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APPLICATION OF DIFFERENTIAL PULSE POLAROGRAPHY IN ANALYSIS OF PECTIN

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Abstract

Differential pulse polarography (DPP) with dropping mercury electrode (DME) was used to determine quantitatively galacturonic acid (GA), methylated polygalacturonic acid (met PGA), pectin (PC) and pectinase (PE) in 0.1 M $NaClO_4$ as supporting electrolyte. Current peaks of GA, met PGA, PC and PE, at -1.55, -1.50, -1.40 and -1.60 V *vs* SCE respectively, were found suitable for quantitative determination in the concentration range considered. Enzymatic reaction between PE and PC was followed and possibility to determine simultaneously of PC and PE was proved.

Introduction

Pectin is an acidic polysaccharide consisting of D-galacturonic acid units with very small quantity of neutral sugars. The monomers are linked together by 1-4 glycosidic linkage. Part of the acid is present as methyl ester. The textures of fruits and vegetables are largely influenced by the pectin content. Many food processors and pectin ingredient suppliers need to determine pectin content to control the quality of their products. Various chemical and instrumental methods, including HPLC, GLC, infrared spectroscopy, HPAEC using pulsed-amperometric detection (PAD), were applied for determination of pectin content in food products. Some of the methods, measuring pectin as galacturonic acid content, are quite accurate, and well-correlated to pectin content. In most cases pectin extraction and hydrolysis are required prior to analysis [1-2]. Until now, electrochemical methods have not been often used in pectin determination. The aim of the presented work was to explore possibility to apply differential pulse polarography (DPP), with dropping mercury electrode (DME) in detection and quantification of pectin and polygalacturonic acid.

Materials and methods

Chemicals Pectin (PC) from citrus fruits, methylated polygalacturonic acid (met PGA) from orange, pectinase (PE) from *Aspergillus niger*, sodium perchlorate (Sigma) and D-(+) galacturonic acid (GA) (Fluka) were used. Analytical grade (99.999) gaseous nitrogen was purchased from Messer-Tehnogas (Belgrade). Deionized water was used for preparing all experimental solutions.

Instrumentation. The current-potential (*i-E*) curves were recorded by polarographic analyzer PAR (Princeton Applied Research) model 174A, equipped with X-Y recorder (Houston Omnigraphic 2000). Dropping mercury electrode (DME) with a programmed dropping time of 1 s as working electrode, saturated calomel electrode (SCE) as a reference and a Pt-foil as auxiliary electrode were used. The *i-E* curves were obtained at different direct current intensity with alternative current modulating pulse of $25mV_{pp.}$ Scan rate of potential changes was 10 mVs^{-1}.

Procedure. Supporting electrolyte (0.1 M $NaClO_4$) in the electrolytic cell was deairated by passing nitrogen, than he inert atmosphere was kept constant during *i-E* curve recording. Dry met PGA, GA, as well as dry PC, or PC solution, with or without PE, dissolved in acetate buffer (pH 5.0), were added in defined amount into the cell solution (20 ml). Then, after bubbling of experimental solution 30 s with nitrogen, the corresponding i-E curves were recorded at room temperature.

Results and discussion

Until now, DPP was not applied for quantitative determination of PC as important ingredient of various natural products. Polarographic behavior of monomer of PGA (GA) at DME, in O.1 M $NaClO_4$ as supporting electrolyte, was firstly investigated. A well defined and symmetrical current peak of GA was recorded at potential about -1.55 V *vs* SCE (not shown). Obtained data gave the opportunity to quantify GA from applied solution.

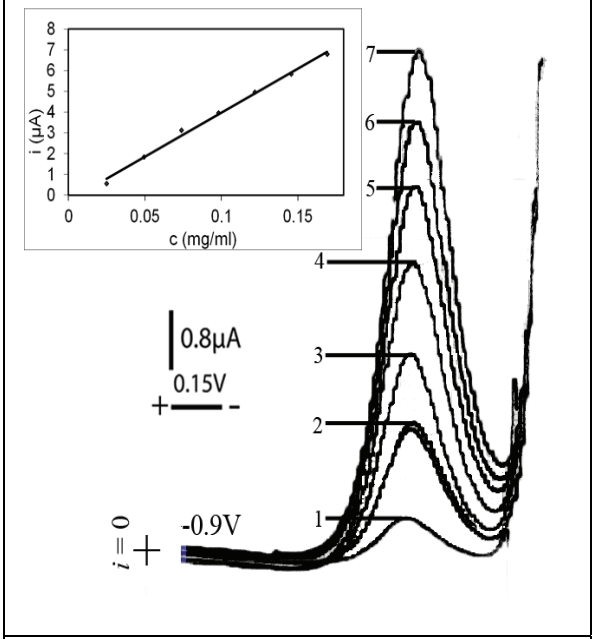

Figure 1. DPP curves of **1**. 0.02 **2**. 0.05 **3.**0.07 **4**.0.10 **5**.0.12 **6**.0.14 **7**. 0.17 mg/ml met PGA in 0.1 M $NaClO_4$.

Also, a well defined DPP peak of met PGA was recorded at somewhat less cathodic potential in respect to GA, i.e. at about -1.5 V *vs* SCE (**Fig. 1).** In concentration range from 0.02 to 0.17 mg/ml, the current linearly depended on PGA concentration (**Fig. 1**., insert).

Possibility for simultaneous determination of PC and PE, an enzyme that breaks PC into simple sugars and GA, was investigated

A well defined peak of PE at potential about -1.6 V *vs* SCE, shown in **Fig. 2.**, was found suitable for quantitative determination. Peak current showed linear dependence in considered concentration range

(**Fig. 2.**, insert). The measurable current of PE was not detected at concentrations bellow 0.05 *mg/ml*, probably due to protein adsorption at the electrode surface.

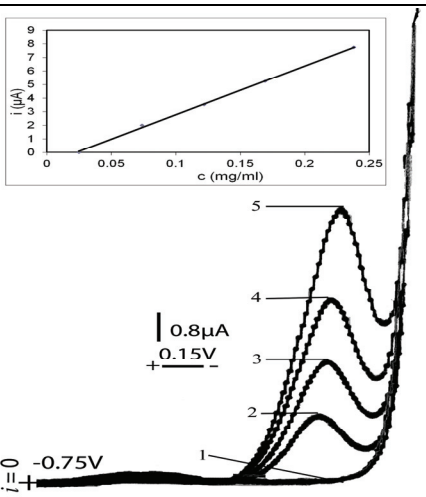

Figure. 2. DPP curves of **1**.0.02 **2**. 0.07 **3.** 0.12 **4**. 0.17 **5**. 0.23 mg/ml PE in 0.1 M $NaClO_4$.

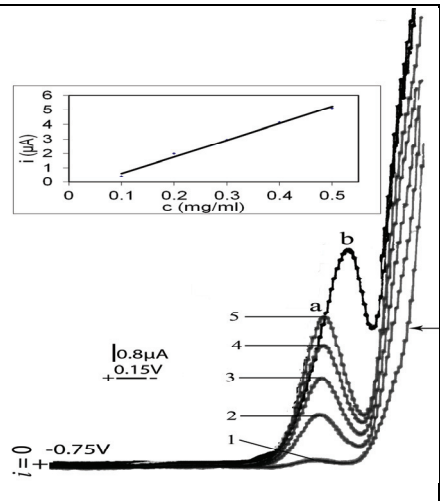

Figure 3. a) DPP curves of 1. 2 2. 4 3. 6 4. 8 5. 10 mg PC in 20 ml of 0.1 M $NaClO_4$; b) solution 5 after addition of PE (0.125 mg/ml).

The solution containing different amount of PC was initially studied (**Fig. 3., curves a, 1-5).** Current peaks recorded at about -1.4 V *vs* SCE depended linearly on applied PC concentrations **(Fig. 3.,** insert). Subsequently, PE was added into solution 5 (**Fig. 3.**, **curve a 5**) and possibility to measure PC and PE simultaneously was proved (**curve b).**

Conclusion

An easy-to-handle, rapid and reproducible method for detection and quantification of galacturonic acid, polygalacturonic acid, pectin and pectinase, based on differential pulse polarography, was developed. Simultaneous determination of pectine and pectinase was shown; it could be widely used to assess contamination of various complex samples after hydrolysis.

Acknowledgements

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References

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