EXCHANGE OF CALCIUM, STRONTIUM AND BARIUM IONS ON PECTIN

R. KOHN and V. TIBENSKÝ

Institute of Chemistry,
Slovak Academy of Sciences, Bratislava

Received September 29th, 1969

Exchange of Ca^{2+}, Sr^{2+} and Ba^{2+} on pectate was examined. The interaction of these cations with carboxyl groups of pectate was estimated from the coefficient of ion exchange selectivity (K_{Ca}^{Sr}, K_{Ca}^{Ba}) and from the stability constant of the corresponding pectates (K_{Ca}, K_{Sr}, K_{Ba}). These characteristic quantities were determined by using tetramethylmurexide as auxiliary ligand. The affinity of cations for the carboxyl groups of pectate increases slightly in the sequence: calcium < < strontium < barium. The results are discussed from the point of view of application of sodium or calcium pectate as a therapeutic agent for decreasing the level of radioactive strontium in an organism.

In the recent years considerable attention has been devoted to the application of alginates for removing radioactive strontium from the gastro-intestinal tract and for decreasing its level in bones (see *e.g.* ref.[1-10]). Application of sodium alginate results in a considerable decrease of the level of strontium in the organism while the level of calcium remains practically unchanged or only a little decreased. During exchange reactions between mono- and divalent cations alginates that are rich in L-guluronic acid show a much higher selectivity toward divalent cations than alginates that are rich in D-mannuronic acid[11-13]. The selectivity of alginate toward strontium ions in solutions containing calcium and strontium is also due to a high content of guluronic acid in the alginate[14-16] which is apparent during application of these preparations *in vivo*[7-10].

Similar to alginates, pectin is a natural ion exchanger of outstanding properties. Pectin has proved its value as a prophylactic in poisoning with heavy metals[17-19]. Fine results were obtained on application of pectin to removal of radioactive strontium from the organism[2,20-25]. Most papers dealing with this problem turned their attention to the prophylactic application of pectin as a component of various foodstuffs. During experiments, common commercial preparations of pectin without

detailed analysis were used. The degree of esterification of pectin was not taken into account.

In cooperation with the Norwegian Institute for Seaweed Research in Trondheim we showed[13] that a fully deesterified pectin (potassium polygalacturonate) shows practically the same selectivity toward calcium ions as alginate which contains mostly L-guluronic acid (potassium polyguluronate). This preparation in the sodium form was found to be most effective for removing radioactive strontium from the organism. From this point of view it appeared useful to elucidate the exchange of Ca^{2+}, Ba^{2+} and Sr^{2+} on pectin. Since the highest selectivity during cation exchange was shown according to our previous experience by fully deesterified pectin[26–30] we restricted our study of exchange equilibria to potassium, calcium, strontium and barium pectates. The affinity of the carboxyl groups of pectate for these cations is assessed from the ion exchange selectivity coefficient and from the stability constant of calcium, strontium and barium pectates in solutions of potassium chloride.

EXPERIMENTAL

Material

Tetramethylmurexide was synthesized[27] from caffeine *via* tetramethylalloxanthin. Solutions of 0·1M-NaOH and 0·1M-KOH were free of carbonates. Other chemicals were of analytical purity. Pectic acid was prepared from citrus pectin, a commercial preparation of Københavns Pektinfabrik, by alkaline deesterification[28]. Pectic acid contained 86·4% polygalacturonic acid and 0·15% sulfate ash in dry weight. Its degree of esterification E was 0%, its molecular weight $\bar{M}\eta$ was 36000.

Calcium, strontium and barium pectates were prepared from pectic acid neutralized with sodium hydroxide to a 1% solution of sodium pectate. Calcium, strontium and barium pectate were precipitated from this solution made slightly acid with acetic acid, by adding 1M solutions of calcium, strontium or barium chloride solutions. The gel precipitate was thoroughly washed with hot distilled water and the gel was homogenized under addition of a small amount of water in a mixer. A stable nonsedimenting thin gel was obtained which contained 10–15 m-equiv. of $[—COOMe_{1/2}]$ groups per kg. For further work, the gel was weighed in the desired amount. As was shown by an analysis the content of Ca^{2+}, Sr^{2+} and Ba^{2+} in the gel was equivalent to the content of pectate carboxyl groups.

Analytical Methods

The concentration of free carboxyl groups of pectic acid was determined by potentiometric titration with 0·1M-NaOH. The concentration of carboxyl groups in the gel-like calcium, strontium and barium pectates was determined with the aid of insoluble copper pectates[31,32]. Gels of these metals were converted quantitatively to copper (II) pectate by washing with 5% $Cu(NO_3)_2$. After a thorough washing of copper (II) pectate with distilled water the gel was dissolved in a slight excess of ammonia and copper was determined chelatometrically using murexide as indicator. The analytical results were in agreement with an acidimetric determination of carboxyl groups in the starting solution used for the preparation of calcium, strontium and barium pectates, with an error of less than $\pm 1\%$. The molecular weight of pectic acid (as Na salt) was determined viscometrically[33].

Concentration of strontium and calcium in combination, concentration of barium and calcium in combination and concentration of free Ca^{2+}, Sr^{2+} and Ba^{2+} in the presence of a substantial excess of potassium chloride were determined spectrophotometrically using tetramethylmurexide as auxiliary ligand[34]. The original method according to Raaflaub[35,36] was designed for determinations of free Ca^{2+} concentrations in solutions of biological origin, for determining the stability constant of calcium salts of organic acids. Its application to the determination of the stability constant of calcium pectates and pectinates was described in detail in our previous publications[26,27]. In view of the fact that Sr^{2+} and Ba^{2+} react with murexide and tetramethylmurexide to a coloured complex, the principle of the method was applied to the determination of stability constant of strontium and barium pectates and to carrying out the above analyses. The spectrophotometric method has been described in detail in our previous paper[34].

In the Ca^{2+}–Sr^{2+} system we determined the absorbance A_1 at 480 nm and A_2 at 540 nm ($\varphi = A_1/A_2 = f[Ca^{2+}, Sr^{2+}]$). In the Ca^{2+}–Ba^{2+} system absorbance A_1 was determined at 490 nm and A_2 at 550 nm ($\varphi = f[Ca^{2+}, Ba^{2+}]$). When determining the stability constants of pectates the absorbances A_1 and A_2 were estimated at the following wavelengths: calcium pectate — A_1 490 nm, A_2 530 nm; strontium pectate — A_1 500 nm, A_2 550 nm; barium pectate — A_1 500 nm, A_2 560 nm. The absorbances were determined in a Uvispek-Hilger spectrophotometer in 1 cm cuvettes with a slit width of 0·1 mm. When determining the coefficient of selectivity of ion exchange (Ca^{2+}–Sr^{2+} and Ca^{2+}-Ba^{2+}) on pectate the concentrations of calcium, strontium and barium in solution were determined with an error of less than ±1%.

Since the determination of concentrations of Sr^{2+} and Ba^{2+} in relatively concentrated solutions of potassium chloride was not described in detail in a previous paper[34], Fig. 1 shows the calibration curves for the determination of Ca^{2+}, Sr^{2+} and Ba^{2+} in solutions of potassium

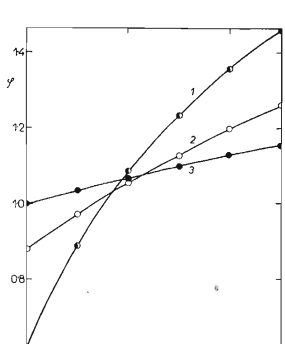

FIG. 1

Calibration Curves for the Determination of Ca^{2+}, Sr^{2+} and Ba^{2+} in a solution of KCl (μ 0·15)

1 $[Ca^{2+}]$; $\varphi = A_{490}/A_{530}$. *2* $[Sr^{2+}]$; $\varphi = A_{500}/A_{550}$; *3* $[Ba^{2+}]$; $\varphi = A_{500}/A_{560}$.

chloride of ionic strength μ 0·15. The calibration curve for Ca^{2+} (curve *1*) is steep which permits a high analytical accuracy. The error of estimation is defined by the slope of the calibration curve for the selected cation concentration and by the error of estimation of the φ quotient. The φ quotient could be estimated in all the cases with an error of less than $\pm$0·0025. Calcium in concentrations of 1, 2, 3 and 4 millimol/l was determined with an error of $\pm$1·1 to $\pm$0·6%. The calibration curve for Sr^{2+} is less steep (curve *2*). Strontium was determined for the same concentrations with the following errors: $\pm$2·9%, $\pm$1·6%, $\pm$1·2% and $\pm$1·0%. The calibration curve for the determination of Ba^{2+} (curve *3*) is rather flat as follows from the closely related absorption spectra of tetramethylmurexide in solutions of barium chloride at concentrations of 1—5 mM Ba in solutions of potassium chloride[34] of 0·15M. In view of the fact that the φ quotient can be determined with a high accuracy when observing all the conditions of analysis, barium can be determined with a fair accuracy in spite of its rather flat calibration curve. At concentrations of 1, 2, 3 and 4 mM Ba^{2+} the error of analysis is $\pm$7·1%, $\pm$3·8%, $\pm$2·6% and 2·0%.

Determination of the Selectivity Coefficient for Cation Exchange in Pectate

Calcium, strontium and barium pectates are water-insoluble and hence the exchange of these cations in pectate was followed by batch method. When studying the exchange equilibrium for each pair of divalent cations Me(I) and Me(II) two opposite directions of approach were used as shown in the scheme:

$$\text{P-Me(I)} + \text{Me(II)Cl}_2 , \qquad (A)$$

$$\text{P-Me(II)} + \text{Me(I)Cl}_2 , \qquad (B)$$

where P-Me is the pectate of the corresponding divalent ion.

We prepared three parallel suspensions according to (*A*) and three according to (*B*). All the suspensions of pectate contained 8·0 milliequivalents of $[-\text{COOMe}_{1/2}]$ per liter and an equivalent amount of the second cation. The suspensions were left to stand for 24 h at room temperature with occasional shaking, they were centrifuged and, in the supernatant, the concentrations of Me(I) and Me(II) were determined spectrophotometrically.

The selectivity coefficient was determined from

$$K^{\text{Me(I)}}_{\text{Me(II)}} = \frac{X^{\text{p}}_{\text{Me(I)}} \, X^{\text{s}}_{\text{Me(II)}}}{X^{\text{p}}_{\text{Me(II)}} \, X^{\text{s}}_{\text{Me(I)}}} ,$$

where X stands for an equivalent fraction of the corresponding cation in insoluble pectate (index *p*) and in solution (index *s*).

Determination of the Stability Constants K

The stability constants of calcium, strontium and barium pectates were determined in the same way as described in detail previously[26,36,37]. The measurements were done in suspensions containing 4 milliequivalents of $[-\text{COO}^-\text{K}^+]$ per liter, together with 2, 3, 4 and 5 mM $MeCl_2$ and $4 \cdot 10^{-5}$M tetramethylmurexide. The ionic strength of the solution μ 0·15 was adjusted by an addition of potassium chloride. After adding tetramethylmurexide and adjusting the volume of the suspension it was stirred vigorously for 1 h, centrifuged and the concentration of free cations Me^{2+} in the supernatant was determined spectrophotometrically as shown above. For spectrophotometric blank sample we used solutions obtained by centrifugation of the correspond-

ing suspensions without adding tetramethylmurexide. A correction for cation binding to the auxiliary ligand tetramethylmurexide was calculated for calcium and strontium pectates according to the procedure described in ref.[37]. With the barium pectate suspension the correction was not done due to the relatively low stability constant of the complex barium–tetramethylmurexide[34].

When calculating the stability constant K we chose as ligand unit a segment of the pectate macromolecule involving two adjacent carboxyl groups, *i.e.* a unit of digalacturonic acid which binds a single Me^{2+} cation. The stability constant is hence referred to the 1 : 1 complex; it was calculated as shown in greater detail in ref.[26]. The stability constant and the selectivity coefficient for ion exchange were determined at 22—25°C.

RESULTS AND DISCUSSION

A prolonged application of sodium pectate as a therapeutic agent for removing radioactive strontium from the gastrointestinal system and for decreasing its level in bones can result in undesirable secondary effects, *i.e.* a decrease of calcium level in the organism. From this point of view it appears useful, similary as with the alginates[5], to employ calcium pectate instead of sodium pectate. The effectivity of this therapy will in addition depend on the selectivity of exchange of Sr^{2+} for calcium in calcium pectate. Therefore, we compared the affinity of Ca^{2+}, Sr^{2+} and Ba^{2+} for carboxyl groups of pectate. We determined the selectivity coefficient for exchange of Ca^{2+} for Sr^{2+} and Ca^{2+} for Ba^{2+} in pectate and the stability constants of calcium, strontium and barium pectates in solutions of potassium chloride.

The exchange of cations in synthetic and natural ion exchangers is best expressed by an exchange isotherm which characterizes the change of the selectivity coefficient with composition of solutions. Instead of the time-consuming determination of the whole exchange isotherm the exchange equilibrium is often characterized by a selectivity coefficient determined at an exactly defined ratio of solution components. Thus, *e.g.* it is recommended to determine the selectivity coefficient for cation exchange under conditions when, after reaching equilibrium, the concentration ratio of both cations in solution is 1 : 1 (ref.[38]). Since only small differences in affinity of Ca^{2+}, Sr^{2+} and Ba^{2+} for the pectate carboxyl groups were observed, the concentration ratio of the pair of selected cations in an equilibrium solution was near 1 : 1. For this reason we did not proceed to determine the exchange isotherm and the exchange of cations was characterized merely by the selectivity coefficient.

TABLE I

Selectivity Coefficients for Exchange of Ca^{2+}, Sr^{2+} and Ba^{2+} in Pectate

Starting gel	K_{Ca}^{Sr}	K_{Ca}^{Ba}
Calcium pectate	0·86 ± 0·04	1·21 ± 0·12
Strontium pectate	1·36 ± 0·03	—
Barium pectate	—	1·56 ± 0·00

As was mentioned above the exchange equilibria were investigated in such a way that in each of the cation pairs two opposite directions of approach were employed. In case of an ideal exchange of cations the results of analysis by both procedures should be identical. The selectivity coefficients of cation exchange are summarized in Table I. Since in the study of cation exchange of Ca^{2+} for Sr^{2+} we proceeded from calcium pectate, a lower value of K_{Ca}^{Sr} was obtained. If, in the opposite direction, strontium pectate was the starting material the corresponding value was higher. The same holds for the cation pairs Ca^{2+}–Ba^{2+}. It follows from the results that the equilibrium state of cation exchange is somewhat affected by the type of the starting pectate. The exchange equilibrium is somewhat shifted toward the cation bound in the starting pectate. The point was made to make sure that the selectivity coefficients summarized in Table I correspond to an equilibrium state.

The above measurements were done with pectate of gel consistency, finely dispersed in suspension. During orientation experiments conducted with pectates in powdery form which swell only very little a considerable shift of the exchange equilibrium was found depending on the type of starting pectate. This phenomenon will be investigated in the future.

It follows from the results summarized in Table I that the affinity of the carboxyl groups of pectate toward Ca and Sr ions is practically identical. In the case of the pair of cations Ca and Ba pectate shows a slight selectivity for Ba^{2+}. If one considers all the results irrespective of the working procedure the affinity of the carboxyl groups toward divalent cations very gently rises in the series Ca < Sr < Ba.

The other procedure used for a mutual comparison of the affinities of Ca^{2+}, Sr^{2+} and Ba^{2+} for the carboxyl groups of pectate was the determination of the stability constants of calcium, strontium and barium pectates in solutions of potassium chloride of a constant ionic strength, μ 0·15. The measurements were conducted in a series of samples with increasing cation contents, using the following ratios of equivalent concentrations of $[-COO^-K^+]$ and $[Me^{2+}]$: 1 : 1, 1 : 1·5, 1 : 2, 1 : 2·5. The stability constants K determined with the individual samples of the series decreased with increasing concentrations of Me^{2+} in the system roughly in the ratios of following numbers 100, 73, 58, 50.

According to the law of multiple equilibria the stability constant K does not depend at a constant ionic strength of solution on the counterion concentration of Me^{2+}. In our previous papers we presented a large number of measurements using pectin of different origin and showed that the interaction of Ca^{2+} with free carboxyl groups of pectin follows the same law[26]. Most of the measurements were conducted with pectin samples where the degree of esterification E was greater than 20–25% and hence solutions were involved. Further measurements showed that a deviation from this law occurs only when working with pectin of a very low degree of esterification, *i.e.* in cases when during binding of divalent cations a greater amount of gel is formed. From the drop of the stability constant K of calcium, strontium and barium pectates with rising cation concentration one can conclude that the first fractions

of Me^{2+} are bound to carboxyl groups more firmly than further fractions. A certain deviation from the law of multiple equilibria thus occurs. The observed drop of the stability constant with gel suspensions of pectate is not due to the method used. The auxiliary ligand tetramethylmurexide attains even after a short period of suspension shaking the same concentration in the gel phase as in the surrounding solution.

It follows from the above said that the stability constant of the gel pectate is not a true constant but only a characteristic value corresponding to exactly defined experimental conditions. For this reason, the stability constants K shown in Table II are expressed in two ways: *1.* By average values of K_1 calculated from all the measurements irrespective of the Me^{2+} concentration in the system, *2.* by showing stability constants K_2 determined only at a given ratio of equivalent concentrations $[-COO^-K^+]$ and $[Me^{2+}]$, *viz.* 1 : 1·5. Both values K_1 and K_2 offer the same picture of the affinity of Ca^{2+}, Sr^{2+} and Ba^{2+} for the pectate carboxyl groups, even if the absolute values of K_1 and K_2 differ. The stability constants of pectates of alkaline earth metals rise in the series Ca < Sr < Ba, at ratios 1 : 1·3 : 3·1. The result is in good agreement with the observation of Schweiger[39] who, on the basis of special viscometric measurements, concluded that the stability of these pectates rises as follows: calcium < strontium < barium pectate.

The stability constants of pectates determined in solutions of potassium chloride of a relatively high concentration thus lead to the same conclusion as the exchange coefficients K_{Ca}^{Sr} and K_{Ca}^{Ba}.

The small difference in the stability constants of calcium and strontium pectates is not significant from the point of view of application of pectate. The affinity of the carboxyl groups of pectate is practically identical toward the two cations, Ca^{2+} and Sr^{2+}. A greater difference was found for Ba^{2+}. In solutions of potassium chloride (μ 0·15) the selectivity of pectate toward Ba^{2+} is greater than in dilute solutions as were used for the determination of the selectivity coefficient for cation exchange.

The stability constant K of calcium pectate is according to our experience rather dependent on the ionic strength of the solution. While in solutions of potassium chloride of ionic strength μ 0·15 the stability constants were about 600 to 800,

TABLE II

Stability Constant K of Calcium, Strontium and Barium Pectates (μ 0·15, KCl)

Pectate	K_1	K_2
Calcium pectate	700	730 ± 10
Strontium pectate	940	1 030 ± 70
Barium pectate	2 160	2 240 ± 240

in solutions with μ 0·02 the stability constants were more than ten times higher (6000 to 10000; ref.[26]). The ionic strength of the solution affects in a similar way the binding of Sr^{2+} and Ba^{2+} to the carboxyl groups of pectate.

From the results of the stability constant determination it is evident that the selectivity of potassium pectate toward Ca^{2+}, Sr^{2+} and Ba^{2+} ions is rather high. A similar situation is probable with sodium pectate. It can be therefore expected that in the application of sodium pectate as prophylactic against intoxication by radioactive strontium or barium a high therapeutical effectivity could be obtained.

In the solutions of calcium and strontium salts, pectate does not show any selectivity toward Sr^{2+} ions, in contrast to alginates, containing mainly L-guluronic acid. These results are in good agreement with the work of Patrick, who demonstrated, in studying the rat duodenal slices *in vitro*, that pectate inhibits the binding of strontium and calcium equally, whereas Waldron-Edward and coworkers[2] found, when applying pectate *in vivo*, that the degree of elimination of strontium from organism is considerably higher than that of calcium.

In long-term application of calcium pectate gel a positive result can be expected even though its effectivity will probably be lower than that of alginate. The use of calcium pectate for decreasing radioactive strontium *in vivo* will necessitate further studies, before any definite conclusions can be drawn.

REFERENCES

1. Skoryna S. C., Paul T. M., Waldron-Edward D.: Can. Med. Assoc. J. *91*, 285 (1964).
2. Waldron-Edward D., Paul T. M., Skoryna S. C.: Nature *205*, 1117 (1965).
3. Moore W. jr, Elder R. L.: Nature *206*, 841 (1965).
4. Hesp R., Ramsbottom B.: Nature *208*, 1341 (1965).
5. Paul T. M., Skoryna S. C., Waldron-Edward D.: Can. Med. Assoc. J. *95*, 957 (1966).
6. Kostial K., Maljković T., Kadić M., Manitašević R., Harrison G. E.: Nature *215*, 182 (1967).
7. Tanaka Y., Waldron-Edward D., Skoryna S. C.: Can. Med. Assoc. J. *99*, 169 (1968).
8. Hodgkinson A., Nordin B. E. C., Hambleton J., Oxby C. B.: Can. Med. Assoc. J. *97*, 1139 (1967).
9. Sutton A.: Nature *216*, 1005 (1967).
10. Patrick G., Carr T. E. F., Humphreys E. R.: Intern. J. Radiat. Biol. *12*, 427 (1967).
11. Haug A.: Acta Chem. Scand. *13*, 1250 (1959).
12. Haug A., Smidsrød O.: Acta Chem. Scand. *19*, 341 (1965).
13. Kohn R., Furda I., Haug A., Smidsrød O.: Acta Chem. Scand. *22*, 3098 (1968).
14. Haug A., Smidsrød O.: Nature *215*, 757 (1967).
15. Smidsrød O., Haug A.: Acta Chem. Scand. *22*, 1989 (1968).
16. Triffitt J. T.: Nature *217*, 457 (1968).
17. Kertesz Z. I.: *The Pectic Substances*, p. 572. Interscience, New York 1951.
18. Bezzubov A. D.: USSR Pat. 104. 801 (1957).
19. Archipova O. G., Zorina L. A.: Prof. Zabolevanija Chim. Prom. *1965*, 210.
20. MacDonald N. S., Nusbaum R. E., Ezmirlian F., Barbera R. C., Alexander G. V., Spain P., Rounds D. E.: J. Pharmacol. Exptl. Therap. *104*, 348 (1952).
21. Bezzubov A. D., Chatina A. I.: Gigiena Truda Prof. Zabolevanija *5*, No 4, 39 (1961).

22. Rubanovskaja A. A.: Gigiena Truda Prof. Zabolevanija *5*, No 4, 43 (1961).
23. Patrick G.: Nature *216*, 815 (1967).
24. Rozumovskij N. O., Torchinskaja O. L.: Med. Radiol. *12*, (4), 88 (1967).
25. Frolova E. I., Dubrovina Z. V., Bachareva Z. A., Burov N. I.: Bull. Eksp. Biol. Med. *1968*, 66, (8), 59.
26. Kohn R., Furda I.: This Journal *32*, 4470 (1967).
27. Kohn R., Furda I.: This Journal *32*, 1925 (1967).
28. Kohn R., Furda I.: This Journal *33*, 2217 (1968).
29. Tibenský V.: Chem. zvesti *22*, 401 (1968).
30. Tibenský V.: Listy cukrov. *83*, 25 (1967).
31. Tibenský V., Rosík J., Zitko V.: Nahrung *7*, 321 (1963).
32. Kohn R., Tibenský V.: Chem. zvesti *19*, 98 (1965).
33. Owens H. S., Lotzkar H., Schultz T. H., Maclay W. D.: J. Am. Chem. Soc. *68*, 1628 (1946).
34. Kohn R.: Chem. zvesti *23*, 721 (1969).
35. Raaflaub J.: Z. Physiol. Chem. *288*, 228 (1951).
36. Raaflaub J.: Z. Physiol. Chem. *328*, 198 (1962).
37. Buddecke E., Drzeniek R.: Z. Physiol. Chem. *327*, 49 (1962).
38. Štamberg J., Rádl V.: *Ionexy*, p. 73. Published by SNTL, Prague 1962.
39. Schweiger R.: Kolloid - Z. *208*, 28 (1966).

Translated by A. Kotyk.