganometric	plumbometric
7,029 mg/ml	6,3034 mg/ml		6,400	0,629	0,0966
6,522 „	6,303 „		6,400	0,122	0,097
0,735 „	6,303 „		6,400	0,335	0,097

The mean square error in the plumbometric determination amounts to 0,0012 mg/ml.

The abovementioned figures indicate that the plumbometric determination reaches extraordinary precise results. The permanganometric determination exhibit a certain dissemination. The results are, however, quite identical.

IV. Determination of phosphate ions

1. Weight determination in the form of ammonium phosphomolybdate.

M a t e r i a l s: Model solution, containing PO_4^{3-} 0,98 mg/ml, solution of ammonium molybdate (120 g/l), ammonium nitrate, nitric acid with specific gravity 1,153.

Mode of work: After the method of Dr. Woy (6), which consists in sedimentation of PO_4^{3-} with ammonium molybdate in a nitric acid medium.

Calculations: 1876,5 mg ammonium phosphomolybdate corresponds to 99,98 mg PO_4^{3-}.

2. Titrimetric determination of PO_4^{3-}. The titrimetric determination of PO_4^{3-} in a solution which contains at the same time Ca^{2+}, Mg^{2+}, $C_2O_4^{2-}$ ions requires preliminary information whether PO_4^{3-} concentration is below or above 1 mg/ml. This is ascertained in preliminary experiments.

a. D i r e c t   c o m p l e x o m e t r i c   d e t e r m i n a t i o n   of PO_4^{3-} at a c o n c e n t r a t i o n below 1 mg/ml (10).

M a t e r i a l s: Model solution (as the abovementioned); (8) ammonia, 95% ethanol, 0,002 M solution of complexon III, eryochromschwarz T, 0,01 M solution of $MgSO_4$, ammonium buffer $NH_4.OH—NH_4Cl$ 1 : 5.

Mode of work: A sample of 2 ml model solution is alcalified with 8% ammonia up to pH — 12,5 ml of the buffer is added, also eryochromschwarz T and 0,002 M solution of complexon III by means of a titration pipette until the colour changes from rosy to a pale grayish blue. Under these conditions Ca^{2+} and Mg^{2+} which are found in the solution under the form of complexonates. 5 ml 95% ethanol is added, it is titrated with 0,01 M solution of $MgSO_4$, energetic stirring up is continued until red colour appears and remains unchanges for 5 minutes.

This is due to free Mg^{2+} after all PO_4^{3-} ions of the sample are already connected with magnesium ammonium phosphate.

Calculation: 1 ml 0.01 M solution of $MgSO_4$ corresponds to 0,95 mg of PO_4^{3-}. The mean square error amounts to 0,0022 mg/ml.

b. Determination of PO_4^{3-} at concentrations of about 1 mg/ml. $PO_4^{'-}$ in concentrations of about 1 mg/ml are precipitated as magnesium-ammonium-phosphate and the determination is reduced to a complexometric determination of Mg^{2+}.

M a t e r i a l s: In addition to the abovementioned: n ammonia, 1 n hydrochloric acid, 3% ammonia, NH_4NO_3, nitric acid with a specific gravity 1,153, sedimentation mixture: 2 g $MgCl_26H_2O$; 3 g NH_4Cl, 0,2 ml mixture of equivalent amounts of Mg Cl_2 solution and complexon III, 20 ml 25% ammonia to make 100 ml water.

Mode of work: In a centrifugal test tube 2 ml are pipetted from the model solution, an equal volume of the sedimentation mixture being added. Stirring up with a glass rod is done until sediment appears; then it is allowed to stay for 10 minutes. The rod is washed with some distilled water, while the suspension is centrifugated for 15 minutes at 3000 rev/min. Then the supernatant fluid is decantated. 2 ml of 3% ammonia is added to the sediment, it is then well stirred up and again centrifugated. This manipulation is repeated another two times. After the last centrifugation the supernatant fluid is decantated while the sediment is dissolved in 1,5 ml of 1 n hydrochloric acid. The resulting solution is then poured in a cup and the test tube is wasted fourfold with distilled water, the washings being added to the solution, containing PO_4^{3-}.

Before titration the solution is alcalified with 3 ml 3% ammonia 3 ml of ammonia buffer being added and titration with 0,002 M solution of complexon III is performed using eryochromschwarz T as indicator until a grayish blue colour appears, which persists for 1 minute.

The aforegoing method gives good results (the square error amounts to 0.057 mg/ml) but it is a slow one. Centrifugation in a high power centrifuge is preffered.

Calculations: 1 ml 0,002 M complexon III corresponds to 0,00019 g PO_4^{3-}.

The results from the calculation of PO_4^{3-} after the abovementioned three methods are presented on Table 4.

Table 4

Method	Taken	Found	Error
Weight determination Titrimetric	0,9800 mg/ml	0,9804 mg/ml	0,02
a) Direct	0,9800 „	0,9798 „	—0,0022
b) Sedimentation of PO_4^{3-} and titrating Mg^{2+}	0,9800 „	0,9625 „	—0,057

c) Determination of PO_4^{3-} at concentrations above 1 mg/ml. When concentrations of PO_4^{3-} in the examined solutions are higher than 1 mg/ml, PO_4^{3-} impede the precise determination of Ca^{2+} and Mg^{2+}. This necessitates the removal of PO_4^{3-} by means of sedimentation under the form

of ammonium phosphomolybdate after the method of Voy. In this case determination of $C_2O_4^{2-}$ is performed previous to sedimentation.

The obtained sediment of ammonium phosphomolybdate is quantitatively filtrated, dissolved in 8% ammonia and used for the determination of PO_4^{3-} after the aforegoing method. Ca^{2+} and Mg^{2+} which are found in the filtrate are detected after some of the abovementioned methods.

The evaluation of the results of various methods for the determination of PO_4^{3-} thus presented leads to the conclusion that the direct complexometric determination with 0.01 M solution of $MgSO_4$ is the most rapid, most convenient and sufficiently precise.

Conclusion

From the investigated methods for quantitative determination of Ca^{2+}, Mg^{2+}, $C_2O_4^{2-}$ and PO_4^{3-} ions in model solutions of concrements, we consider the following as most appropriate:

1. For Ca^{2+} — complexometric determination with 0,002 M solution of complexon III using a mixed indicator — Fluorexon-thymolphthalein.

2. For Mg^{2+} — a simultaneous determination of Ca^{2+} and Mg^+ in the examined solution with 0,002 M solution of complexon III and eryochromschearz as indicator; separate determination of Ca^{2+} and detection of the amount of Mg^{2+} by the existing difference.

3. For $C_2O_4^{2-}$ — plumbometric determination with 0,01 M solution of $Pb(No_3)_2$.

4. For PO_4^{3-} — direct complexometric determination with 0,01 M solution of $MgSO_4$.

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К ВОПРОСУ О КОЛИЧЕСТВЕННОМ ОПРЕДЕЛЕНИИ КОНКРЕМЕНТОВ I-ОЕ СООБЩЕНИЕ

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РЕЗЮМЕ

Описываются результаты сравнительного изучения методов для определения кальциевых, магниевых, оксалатных и фосфатных ионов, которые встречаются чаще всего в составе конкрементов. Подбор методов был произведен на соответствующих модельных растворах, похожих по составу на нативные конкременты, преимущественно почечные и пузырные. В процессе испытания, известные методы приспособлены к особым случаям, причем в то же время уточнены количественно условия для этих определений. Цель настоящей работы — выработка метода, позволяющего количественный анализ конкрементов.