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Determination of reducing carbohydrates in natural honey samples by optical micrometry method

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Abstract

The present article deals with the determination of the total amount of glucose and fructose in natural honey samples by the optical micrometry (OM) method. (Poly)vinyl alcohol spherical granules impregnated with a 0.05 mol/l borax solution were chosen as a sensitive element. It was shown that the formation of chelate esters of boron with polymer and carbohydrates is a pH-dependent process, and that the pH range 8.5–10.0 is the most appropriate for quantifying the total amount of reducing carbohydrates because glucose and fructose are undiscriminated. The impregnated polymer is not sensitive to the sucrose presence in the solution due to the absence of cis-diol fragments in it. Subsequently, the OM method was tested in the analysis of natural honey samples. The relative standart deviation in the case of OM method is less than 6%, and the results are similar to those obtained by the iodometric titration method. This makes the OM method suitable for laboratory-scale applications.

Keywords

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sensitive polymers
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analysis of honey

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Key findings

- The OM method is based on direct measurements of swelling degree of a sensitive polymer.
- An application of (poly)vinylalcohol spherical grains impregnated with borax solution leads to higher selectivity and sensitivity to carbohydrates.
- Weak alkaline medium of solution is appropriate for the determination of total amount of glucose and fructose.

1. Introduction

The determination of carbohydrates in food samples is an important step in their quality control. Nowadays, methods used for this purpose, such as redox titration [1–3], IR- and Raman spectroscopy [4–6], UV-vis-spectrometry [7, 8], liquid chromatography [9–11] and capillary electrophoresis [12, 13], are widely applied in laboratories. Their main drawbacks include the complicated sample treatment, the high cost of the equipment and the requirement for a highly qualified operator.

These problems can be solved by the application of modern chemical sensors or test-systems. Sensors for the determination of sugars are often based on enzymatic reactions [14–16]. However, over the last decades, a new class of carbohydrate sensors based on chelation of sugars with boron has been investigated. The nature of analytical signal coming from them is related to a change in the swelling degree of a sensitive polymer. In the works [17–20], the authors used polymers functionalized with phenylboronic acid or its derivatives for the determination of glucose in blood, tears and serum [21, 22]. Nevertheless, the difficulty of synthesis of such materials and a high cost of precursors limit their application for the analysis of natural samples. Besides, there is only a small number of works dealing with the determination of other sugars that prevents creating a method for the determination of the sugar content in food samples.

The present article is devoted to the application of the optical micrometry (OM) method for the analysis of natural honey samples. The OM method is based on direct measurements of the swelling degree of a spherical granule made of a sensitive polymer [23]. The device consists of an optical microscope equipped with a camera connected to a personal computer. Special software for obtaining and treatment of granule images makes the quantitative analysis both with the equilibrium swelling degree [24, 25] and the kinetic data [26, 27] possible. This method can be an alternative to the fabrication of photonic crystal matrix, because traditional methods of fabrication of photonic crystals are more expensive and difficult to implement. Besides, OM method can be simply miniaturized and used out of laboratories, whereas the most important limitation of photonic crystal sensors is a strictly fixed angle angle between the sensitive element and the measurement device. Nevertheless, the main limitations of the existing methods for the concentration measurements are low sensitivity and non-selectivity. To improve the proposed method, a cross-linked (poly)vinylalcohol that had been well-studied earlier was impregnated by a borax solution. As it is described in [28], functional groups of PVA are able to react with the $[B(OH)_4]^-$ solution forming chelate esters. The presence of glucose molecules in the analyzed solution causes the desorption of boron, and the volume of the polymer gel decreases due to the formation of additional cross-linking fragments. To apply the polymer for the analysis of natural honey samples it is necessary to have information about changing of the swelling degree of the impregnated PVA in glucose, fructose and sucrose solutions.

2. Experimental

2.1. Materials and methods

All necessary reagents and solution preparation methods are described in [29]. An experimental appliance for the determination of the carbohydrate concentration from a 48-well plate for biochemical research is equipped with a cover glass and an optical microscope. For obtaining and treatment of granule images, such programs as “Webcam Screen Video Capture” and “Image Treatment”, which is the improved version of “Grain Size Treatment” created by M.G. Tokmachev [30] were used.

2.2. A sample treatment

3.000 g of natural honey was put into a beaker and dissolved in a small amount of an appropriate stock solution. The obtained solution was transferred into a volumetric flask and diluted up to 50.0 ml with the stock solution. 2.5 ml of the resulting solution was transferred into a volumetric flask and diluted with the stock solution up to 25.0 ml.

2.3. A general procedure

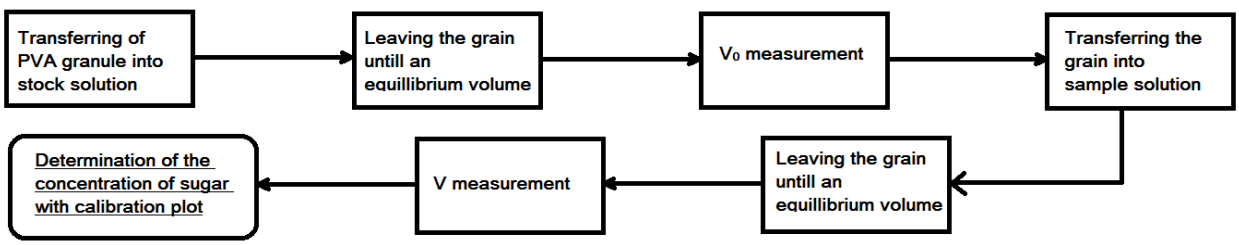
The determination of sugar concentration in analyzed solutions was carried out according to Scheme 1. PVA granule, held in the stock solution until an equilibrium state had been reached, was put to the measurement cell of the plate. The cell was filled up with the stock solution and covered with a glass to avoid the appearance of air bubbles inside the cell. Then, the granule was captured and measured. The initial volume (V_0) of it was calculated. After that, the background solution was replaced with the analyzed solution, the granule was kept for about 30 minutes until the equilibrium had been reached, and then the granule volume (V_{eq}) was determined. The data obtained were used for the determination of the concentration of carbohydrate according to the calibration plot ($V_{eq}/V_0 = f(C)$).

3. Results and discussion

3.1. Influence of pH on PVA swelling

It is known that the chelation of elements with ligands-weak acids is a pH-dependent process. This reaction is usually described with conditional constants of chelation that take into account the protonation of a ligand and the hydrolysis of a complexing element. In the present work, the influence of pH of phosphate buffer on the swelling degree change of investigated PVA was studied (Figure 1).

It was shown that the swelling degree of PVA in a weak-alkaline solution of glucose and fructose decreases at the pH values from 6.5 to 8.5 and keeps constant from 8.5 to 10.0 of pH. It is important to note that in this pH range glucose and fructose are undiscriminated, whereas at the pH value of 6.5 the analytical signals of glucose and fructose are different. It is supposed to be related to a possible mechanism of the reaction between boron and PVA. Acidic and alkaline solutions are supposed to facilitate the formation of complexes of compositions 1:2 and 1:1, respectively [31]. However, the presence of both glucose and fructose in the solution leads to the desorption of boron due to the ligand exchange. Sucrose is supposed not to react with boron due to the absence of cis-diol fragments; thus, the swelling degree of impregnated PVA in the sucrose solution does not change in the whole studied pH range.



Scheme 1 An algorithm of the determination of carbohydrate in solution by OM method with equilibrium swelling degree of PVA.

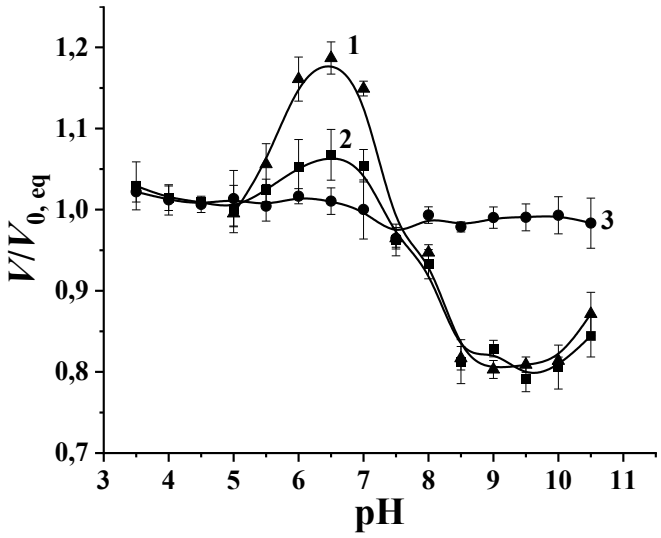


Figure 1 Dependense of swelling degree of impregnated PVA from pH of buffer solution in presence of fructose (1), glucose (2) and sucrose (3): phosphate buffer solution, C_{borax} = 0.05 mol·L^{-1}, C_{sugar} = 0.04 mol·L^{-1}.

3.2. Analysis of honey samples

Since glucose and fructose are undiscriminated at pH value from 8.5 to 10.0, it becomes possible to use the impregnated PVA for the determination of the total amount of glucose and fructose that are often called «inverted sugar» in natural honey samples. It is also known that the time for getting an equilibrium swelling degree is about 30 minutes [29].

Earlier, for the determination of carbohydrates in real samples by the OM method, some ion-exchangers in different forms [32] and non-modified PVA [33] were suggested as a sensitive matrix. But limits of detection of carbohydrates were very high, and there was no possibility for the determination of sugar using the equilibrium data. For example, the limits of detection for non-modified PVA were about 0.2 mol·L^{-1} for glucose and 0.1 mol·L^{-1}. M for sucrose. Impregnation of PVA by borax solution led to the decrease of LOD of glucose from 0.2 mol·L^{-1} to 6.9 mmol·L^{-1} and made it possible to determine the concentration of glucose and fructose in the presence of sucrose.

Using the calibration plot that was obtained earlier at pH = 8.5, we analyzed some samples of honey by the method proposed. To confirm the accuracy of this method, all the same samples were analyzed by the iodometric titration method according to [34]. The results obtained are presented in the Table 1**.** As seen, the relative standard deviation in the case of OM method is less than 6%, and the results of the analysis of honey samples by both methods are similar. Therefore, the OM method is appropriate for the analysis of natural honey samples and can be used in laboratories.

4. Limitations

It is known [35] that the equilibrium swelling degree of polymers depends on their degree of cross-linking. To avoid high standard deviation of the analytical signal and ensure good reproducibility of obtained results, all used polymer granules should have the same degree of cross-linking. The existing method of synthesis of cross-linked PVA [29] makes it impossible to follow this requirement. One of possible ways to overcome this drawback is working with one granule during the calibration and the analysis of the sample. Another possible way for elimination of error is the application of some hydrophilic cross-linking agent instead of epichlorhydrine.

The equilibrium swelling degree does not depend on the granule size, but the granule size has an impact on the kinetics of polymer swelling. If the granule diameter is known, it can be used for calculation of the concentration of solution through the application of kinetic constants [27] in simple systems. However, the heterophase model of polymer swelling degree does not take into account the chemisorption of analytes. Thus, it can not be used for the description the swelling of PVA of impregnated with borax in carbohydrate solutions. The size fractionation of synthesized grains can help to improve the accuracy of analysis with kinetic data and even to create a method for the simultaneous determination of analytes without the pre-separation stage.

Another way for the improvement of the OM method is the modification of the sensitive polymer by magnetite nanoparticles. This approach was tested in the work [36]. The image of a polymer granule coated with Fe_3O_4 has more contrast in comparison with the uncoated granule, and thus its measurement is more precise. Besides, such granule can be simply fixed in a measurement cell with a magnet. This opens the possibility for the more accurate description of the initial part of the kinetic curves.

5. Conclusions

The OM method was used for the first time for the analysis of natural samples. The proposed method is inexpensive, fast and simple; it does not require a large number of reagents. It was demonstrated that the modification of PVA with borax increases selectivity and sensitivity of swelling granules to reducing sugars such as glucose and fructose. The study of the pH influence showed that weakly alkaline solutions where the pH ranges from 8.5 to 10.0 are the most appropriate for the determination of the total amount of glucose and fructose. Subsequently, the OM method was tested out in the analysis of natural honey samples, and the results obtained were highly accurate. In the future, it is planned to investigate the behavior of this polymer at pH 6.5 to study the possibility of using the impregnated PVA for the separate determination of glucose and fructose by special mathematical algorithms and to find a way to determine sucrose in honey samples by the OM method. Moreover, this method can be used for the determination of sugar in other food products and even in pet food, and can replace a number of more complex techniques used in laboratories carrying out veterinary and sanitary analyses. This research demonstrated that modification of a sensitive polymer with appropriate functional groups makes the OM method appropriate for the analysis of real samples.

Table 1 Results of analysis of honey samples.

Sample	Iodometric titrarion, %	OM, %	Sr, % [a]	*R*, %
Linden honey	71.8	70.5	2.85	98.2
Mixed honey	72.9	68.5	5.69	94.0
Honeydew honey	69.0	64.5	2.52	93.5
Buckwheat honey	59.4	61.5	1.82	103.5

[a] $n = 3$

Supplementary materials

No supplementary materials are available.

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Conflict of interest

The authors declare no conflict of interest.

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