

1-1-2013

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DÜRÜST, NEDİME and KAYANSALAN, ALİ (2013) "Potentiometric determination of some food additives and their binding to a polycationic species using polyion sensors," *Turkish Journal of Chemistry*. Vol. 37: No. 2, Article 14. <https://doi.org/10.3906/kim-1208-10>
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Potentiometric determination of some food additives and their binding to a polycationic species using polyion sensors

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Received: 06.08.2012 • Accepted: 04.03.2013 • Published Online: 17.04.2013 • Printed: 13.05.2013

Abstract: Polycation-sensitive membrane electrodes based on dinonylnaphthalene sulfonate (DNNS) were examined for the determination of some polyanions, namely xanthan (Xanth), alginate (Alg), carboxymethyl cellulose (CMC), and carrageenan (Carr). The potentiometric titrations of polyanionic analytes yielded sharp end points and correlative stoichiometries. After the determinations were successfully performed in saline, these polyanions were also determined in some food products consumed in Bolu (Turkey). In addition, the titration data were used to estimate the interactions between titrant and polyanionic analytes. The equilibrium constants, K_{eq} , and the number of titrant binding sites (n) per mole of analyte polyanion were calculated using Scatchard plots. The accuracy of the assayed method was confirmed by recovery experiments in spiked samples. Dynamic ranges for the determination of polyanionic analytes were 2–20 $\mu\text{g/mL}$ for CMC, 1–8 $\mu\text{g/mL}$ for Xanth, 0.5–4 $\mu\text{g/mL}$ for Alg, and 2–20 $\mu\text{g/mL}$ for Carr. LOD values were $< 2 \mu\text{g}$ for CMC and Carr, $< 1 \mu\text{g}$ for Xanth, and $< 0.5 \mu\text{g}$ for Alg.

Key words: Xanthan, alginate, carboxymethyl cellulose, polycation-sensitive, potentiometric sensors, Scatchard plot, binding constant

1. Introduction

Polymer membrane-based electrodes have been devised to detect highly charged clinically important polyions, most notably heparin and protamine, 2 drugs commonly used during cardiopulmonary bypass surgery.^{1–5} These electrodes have been called polyion-sensitive membrane electrodes (PSEs). The pioneering work on the development of electrochemical polyion-sensitive electrodes as a novel analytical tool was initiated by Meyerhoff's group, who adopted the ion-selective electrode's diagram to design such a device. A number of published works focusing on better characterization and applications for monitoring enzymatic activities of these unique electrochemical sensors can be found in the literature.^{6–12}

Xanthan (Xanth), alginate (Alg), carboxymethyl cellulose (CMC), and carrageenan (Carr) are polyionic species and food additives used as thickening agents (also called food hydrocolloids) and widely used to viscosify various food products such as dairy products, puddings, ice cream, sauces, soups, jams, and sausages, and some pharmaceuticals. They are also employed in toothpaste, drugs, and cosmetics for filling, homogenizing, gel forming, and strengthening. As it is known that they can be harmful to the digestive system, their excessive usage in foods was prohibited and, in this regard, the determination of the above-mentioned polyions is important. Various methods are currently applied for the determination of these polyanions. The colorimetric

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determination of Carr with 2-thiobarbituric acid,¹³ acridine orange,¹⁴ methylene blue,¹⁵ alcian blue,¹⁶ and hyamine,¹⁷ and determination of CMC^{18–20} have been reported. To the best of our knowledge, there are no reports on the determination of Xanth and Alg by using a polyion-sensitive film sensor except for our previous spectrophotometric work.²¹ Hassan et al. firstly reported⁸ the potential analytical utility of electrochemical sensors for the determination of Carr only.

These polyanions are polysaccharides. Among them, Xanth is a very high molecular weight structure consisting of repeating units. Alginates with more extreme compositions containing up to 100% mannuronate can be isolated from bacteria. Carr is not a uniformly composed single biopolymer; instead, it is a mixture of water-soluble, linear, sulfated galactans and they are composed of alternating 3-linked β -D-galactopyranose (G-units) and 4-linked α -D-galactopyranose (D-units) or 4-linked 3,6-anhydrogalactose (DA-units), generating the ideal disaccharide-repeating unit of carrageenans. CMC or cellulose gum is a cellulose derivative bearing carboxymethyl groups (-CH₂-COOH) bound to a couple of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone.²²

Herein, we firstly demonstrate that food additives like Xanth, Alg, and CMC can be determined by using polycation-sensitive membrane electrodes. We introduce a simple, more rapid, and reliable alternative method of determination of these polyanions. This is also the first report on the interaction of these polyanionic analytes and Carr with the polycationic titrant protamine. Thus, the binding constants are determined by recasting the titration data in the form of a Scatchard plot. After reproducible results were obtained for each polyanionic analyte in saline, some of these food additives were determined in real samples purchased from local markets. The method was verified by studying spiked samples.

2. Experimental

2.1. Apparatus and chemicals

2-Nitrophenyloctyl ether (NPOE), tetrahydrofuran (THF), protamine sulfate (from herring, grade III, average MW: 4500 Da), and CMC (MW: 982 Da) were purchased from Sigma-Aldrich Chemical Co. Xanth, Alg, λ -Carr, and polyurethane (PU M48) were obtained from Fluka. Dinonylnaphthalene sulfonate (DNNS) was kindly provided by Prof ME Meyerhoff. The other reagents were analytical grade. The buffer solution used in all experiments was 50 mM tris-HCl, pH 7.4, containing 120 mM NaCl. All solutions were prepared using deionized water (0.055 μ S cm⁻¹) produced by a TKA Smart2Pure water purification system. An Orion Model 720 A Plus ionmeter was used for potentiometric titrations. Food products containing the target polyanions as food additives were obtained from local markets.

2.2. Preparation of polycation-sensitive electrodes and titration

Polycation-sensitive membrane electrodes were prepared as previously described.⁷ The sensor film composition was 1 wt.% DNNS, 49.5 wt.% NPOE, and 49.5 wt.% M48. The cocktail solution containing a total of 1 g of the components was prepared by dissolving in 8 mL of THF. Electrodes were prepared by dip coating Tygon tubes (i.d.: \sim 1.3–1.5 mm) in this solution (12 times at 25-min intervals) and drying overnight. After soaking in 15 mM NaCl for about 6 h, the tubes were filled with 50 mM tris-HCl containing 120 mM NaCl (pH 7.4). A Ag/AgCl wire was inserted into the inner bore of the Tygon tube to serve as the internal reference electrode. These cylindrical electrodes were used in all experiments.

Potentiometric responses of the disposable electrodes were measured versus an external Ag/AgCl reference

wire using a Thermo Orion model 720 A Plus ionmeter. All measurements were conducted with constant stirring of the sample solution at room temperature. The potential changes were recorded after 2 min following each sequential addition of polycationic titrant solution. Potentiometric titrations of analytes were performed by adding small volumes of a standard, concentrated polycationic solution (0.1–10 mg/mL) to 5 mL of well-stirred 50 mM tris-HCl containing fixed amounts of polyanions. Proportional concentrations of each polyanionic thickening agent were then titrated and proportional end points were determined. Blank and polyanion curves were prepared by plotting the total potential changes (Δ EMF) versus the concentration (or log concentration) of the titrant protamine.

2.3. Interaction of polyanions and binding affinities

Polycation-sensitive membrane electrodes were also used to study the binding interactions of the target polyanions (CMC, Xanth, Alg, and Carr) with the titrant protamine. A Scatchard plot for each analyte was obtained as previously described.¹⁰ The binding constants and number of polycation binding sites per mole of polyanionic analytes were estimated by using individual Scatchard plots. For this purpose, the polycation sensor was first calibrated by adding increasing concentrations of polycation to buffer solution. The potentiometric titration of the target polyanion was carried out by titrant protamine in tris buffer solution. Then the calculations regarding each polycation–polyanion binding constant (K_{eq}) and the number (n) were performed according to previously published work.⁷

2.4. Analysis of some food product samples containing polyanionic additive

Known quantities of food products (10–500 mg) were dissolved in 10 mL of 50 mM tris-HCl containing 120 mM NaCl (pH 7.4) solution, and titrated with a standard protamine solution. A polycation-sensitive membrane electrode based on DNNS was used to monitor the titration end point. The potential change in the membrane electrode was recorded after 2 min following each sequential addition of standard polyion solution. The end points were calculated from sigmoidal titration curves. Titrations were repeated 3 times for each food product. Polyanion concentrations were calculated from the end points of the sample and blank titration curves. The ability of the titrimetric method to detect the thickening agents in food product samples was assessed by spiking sample solutions with varying levels of polyanions. Thus, the polyanion content of food product samples was tested for recovery experiments.

3. Results and discussion

3.1. Titration of polyanionic additives

Polyanions like CMC, Xanth, Alg, and Carr are used as thickening, homogenizing, gel forming, and strengthening materials for food products. Since excess doses of food additives are known to cause harmful gastrointestinal effects, determination of this kind of reagents is quite important in food science, and DNNS-based polycation-sensitive membrane electrodes and polycationic titrant protamine sulfate solution were used for the determination.⁸ Typical potentiometric titration curves obtained for varying levels of CMC, Xanth, Alg, and Carr are shown in Figures 1–4, respectively.

Levels of CMC greater than 2 μ g/mL can clearly be perceived from the blank titration (0 μ g/mL analyte). The data obtained from the titration curves of all analytes are summarized in Table 1. The stoichiometries between CMC, Xanth, Alg, Carr, and a polycationic titrant such as protamine were calculated by means of these titration curves and are tabulated in Table 1, indicating consistency with each concentration of each polyanion

examined in this work. The end points were found to be reproducible. Proportional end points were obtained from the titrations of corresponding proportional amounts of each analyte. The results were compiled as an average of 3 measurements.

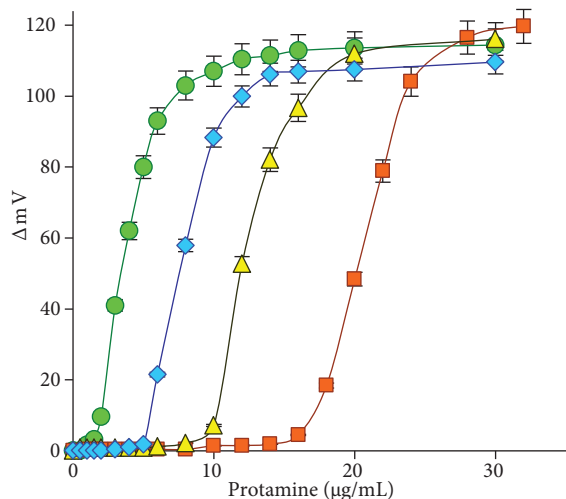


Figure 1. Potentiometric titration curves of 0 (●), 5 (◆), 10 (▲), and 20 (■) $\mu\text{g/mL}$ of CMC with titrant protamine solution.

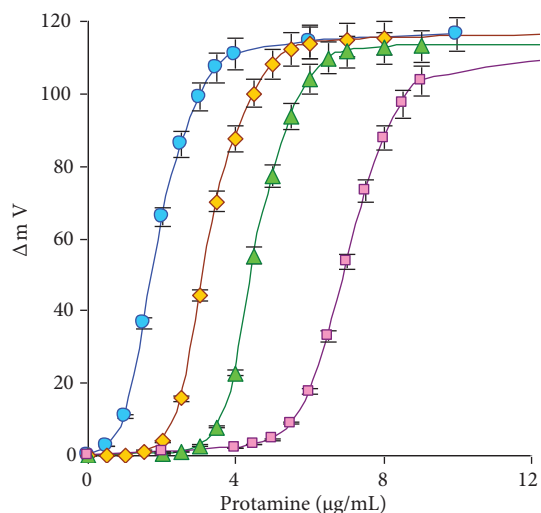


Figure 2. Potentiometric titration curves of 0 (●), 2 (◆), 4 (▲), and 8 (■) $\mu\text{g/mL}$ of xanthan with titrant protamine solution.

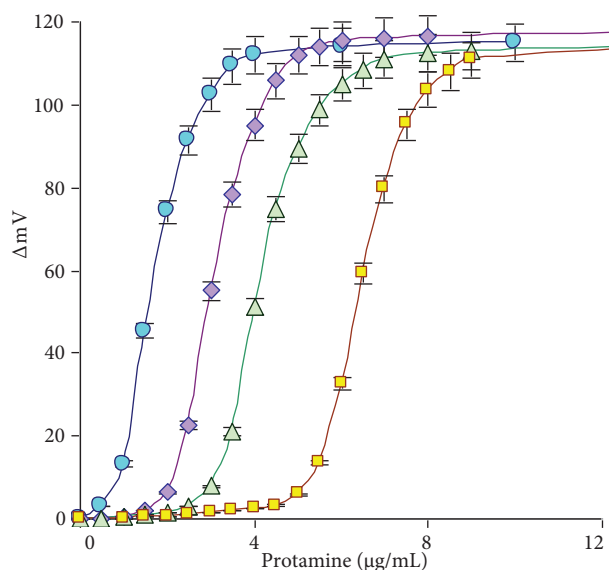


Figure 3. Potentiometric titration curves of 0 (●), 1 (◆), 2 (▲), and 4 (■) $\mu\text{g/mL}$ of alginate with titrant protamine solution.

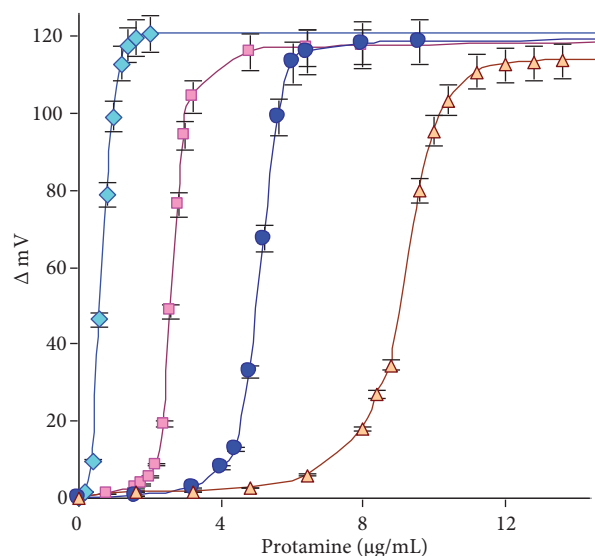


Figure 4. Potentiometric titration curves of 0 (◆), 5 (■), 10 (●), and 20 (▲) $\mu\text{g/mL}$ of carrageenan with titrant protamine solution.

3.2. Scatchard plot analysis of polyanions

In addition to the quantification of polyanion levels, polycation-sensitive electrodes were also used to obtain fundamental information regarding the strength of the interaction of target polyanions with protamine. Figure 5 shows the Scatchard plot obtained for the interaction of Alg with protamine. The polyanion-polycation binding

constant (K_{eq}) and number of protamine binding sites per mole of polyanion (n) were estimated by recasting the data in the form of a Scatchard plot of B' versus B'/F (B' , the moles of bound protamine per mole of polyanion). The slope of the line is the negative value of the equilibrium constant (K_{eq}), and the x-intercept provides the values for n , the number of protamine binding sites per mole of polyanion molecule. The results of the Scatchard plot analysis of all polyanionic additives are shown in Table 2. The observed increase (approximately 500-fold) in the number of carrageenan binding sites per mole protamine is in good accordance with the values expected, given the higher average MW of the carrageenan (500,000 Da)^{22,23} compared to CMC (982 Da).

Table 1. Potentiometric titration data of different levels of polyanions with protamine as monitored by polycation-sensitive membrane electrode detection.

CMC (μg)	Neutralization end point ^a (μg)	Mass ratio (Prot/CMC)
0.0	3.8	-
5.0	7.9	0.85
10.0	12.3	0.82
20.0	20.8	0.85
		Average: 0.84 (± 0.02) ^b
Xanthan (μg)	Neutralization end point ^a (μg)	Mass ratio (Prot/Xanth)
0.0	1.8	-
2.0	3.2	0.70
4.0	4.6	0.70
8.0	7.1	0.69
		Average: 0.70 (± 0.01) ^b
Alginate (μg)	Neutralization end point ^a (μg)	Mass ratio (Prot/Alg)
0.0	1.8	-
1.0	3.1	1.30
2.0	4.1	1.15
4.0	6.5	1.18
		Average: 1.21 (± 0.08) ^b
Carrageenan (μg)	Neutralization end point ^a (μg)	Mass ratio (Prot/Carr)
0.0	1.8	-
5.0	7.0	1.04
10.0	13.0	1.12
20.0	23.0	1.06
40.0	45.5	1.09
		Average: 1.08 (± 0.04) ^b

All experiments were carried out in 0.12 M NaCl solution.

^a Each neutralization end point is the average value of 3 measurements.

^b Average mass ratio values \pm SD.

3.3. Analysis of some real samples and recovery experiments

After determining the stoichiometries between each polyanionic additive and titrant in saline solution, the titrations were further performed to determine total polyanion in the real food products making use of these stoichiometries. Eight different food product samples, i.e. salep, fruit yogurt, salad sauce, ice cream, chocolate cream, tiramisu cream, banana milk, and vanilla pudding, were tested for these additives. Total amounts of polyanions in these samples were measured by the potentiometric titration method using DNNS-based polycation-sensitive membrane electrodes and the results are expressed as grams of additive per 100 grams

of food sample (Table 3). A representative titration curve of salep sample solution is illustrated (Figure 6). The accuracy of the method was confirmed by recovery studies. For this purpose, the food product sample solutions were spiked with proportional amounts of the related polyanion and the solutions were titrated with the same titrant (Figure 7).

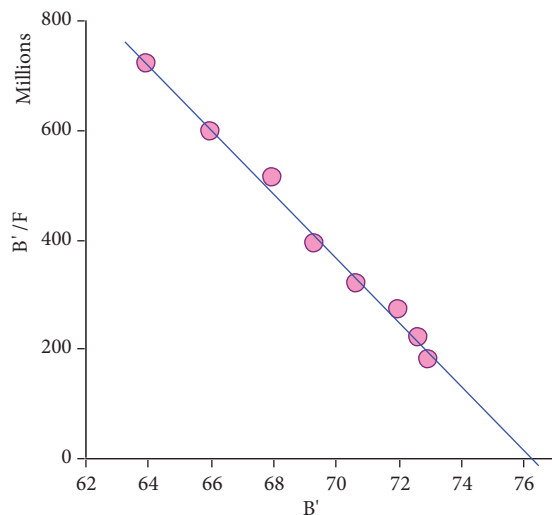


Figure 5. Typical Scatchard plot for the binding of alginate to protamine. B' and B'/F values were calculated from the potentiometric titration data of blank and 10 $\mu\text{g}/\text{mL}$ alginate.

Table 2. Binding constants (K_{eq}) and number of polycation binding sites (n) per mole of each polyanion as measured by potentiometric titrations.

Titrant – Analyte	$K_{eq}, (\mu\text{M})^{-1}$	n
Prot – CMC	2.25 (± 0.12)	0.40 (± 0.02)
Prot – Carrageenan	6.95 (± 0.23)	180 (± 9)
Prot – Xanthan	48.2 (± 1.2)	1300 (± 40)
Prot – Alginate	58.6 (± 2.3)	76.2 (± 1.4)

All experiments were carried out in 0.12 M NaCl solution.
Average value \pm SD of 6 measurements.

Table 3. Thickening agent concentration levels measured in some food products using polycation-sensitive electrodes.

Food products	Polyanion as additive (% m/m)*	
	Found	Nominal ^a
Salep	0.592 (± 0.027)	-
Fruit yogurt	0.233 (± 0.066)	-
Salad sauce	0.189 (± 0.009)	-
Ice cream	0.606 (± 0.058)	0.5–0.8
Chocolate cream	1.996 (± 0.346)	1.5–2.5
Tiramisu cream	0.293 (± 0.057)	-
Banana milk	0.440 (± 0.034)	-
Vanilla pudding	0.472 (± 0.010)	0.2–0.4

*Average value \pm SD of 3 measurements.

^aRef. 8.

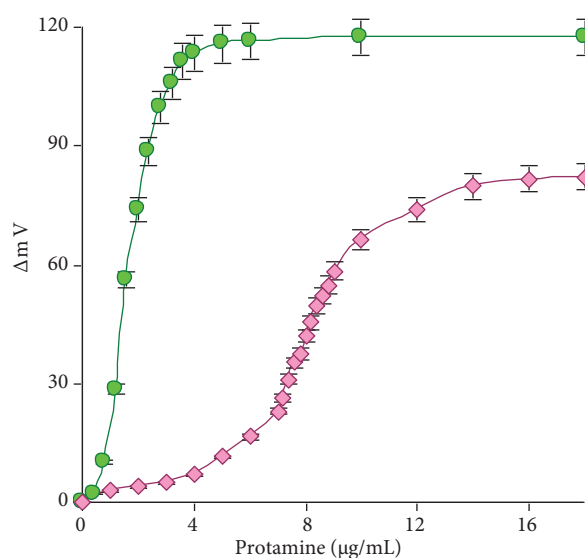


Figure 6. A typical potentiometric titration curve for salep containing polyanionic additive with standard protamine: blank (●) and 1000 $\mu\text{g/mL}$ sample solution (◆).

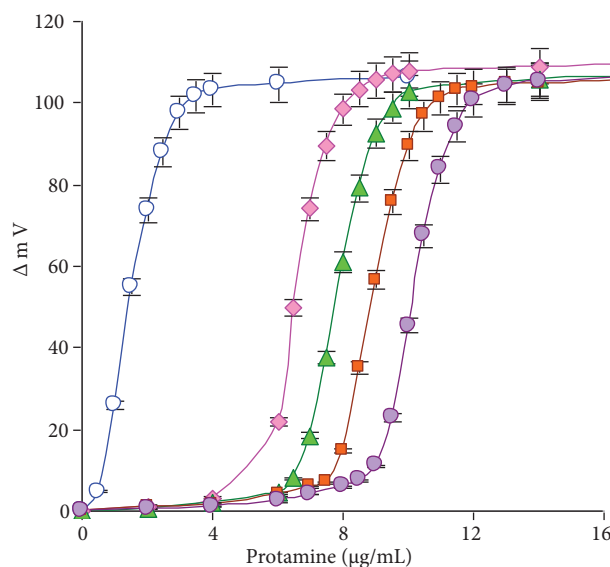


Figure 7. Potentiometric titration curves obtained from recovery studies of pudding sample containing polyanion with protamine solution. Blank (○), pudding solution (◆), pudding solution + 1 $\mu\text{g/mL}$ Carr. (▲), pudding solution + 2 $\mu\text{g/mL}$ Carr. (■), pudding solution + 3 $\mu\text{g/mL}$ Carr. (●).

For example, from the titration data of pudding sample containing both Carr and Carr-spiked solution, the polyanion recoveries were 107(± 2)% for 1 $\mu\text{g/mL}$ Carr added, 103(± 5)% for 2 $\mu\text{g/mL}$ Carr added, and 107(± 4)% for 3 $\mu\text{g/mL}$ Carr added. The recoveries of polyanion added were quantitative. Thus we report that this method is quite simple, accurate, and applicable for routine analysis of these food additives.

4. Conclusions

The present investigation clearly demonstrated the potential analytical applications of potentiometric polyion sensors for the determination of some thickening agents in a variety of food products. Simple potentiometric titration of these polyanions with titrant protamine as monitored by a polycation sensor provides a rapid and convenient method to quantitate target polyanions at $\mu\text{g/mL}$ levels.

Acknowledgment

We are extremely grateful to the Abant İzzet Baysal University Directorate of Research Projects Commission (BAP Grant 2002.03.03.120) for its financial support.

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