

Ascertainment of the Inner Coordinating Water Content in Insoluble Metallic Derivatives of Pectin's

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Abstract

By derivatography in insoluble pectin's Cu^{2+} (PCu²⁺) and Pb²⁺ (PPb²⁺) the presence of «a high-temperature component» (150-165°C) is established. During potentiometric alkalimetric titration of PCu²⁺ and PPb²⁺ endpoints are established at pH accordingly 4.87 and 4.95, proving acidic properties of PMe. Obtained data show the presence of water in the internal sphere of PMe. Considering the loss of this water and the known ratio of metal cations and monomers of pectin (L⁻), the simplest formulas of pectin's are established: [Cu (L⁻)₂ (H₂O)₂], [Pb (L⁻)₂ (H₂O)₄].

Keywords: Coordination connections; Pectinates of metals; Ions of copper (II); Ions of lead (II); Composition; Derivatography; Potentiometry

Introduction

Toxic and heavy metal intoxication made it necessary to use different antidotes the effect of which should be selective to remove the cation of an undesired metal not touching the cations of the metals which are necessary for the organism. The following presents the demands placed on the antidotes [1].

The formation of reliable coordination compounds (CC) with toxic and heavy metals in order to ensure their removal from the active center of the ferment and excretion from the organism; this requirement is compiled by the antidotes with 5-6-member cycles containing several electron-donating groups, preferably chromophores, which ensure reliable, almost complete binding of metals.

The ability of antidotes and their CC with toxic and heavy metals to penetrate through the cellular membrane, to perform this they should be uncharged or bearing slight charge in order to dissolve in the membrane lipids. The non-toxicity of antidotes and CC with toxic and heavy metals which they form different stability of CC formed by the antidotes and CC formed with

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toxic and heavy metals must be more stable than those formed with biogenic metals in order to avoid the excretion of the latter from the biological systems.

Pectin's are the centerpiece in connection with their specific structural and physicochemical properties [1-5] in their molecules there are more coordination groups (carboxyl, hydroxyl groups, glyosidic linkage, oxygen atom of the pyranose cycle) than it is necessary to bind toxic and heavy metals which obtain the possibility of the choice of group with the aim of forming a more resistant CC less strong tendency to forming CC (and as a result the reliability of products) in comparison with regular complexions due to the rigidity of chain limiting its bending and twisting solubility or insolubility in water of CC with metals formed by pectin's depends directly on the degree of polymerization and concentration of pectin's, in this connection pectin's may act both in gastrointestinal tract and biological fluids.

Despite the fact that the structure of such CC as metal pectinates (PMe) [1] is rather well-examined, it is still a problem to determine the content of the inner coordinating water in them able to influence the solubility, biological availability of PMe and the therapeutic dose of pectin's as antidotes. In PMe obtained in aqueous reaction medium [6] the molecules of water may be contained in the inner or outer sphere of CC and be bound (adsorbed) capillary [7]. The determination of the content of the inner coordinating water is of interest from the point of view of the calculation of the dosage of pectin's, the reagents binding toxic and heavy metals. Ignoration of the presence of water molecules in any CC may result in underrating of doses of the antidote lacking in comparison with toxic and heavy metals.

The methods of analysis often used for studying CC (electron paramagnetic resonance spectroscopy [8,9] UVspectrophotometry [1], compleximetry [1], trilonometry [1], ion-exchange chromatography [1]) make it possible to solve many problems: to determine the geometric configuration and the type of CC, the coordination number of the central atom, the ratio of the metal cation and pectin, the stability of CC, the constants of the metal ions interchange at PMe. However, these methods cannot be used to determine the presence and content of the water molecules in PMe which may be contained in the coordinating ion, in the outer sphere of CC or be bound (adsorbed) capillary [7].

Among few methods of water detection in CC one can mention polarography [9], infrared spectroscopy [10, 11]. However their application does not allow not just to determine the water amount in terms of quantity but also to ascertain its presence or differentiate its position in the structure of CC. So, the detection of stripes referring to the deformative two-dimensional fluctuations of water at infrared spectra (1600 sm⁻¹, 590 sm⁻¹) of pectin and PMe does not let us determine their belonging to CC. The dehydration of PMe (10⁻⁵ mm of the mercury column, 185°C) [11] contributes to the loss of both adsorption and coordinating water and the following processing of CC with water does not let estimate objectively the participation of the water molecules in the formation of PMe. Besides, the use of the mentioned methods is limited by the interfering influence of other structural components of CC.

To our mind the methodological approach is the most interesting; this approach was suggested by Schwarzenbach who proved the presence of inner coordinating water in ethylene diamine tetraacetate of chrome (III) by revealing it using the method of derivatography of a "high-temperature" component (150°C and more) which being titrating by alkali forms a

hydroxocomplex in an acidic medium [12]. Such combination of the methods of derivatography and alkalimetry for determining the content of the water molecules in PMe determined the objective of our research.

The objective of the research is the interpretation of presence, position and quantity of the water molecules while establishing the molar composition of insoluble pectinates Cu^{2+} (PCu²⁺) and Pb²⁺ (PPb²⁺) using the methods of derivatography and potentiometry.

Experimental Part

The object of the research is beet pectin (meeting the requirements of the temporary certified pharmacopeial description 42-3433-99 «pectin») with average molar mass of 3200 kilogram-molecule and constant dissociation in water 3.2×10^{-4} containing 14.4% of free carboxylic groups, 9.2% methylated carboxylic groups [13]. Acetates Cu²⁺ (ACu²⁺) μ Pb²⁺ (APb²⁺) ("pure for the analysis" qualifications) were used in the research.

For obtaining PMe, 2.0 mol/l of aqua ammonia was added to $1 \ 17.8 \times 10^{-4} \text{ mol/l}$ of water solution of beet pectin (pH 3.5) drop by drop stirring with a magnetic agitator till reaching pH 8.0 (potentiometrically), then 100 ml 0.1 mol/l of water solution AMe (ACu²⁺ или APb²⁺) was added. Immediately formed jellous sediments of PMe (in order to guarantee practically absolute deposition) were treated with 96% ethanol in 1:2 volumetric proportion, then held at temperature 20°. C during 2 hours. The supernatant fluid was decanted, the sediments were filtered with dense blue band filters, then washed with 100 ml water for three times, then the sediments were dried in the drying chamber at temperature 70°. C during 3 hours' till obtaining fixed mass [6]. PCu²⁺ is of green colour, PPb²⁺ is of light brown colour. Defined by the method of viscometer [14] average molar masses of PCu²⁺ и PPb²⁺ are 4046 g/mol и 5340 g/mol correspondingly. The elemental composition (in %) of PCu²⁺: C 32.0, H 4.0, O 49.8, Cu 14.2 и PPb²⁺: C 24.3, H 3.0, O 37.8, Pb 34.9 was determined with the elemental analyzer and STE-1 spectrograph thus allowing to derive empirical formulae: Cu₉C₁₀₈H₁₆₂O₁₂₆, Pb₉C₁₀₈H₁₆₂O₁₂₆.

The study of the composition of $PCu^{2+} \mu PPb^{2+}$ in comparison with pectin and AMe was conducted step by step. With the use of the variants of thermal analysis: differential thermal analysis (DTA), differential thermogravimetric analysis (DTGA) and thermogravimetric analysis (TGA) at «Q-1500» derivatograph (Hungary, «MOM») in the 20°C to 1000°C temperature range in dynamic air with the rate of substance heating 10° per minute, speed of paper movement 5 mm per minute, by using the aluminum oxide (standard) the presence of "high-temperature" component in solid PMe with the mass about 0.5 g to 0.6 g (precise sample) was determined. The choice of high speed of heating was due to the necessity to prevent while recording the thermal curves of the structural rearrangement of PMe: the transition of the water molecules into the sphere of the coordinating ion [12]. In order to ascertain the possibility of formation of hydroxocomplexes by pectinates, using the method of potentiometric titration at «pH-3.4» pH-meter (reference electrode-silver chloride, indicator electrode-glass) 50 ml of aqueous suspensions of PCu²⁺ (8.6 × 10⁻⁴ mol/l) and PPb²⁺ (7.3 × 10⁻⁴ mol/l) prepared from samples dried at temperature 120°C during 8 hours were titrated with 0.1 mol/l sodium hydrate solution. The equivalence point was determined in the graphics system "(Δ pH/ Δ V)-Vtitrant" [15] using the differential method. For the comparison water solutions (mol/l) of pectin (6.3×10^{-4}), ACu²⁺ (5.1×10^{-3}), APb²⁺ (6.1×10^{-3}), were used which had been prepared from the substances previously cleaned from adsorption water (120°C, 8 hours). While ascertaining (in accordance with the thermal curves) the presence of "hightemperature" component (with the temperature >150°C) and the formation (in accordance with the potentiometric curves) of the hydroxocomplex (pH in the equivalence point <5) indicating the presence of the inner coordinating water, its content in the PMe dried at 120°C during 8 hours was determined by TGA presented in the graphics system "Loss of water, mol-Dehydration temperature, °C", after this the PMe molar composition was calculated.

The results and the discussion of them. The analysis of PCu^{2+} composition. The comparative analysis of thermograms of pectin (FIG. 1), ACu^{2+} (FIG. 2) and PCu^{2+} (FIG. 3) indicating essential differences of thermal effects, the nature of which [11,16-18] is shown in TABLE 1.



FIG. 1. Pectin thermogram.



FIG. 2. Thermogram ACu²⁺.



FIG. 3. Thermogram PCu²⁺

TABLE 1. Thermal characteristics PCu ²⁺ and reagents

Effect of DTA	Nature of the effect	Effect of DTGA	Total weigh	
(T ₁ -T ₂), °C		(T ₁ -T ₂), °C	loss, %	
	Pectin		1	
100-115 (max 113)	↓ desolvation	80-105 (max 105)	98.0	
190-210 (max 200)	↓ destruction of carboxyl groups	210-230 (max 230)	-	
230-260 (max 240)	0-260 (max 240) ↓ destruction for 1,4-glyosidic bonds		-	
420-450	↓ destruction	410-415 (max 415)	-	
	ACu ²⁺			
115-120 (max 118)	↓ desolvation	110-115 (max 115)	68.0	
300-430 (max 400) ↓ destruction with melting		320-450 (max 390)	-	

115-120 (max 120)	↓ desolvation	90-115 (max 110)	75.0
155-160 (max 160)	↓ desolvation	150-165 (max 165)	
200-220 (max 215)	↓ destruction of carboxyl groups	215-230 (max 225)	
240-260 (max 255)	↓ destruction for 1,4-glyosidic bonds	250-265 (max 260)	
470-500	↓ destruction	460-480 (max 475)	

Note here and is an endothermic effect; «max» is the maximum point of the thermal effect; (T1-T2) is a temperature interval of beginning and ending of the effect.

The first thermal effect (endothermic) observed for all substances refers to the temperature range 80°C to 115°C (DTGA), 100°C to 120°C (DTA). The results of the quantitative determination of water by drying (120°C, 8 hours): loss in mass of pectin from 0.60802 g to 0.50344 g (loss of water 17.2%), ACu²⁺ from 0.52315 g to 0.47607 g (loss of water 9.0%), PCu²⁺ from 0.58683 g to 0.54340 g (loss of water 7.4%) indicate that the first thermal effect is connected with the loss of capillary-bound (adsorption) water. Unlike pectin (FIG. 1) and ACu²⁺ (FIG. 2), the second endothermic effect was discovered for для PCu²⁺ (FIG. 3): (DGTA: 150°C to 165°C; DTA: 155°C to 160°C), possibly also connected with the loss of water. Other observed effects, being also endothermic, are conditioned by the destruction of the organic part of pectin and PCu²⁺ concerning carboxylic groups, glyosidic linkage. The following temperature rise led to complete decomposition of all substances. Thus, the analysis of thermal curves showed that pectin, ACu²⁺ and PCu²⁺ contain adsorption water which segregates at lower temperature and PCu²⁺ also contains a "high-temperature" component.

Supposing that the "high-temperature" component in PCu^{2+} are the molecules of inner coordinating water, PCu^{2+} being practically completely dehydrated of adsorptive water at temperature 120°C (the upper limit of the endothermic effect) during 8 hours, in comparison with pectin and ACu^{2+} , was subjected to alkalimetric titration with 0.1 mol/l sodium hydrate solution. If while the titration of pectin (FIG. 4) the point of equivalence is reached at pH 9.14 ($\Delta pH/\Delta V=28.70$), ACu^{2+} (FIG. 5, curve 1)- pH 6.42 ($\Delta pH/\Delta V=2.78$), the result while the titration of PCu²⁺ (FIG. 5, curve 2) is pH 4.87 ($\Delta pH/\Delta V=1.62$).



FIG. 4. The curve of alkalimetric pectin titration.



FIG. 5. The curves of alkalimetric titration ACu²⁺ (curve 1) and PCu²⁺ (curve 2).

Among all substances only PCu^{2+} is characterized by a point of equivalence in acidic medium having pH which is significantly lower than pH of pectin and ACu^{2+} . The obtained data testifies the manifestation of PCu^{2+} acidic properties while interacting with alkali which is possible only by the use of water molecules the acidic properties of which are intensified as a result of coordination with ions of Cu^{2+} .

Thus, the revelation of "high-temperature" component (150°C to 165°C) in PCu^{2+} and its manifestation of acidic properties proves the presence of water molecules in the inner sphere of PCu^{2+} which is not characteristic of reagents.

The quantities of water removed from the substances under analysis calculated in accordance with the TGA data are presented in TABLE 2.

T, ℃	Amount of removed water					
	Pe	ctin	AC	Cu ²⁺	PC	² u ²⁺
	mg	mmol	mg	mmol	mg	mmol
70	8.4	0.452	26.68	1.482	22.47	1.248
80	9.40	0.522	28.28	1.571	23.90	1.328
90	11.59	0.644	30.28	1.682	25.33	1.407
100	14.78	0.821	31.72	1.762	28.73	1.596
110	17.51	0.973	33.23	1.846	31.34	1.741
120	20.59	1.144	34.96	1.942	33.46	1.859

TABLE 2. Data of	TGA of pectin	, ACu ²⁺ , PCu ²⁺ .
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130	23.81	1.323	37.15	2.064	35.85	1.992
140	26.66	1.481	38.93	2.163	38.54	2.141
150	29.20	1.622	41.49	2.305	40.32	2.240
160	30.96	1.720	44.14	2.452	41.04	2.280
170	33.16	1.842	46.46	2.581	47.80	2.656
180	33.89	1.883	48.31	2.684	52.27	2.904

Unlike pectin (FIG. 6) and ACu^{2+} (FIG. 7), the TGA of PCu^{2+} (FIG. 8) distinctly demonstrated the "area" for inner coordinating water (dehydration temperature 150°C to 165°C), its quantity is 2.42 mmol (or 0.04356 g).



FIG. 6. TGA of pectin dried beforehand (120°C, 8 hours).



FIG. 7. TGA of ACu²⁺dried beforehand (120°C, 8 hours).



FIG. 8. TGA of PCu²⁺ dried beforehand (120°C, 8 hours).

The mass difference of aquacomplex of PCu^{2+} (0.54340 g) and inner coordinating water (0.04356 g) demonstrated the mass of anhydrous PCu^{2+} (0.49984 g).

Preliminarily the molar ratio of Cu^{2+} ions and the remnants of galacturonic acid (pectin monomer, L) in PCu^{2+} was ascertained as 1:2 using the following methods. The method of spectroscopy of the electronic paramagnetic resonance at indoor temperature and the boiling temperature of nitrogen; the coordination number was defined on the following spectrum parameters: the measure of effective magnetic moment of an electron, the factors of effective magnetic moment in different directions of the magnetic field, the broadening of the resonance line for the effective magnetic field, the tensor of hyperfine interaction [8,9].

- the method of emission spectrum analysis of the metal mass [1]
- the method of gravimetry of the ash mass after mineralization [1]
- the method of complementary with the usage of the method of the intersection of curves for the calculation [1-19] in the graphics system of the dependence of the CC stability constant logarithm on the coordination number of the complex former one could observe the intersection of all straight lines in one point which corresponded the abovementioned correlation
- the method of UV-spectrophotometry of equilibrium concentration of pectin's (with the analytical wavelength 292 nm) after partial dissolution of CC [1].

Taking into account the molar ratio of Cu^{2+} ions and L as 1:2 (15.46 mas. %: 84.54 mas. %), the content of cations of Cu^{2+} (0.07728 g or 1.217 mmol) and L (0.42256 g or 2.415 mmol) was calculated. Thus, the composition of PCu^{2+} freed from adsorption water is conveyed by the following correlations of ions of Cu^{2+} , L⁻ and the molecules of coordinating water: by

mass (g)-0.07728: 0.42256: 0.04356; by quantity (mmol) 1.217: 2.415: 2.420 or 1:2:2, i.e. the composition of PCu^{2+} is expressed by an elementary formula [Cu (L⁻)₂ (H₂O)₂]. These results let one think that at the interaction of pectin and Cu₂⁺ ions a partial substitution of the water molecules with L- takes place in the hydration shell of Cu²⁺ ions.

The analysis of the PPb²⁺ composition. The comparison of characteristics of thermograms of Pectin, $APb^{2+} \mu PPb^{2+}$ (FIG. 9, TABLE 3) testifies the presence of the first (endothermic) effect in the temperature ranges 80°C to 115°C (DTGA) and 100°C to 120°C (DTA), with the corresponding loss of mass on TGA curves which is referred to the loss of adsorption water [11,16-18]: 17.2% for pectin, 13.5% for APb^{2+} , 9.4% for PPb²⁺.



FIG. 9. Thermogram PPb²⁺.

TABLE 3. Thermal characteristics of PPb²⁺ and reagents.

Effect of DTA	Nature of the effect	Effect of DTGA	Total weight loss,
(T ₁ -T ₂), °C		(T ₁ -T ₂), °C	%
	Pectin		
100-115 (max 113)	↓ desolvation	80-105 (max 105)	98.0

\downarrow destruction of carboxyl groups	210-230 (max 230)	
↓ destruction for 1,4-glyosidic	255-270 (max 265)	
bonds		
↓ destruction	410-415 (max 415)	
APb ²⁺		<u> </u>
↓ desolvation	100-115 (max 110)	70.4
\downarrow destruction with melting	300-350 (max 310)	
PPb ²⁺		
↓ desolvation	80-110 (max 110)	73.0
↓ desolvation	150-160 (max 157)	
↓ destruction of carboxyl groups	215-235 (max 235)	
↓ destruction for 1,4-glyosidic	250-255 (max 250)	
bonds		
↓ destruction	350-500 (max 380)	
	↓ destruction for 1,4-glyosidic bonds ↓ destruction ↓ desolvation ↓ desolvation with melting PPb ²⁺ ↓ desolvation ↓ desolvation ↓ desolvation ↓ destruction of carboxyl groups ↓ destruction for 1,4-glyosidic bonds	$\downarrow \text{ destruction for 1,4-glyosidic} 255-270 (max 265)$ bonds $\downarrow \text{ destruction} \qquad 410-415 (max 415)$ APb^{2+} $\downarrow \text{ desolvation} \qquad 100-115 (max 110)$ $\downarrow \text{ destruction with melting} \qquad 300-350 (max 310)$ PPb^{2+} $\downarrow \text{ desolvation} \qquad 80-110 (max 110)$ $\downarrow \text{ desolvation} \qquad 150-160 (max 157)$ $\downarrow \text{ destruction of carboxyl groups} \qquad 215-235 (max 235)$ $\downarrow \text{ destruction for 1,4-glyosidic} \qquad 250-255 (max 250)$ bonds

The conclusion about the loss of adsorption water is verified with the results of the quantitative determination of the substance mass after drying (120°C, 8 hours): degrowth of pectin is 17.2% (from 0.57942 g to 0.47976 g), APb²⁺-13.5% (from 0.53274 g to 0.46082 g), PPb²⁺-9.4% (from 0.56358 g to 0.51060 g). Unlike the reagents, a "high-temperature" component has been discovered for PPb²⁺ (150°C to 160°C).

Comparative analysis of the curves of alkalimetric titration shows that while titrating pectin (FIG. 4) the point of equivalence is reached at pH 9.14, APb²⁺ (FIG. 10, curve 1)-pH 7.84 (Δ pH/ Δ V=2.84) and while titrating PPb²⁺ (FIG. 10, curve 2) it is pH 4.95 (Δ pH/ Δ V=1.83). The point of equivalence for PPb²⁺ in acidic medium testifies the manifestation of its acidic properties with the formation of hydroxocomplex.



FIG. 10. The curves of alkalimetric titration APb²⁺ (curve 1) and PPb²⁺ (curve 2).

Thus, the detection of "high-temperature" component (150° C to 160° C) in PPb²⁺ and the manifestation of its acidic properties while interacting with alkali (pH 4.95) testifies the presence of water molecules in the inner sphere of CC which is not true with reagents.

As it can be concluded from the TGA, neither pectin (FIG. 6) nor APb^{2+} (FIG. 11, curve 1) demonstrate the presence of inner coordinating water. Unlike these, the TGA of PPb^{2+} (FIG. 11, curve 2) has an "area" for inner coordinating water (150°C to 165°C), its quantity is 3.25 mmol (or 0.05850 g). The mass difference of aqua complex of PPb^{2+} (0.51060 g) and inner coordinating water (0.05850 g) demonstrates the mass of anhydrous PPb^{2+} (0.45210 g).



FIG. 11. TGA APb²⁺ (curve 1) and PPb²⁺ (curve 2) dried beforehand (120°C, 8 hours).

Preliminarily the molar ratio of Pb^{2+} ions and the remnants of galacturonic acid (pectin monomers, L) in PPb^{2+} was ascertained as 1:2 using the following methods. The method of spectroscopy of the electronic paramagnetic resonance at indoor temperature and the boiling temperature of nitrogen; the coordination number was defined on the following spectrum parameters: the measure of effective magnetic moment of an electron, the factors of effective magnetic moment in different directions of the magnetic field, the broadening of the resonance line for the effective magnetic field, the tensor of hyperfine interaction [8,9].

- the method of emission spectrum analysis of the metal mass [1]
- the method of gravimetry of the ash mass after mineralization [1]
- the method of complexometry with the usage of the method of the intersection of curves for the calculation [1,19] in the graphics system of the dependence of the CC stability constant logarithm on the coordination number of the complex former one could observe the intersection of all straight lines in one point which corresponded the abovementioned correlation.
- the method of UV-spectrophotometry of equilibrium concentration of pectin's (with the analytical wavelength 292 nm) after partial dissolution of CC [1]

Taking into account the molar ratio of Pb^{2+} ions and L- as 1:2 (37.19 mas. %: 62.81 mas. %), the content of ions of Pb^{2+} (0.16814 g or 0.811 mmol) and L- (0.28396 g or 1.623 mmol) was calculated.

Thus, the composition of PPb^{2+} freed from adsorption water is conveyed by the following correlations of ions of Pb^{2+} , L- and the molecules of coordinating water: by mass (g)-0.16814: 0.28396: 0.05850 or 1:2:4; by quantity (mmol)-0.811: 1.623: 3.25, i.e. the composition of PPb^{2+} is expressed by an elementary formula [Pb (L-)₂ (H₂O)₄].

The relative error of definitions is 3.1% to 4.8%.

Conclusion

Using the method of derivatography (DTA, DTGA and TGA) in comparison with beet pectin and ACu^{2+} and APb^{2+} the presence of "high-temperature component" in the composition of insoluble CC-PCu²⁺ (150°C to 165°C) and PPb²⁺ (150°C to 160°C) was ascertained. By potentiometric alkalimetric titration of PMe it was ascertained that it demonstrates acidic properties: for PCu²⁺ pH in the point of equivalence was 4.87, for PPb²⁺ it was 4.95, which is not characteristic of reagents: pectin (9.14), ACu^{2+} (6.42), APb^{2+} (7.84). The presence of "high-temperature" components (>150°C) in PMe and the ability of PMe to demonstrate acidic properties (pH~5) while interacting with alkali proves the presence of "inner coordinating water" in PMe. Taking into account the loss of this water and the known ratio of metal cations and L-, the structures of CC described with formulae [Cu (L-)₂ (H₂O)₂], [Pb (L-)₂ (H₂O)₄] were established.

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