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NEW EVIDENCE FOR FREQUENCY-DEPENDENT PERMITTIVITY RESPONSE OF BIOLOGICAL MATTER PART II: CARBOHYDRATE TESTING BY DIELECTRIC IMPEDANCE SPECTROSCOPY

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ABSTRACT

Present work is a novel investigation focused on detection of some widely used monosaccharides: aldopentoses - xylose and arabinose; aldohexoses - galactose and glucose and oligosaccharaide - rafinose by measuring their dielectric response. The extension of the frequency range down to 0.1 Hz is a novel and very valuable approach. It allows examination of the whole molecules, on the one hand, and on the other, observation of induced 3D dipole structural assemblies under the external alternative electric field. This is indicated by a sharp increase in the effective capacity C'. The investigation of xylose and raffinose proved that DIS technique was able to detect concentration as low as 0.1 %. Concentration of aldohexoses galactose and glucose was studied between 2 - 20 % w/v and showed easily distinguishable concentration curves. Per contra, detection of different concentrations of arabinose was not possible, as the obtained C' curves were overlapping.

Keywords: *dielectric impedance spectroscopy, permittivity spectroscopy, carbohydrate testing.*

INTRODUCTION

Quantitative and qualitative determination of carbohydrates, proteins, and other ingredients that are part of various biological objects is a vital task to ensure the properties of the final products. For example, milk is an essential organic product. It contains various ingredients, the main of which are water, lipids, proteins, carbohydrates and salts. Due to the complexity of the composition of milk, different methods are often used to determine protein and carbohydrate content.

Part I of the study is devoted to the bovine and soy milk as a whole system, as well as the determination of whey (serum) proteins using DIS [1]. The method has demonstrated high sensitivity and selectivity. It is a valuable experimental method that is widely used in scientific research, but has also proved beneficial in the examination of biological tissues, construction materials for investigation of aging processes of concrete, microelectronics [2, 3] and communication technologies [4, 5]. In the present work we focus on the determination of carbohydrates of both animal and plant origin by the method of DIS.

Carbohydrates provide over 40 % of the energy of all living organisms, including human beings [6]. Since different sugars are metabolized in a specific way and have a certain biological impact on different living organisms, the determination of the type and amount of the constituent carbohydrates, in certain biological objects is of paramount importance.

Four are the important forms of carbohydrates for all living forms: monosaccharides, disaccharides, oligosaccharides and polysaccharides. They are produced mainly during the process of photosynthesis in plants, store solar energy and make up about 50 % - 80 % of the dry matter in leaves, fruits and seeds.

Monosaccharides are simple sugars that contain three to seven carbon atoms. They cannot be hydrolyzed and are directly absorbed by enterocytes. Important monosaccharides for nutrition of living organisms, including for the human body are hexoses glucose, fructose and galactose. Glucose ($C_6H_{12}O_6$) is a moderately sweet simple sugar present in free form in honey, ripe fruits and some vegetables. It is the final product obtained from the hydrolysis of starch and is the main carbohydrate that provides energy to cells. Galactose is found in some microorganisms and in almost all products of plant and animal origin. It has the same composition to glucose ($C_6H_{12}O_6$) and similar structure but differs In the position of a hydroxyl group. However, this difference provides galactose with different chemical and biochemical properties. In mammals, glucose is transformed to galactose to provide the necessary lactose to the mammary glands during lactation. The other essential monosacharides are xylose and arabinose which are aldopentoses i.e., they contain five carbon atoms and includes an aldehyde functional group. Xylose is obtained by decomposition of cellulose. Most natural saccharides are more common as the D-form, but L-arabinose is more common than D-arabinose. Arabinose occurs in nature as a component of biopolymers such as hemicellulose and pectin [7].

Disaccharides consist of two monosaccharide residues. For example, galactose binds to glucose by a condensation reaction to produce the disaccharide lactose [8].

Oligosaccharides are carbohydrates containing from three to ten monosaccharide residues. Some are intermediates in the synthesis or degradation of polysaccharides. Raffinose is a trisaccharide composed of fructose, glucose and galactose. It is widespread in most plants. Raffinose is an oligosaccharide that can passes undigested through the stomach and intestines into the human body and is an ideal food source and generating factor for beneficial bacteria in the gastrointestinal tract [9].

Many intermolecular interactions occur in a carbohydrate solution, either within the carbohydrate molecules themselves or between the carbohydrate molecules or those of the matrix [10].

The present work is a novel approach that aims to investigate the discerning ability of DIS technique to study carbohydrates of different origins - animal and plant. In order to accomplish this aim, the DIS frequency range is extended to 0.1 Hz [11 - 15]. As presented in Part I, this extension allows the successful study of the structural behavior of biological molecules [1].

EXPERIMENTAL

Theoretical approach

Dielectric spectroscopy (also known as Permittivity spectroscopy) is a branch of the Impedance Spectroscopy specially tuned for measurements and analyses of dielectrics permittivity properties as a function of frequency.

According to the general theory, pure capacitive impedance follows the dependence [16]:

$$Z(j\omega) = -(j\omega C)^{-1} \tag{1}$$

where *C* is the capacitance. For parallel plate capacitor, it can be expressed as [16 - 19]:

$$\mathrm{C} = \frac{\varepsilon\varepsilon_0 \mathrm{S}}{\mathrm{d}} \tag{2}$$

where $\boldsymbol{\varepsilon}$ is the relative dielectric permittivity, $\boldsymbol{\varepsilon_0}$ is the permittivity of vacuum, S is the surface area of the capacitor electrodes and d is the distance between them.

Taking into account the polarization losses, when AC signal is applied, the relative permittivity can be expressed as a complex number [20, 21]:

$$\varepsilon = \varepsilon' - j\varepsilon'' \tag{3}$$

where $\boldsymbol{\varepsilon'}$ and $\boldsymbol{\varepsilon''}$ denote the real and the imaginary (loss factor) components of the complex value of $\boldsymbol{\varepsilon}$.

Applying equations (2) and (3), the capacitance can be also presented in a complex form:

$$C = C' - C'' \tag{4}$$

If Eq. (1) is expressed as admittance $Y(j\omega) = 1/Z(j\omega)$, then

$$Y(j\omega) = j\omega C = j\omega\ (C' - jC'') = \omega C'' + j\omega C' \quad (5)$$

The real component $\omega C''$ in Eq. (5) can be regarded as dielectric conductivity, which can also be expressed as equivalent resistance $R = 1/\omega C''$ directly corresponding to the polarization ability [16].

The letter equation involves the energy dissipative effects such as ohmic conductivity and dipole's reorientation losses in electric field while the dielectric permittivity C' directly corresponds to the polarization ability.

Measurements

All investigated monosaccharides are studied in the optical isomer form found most frequently in nature i.e., xylose, glucose and galactose, are D-form enantiomers, while the arabinose is L-form.

The complex permittivity measurements were performed on IVIUM-CompactState 10030 at room temperature in frequency range 1 MHz – 0.1 Hz with amplitude of the signal 1V and density 5 points/decade. This expansion of the frequency range to 0.1 Hz extends the capabilities of DIS testing, hence the technique delivers broader information about the specific organic ingredients [1]. The measured data are represented in C'/f or R/f plots, where f stands for frequency [15, 21]. This type of representation is very convenient and permits easy observation of all the investigated phenomena. It is used for studies of permittivity enhancement in oil, foods and ceramic dielectrics. In the present work, it is shown that it is also appropriate for presenting of dielectric behavior of milk and its ingredients [1, 12].

The experimental cell is design in a way to minimize any incorrectness that might arise from electric filed distribution and appearance of parasitic capacitance. It consists of two coaxial stainless steel cylindrical electrodes with coplanar working surface. Thus, complex permittivity measurements were carried out in special spacer with d = 0.4 cm and the same volume of V = 15 µl.

Table 1. Concentrations of carbohydrate aqueous solution used in the article.

	Concentration, aqueous solution, % w/v
Aldopentoses	
L-Arabinose	0.1, 2, 4, 5
D-Xylose	0.1, 0.5, 1, 2, 4, 5, 10, 20
Matrices	
Xylose +Nutrition medium	2
Nutrition medium	pure
Aldohexoses	
D-Glucose (dextrose)	2, 5, 10, 20
D-Galactose	2, 5, 10, 20
Oligosaccharide	
Raffinose	0.1, 2, 4, 5, sterile

RESULTS AND DISCUSSION

DIS is a very sensitive method that can be used to track the frequency behavior of carbohydrate solutions. When carbohydrates dissolve in water, different interactions occur between the two types of molecules. As a result, a number of water molecules coordinate around the carbohydrate molecule, forming a complex with a polar hydrate shell [22 - 25]. Under the influence of an AC signal, these complexes are oriented in the direction of the signal. This allows the DIS technique to capture even small differences in the concentrations of carbohydrate solutions.

The frequency spectrum obtained by diluting an aqueous solution of xylose is represented in Fig. 1. The interactions between polar water and xylose molecules increase the polarization ability of the system (Fig. 1(a)). The change in C' varies for different concentrations being lowest at 0.5 % and highest at 20 %. The sharp increase of C' starts at lower water content and reaches much higher values at 1 Hz, the enhancement is 4-5 orders of magnitude. This exceptional enhancementis related to the formation of organized dipole 3D structures. This confirms that the polarization effect is strongly related to the interactions between carbohydrate and water molecules.

Since the nutrient medium is often used in biological research including monitoring of the changes in carbohydrate concentrations, our studies have also been conducted in polar water-based such matrix. The results demonstrate that the DIS method is also very receptive for both the "pure" microbial medium De Man Rogose of Sharp matrix and the same matrix with dissolved xylose (Fig. 1(b)).

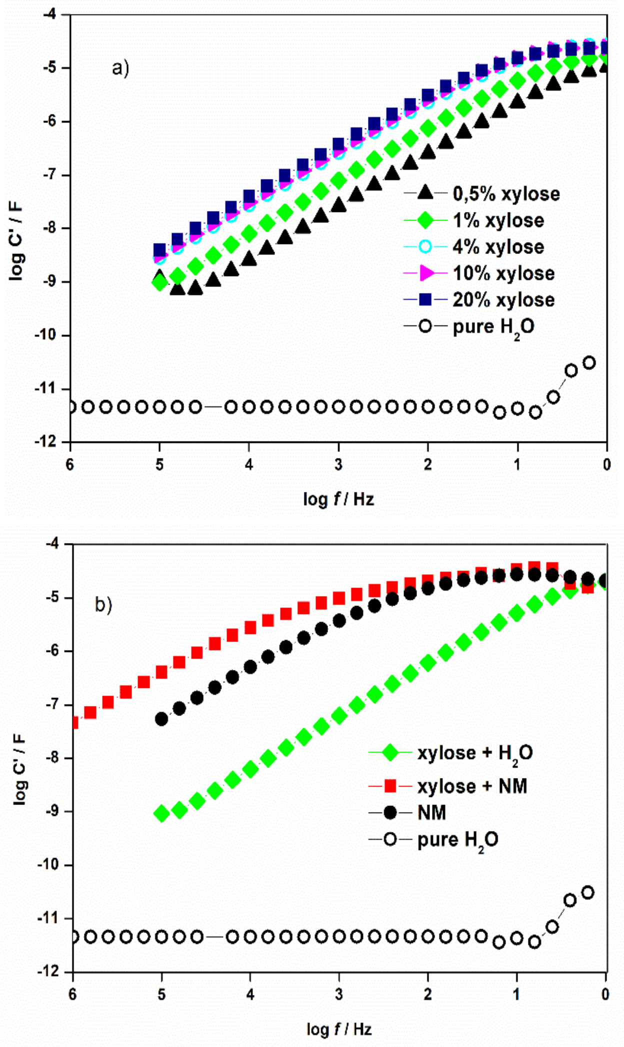

Fig. 1. Frequency dependence *logC′* of: a) different concentrations of xylose diluted in water and a control of pure deionized water; b) xylose dissolved in water and in nutritional medium (NM), pure nutrient medium and a control of pure deionized water.

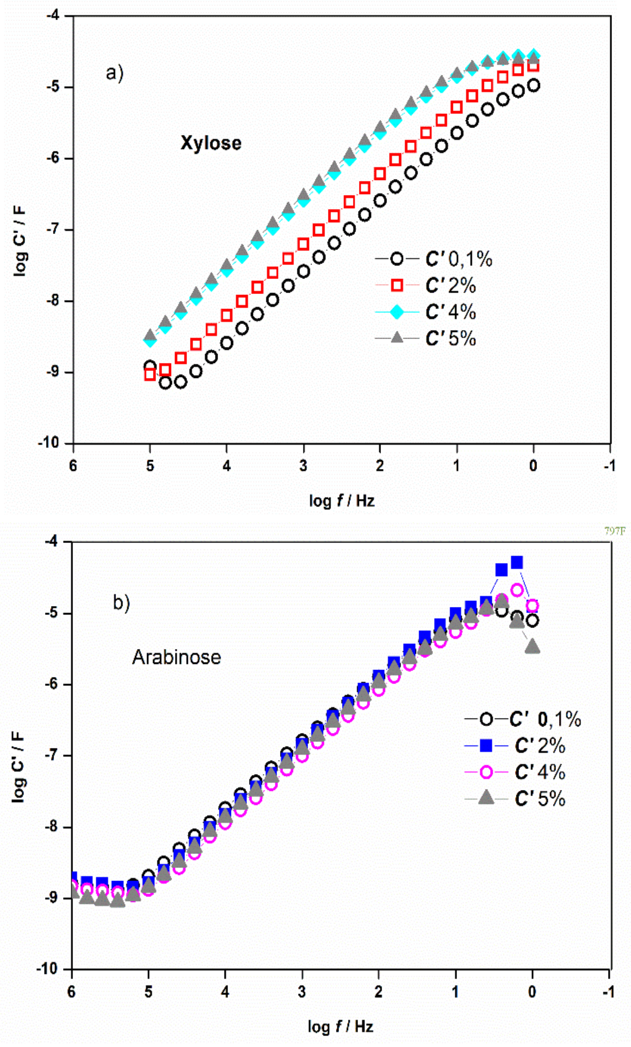

Fig. 2. Frequency dependences of the real part of the capacity *logC'*of aqueous solutions with different concentrations of aldopentoses: a) xylose and b) arabinose.

The frequency dependence of *logC′* of the „pure“ nutrient solution is approximately 2.5 orders of magnitude, while in the presence of hexose in it the frequency dependence of *logC′* is 2 orders of magnitude This diverse behavior of NM and NM + xylose reveals the influence of the inherent nature of the environment, as well as reflects the interactions between the individual components with xylose.

In addition, being a universal polar solvent that is involved in all biochemical processes, water is important starting point for all investigations which study biological matter. In the applied frequency range, the DIS spectra of pure water show plateau with a single band appearing at 82 at 1 kHz (Fig. 1) [21, 26]. This lack of frequency dependence makes water appropriate for a reference sample.

In order to investigate the reproducibility of the results, for all samples 5 consequently measurements were conducted. The deviation of the obtained values for all of them is between 1-2 %.

Studying of pentoses xylose (Fig. 2(a)) and arabinose (Fig. 2(b)) reveals an unequal sensitivity of the DIS technique to the two aldopeptoses. Investigation of xylose show that the frequency behavior of the solutions with concentration up to 4 % are quite distinguishable, hence the DIS method is susceptible enough (Fig 1(a),

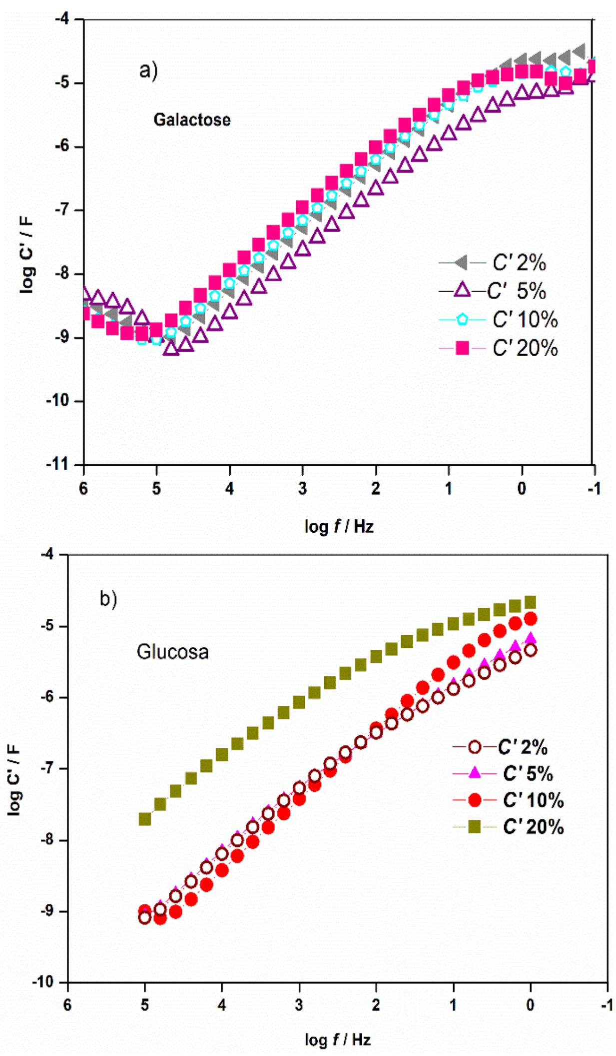

Fig. 3. Frequency dependences of the real part of the capacity *logC'* of aqueous solutions with different concentrations of hexoses: (a)galactose and (b)glucose.

Fig. 2(a)). However, the concentration of 5 % xylose is not so easily distinguishable from the 4 % one. For concentrations of 0.1 % and 5 % are *logC'*=-7.5631F and *logC'* =-6.5147F, respectively. In contrast, the frequency dependence of the real part of the capacity *C'* of arabinose practically overlaps for all studied concentrations, i.e. the DIS method seems indifferent to concentration changes. Nevertheless, the numerical values are quite different. For instance, at a frequency of 1 kHz and a concentration of 0.1 %, the value of *logC'* = -7.0797F, and for 5% *logC'*= - 6.9025 F. One possible explanation is related to the nature of the two carbohydrates and the way they interact with polar water

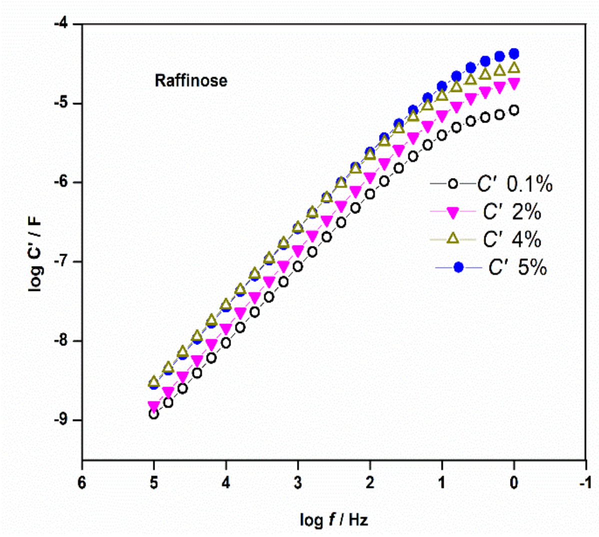

Fig.4. Frequency dependences of the real part of the capacity *logC'* of aqueous solutions with different concentrations of raffinose.

molecules response to AC signal.

In contrast to the frequency behavior of pentoses, hexoses response in a different manner, thus, permitting investigation of more concentrated aqueous solutions, i.e. from 2 % to 20 % (Fig. 3(a) and 3(b)).

The curves that represent the behavior of different concentrations of galactose begin to change at frequencies of 1 MHz. It is followed by a minimum, observed at 100 kHz and then there is a sharp increase in the frequency dependence of *logC'* reaching values of -5 F around 0.5 Hz. A second characteristic kink is observed for all concentartions at different frequencies. At lower concentrations, it is observed at frequencies around 10 Hz, whereas at higher ones it is found between 1 Hz - 0.1 Hz. The frequency dependence of *C'* for different solutions of glucose displays minimum at lower frequencies - at 100 kHz, and the characteristic kink is at about 1 Hz.

The internal reform is remarkably dynamic and can be depicted as follows: at lower frequencies, the initially small 3D dipole structures start to grow. When the half cycle of the AC signal is large enough, all small 3D dipole units organize and form mycelium-like assemblies throughout the whole volume. This leads to a sharp increase of *C'* . A characteristic bend in the curves is observed (Fig. 1(a), Fig. 2 and Fig. 3). In the next half cycle of the AC signal, the micelle-like agglomerates reorganize.

The frequency behavior of aqueous solutions of raffinose is represented in Fig. 4. At high frequencies (around the 1 MHz range) the curves that correspond to lower concentrations are located close to each other, while the two highest concentrations 4 % and 5 % overlap. However, at low frequencies the *logC'* values are clearly differentiated. For instance, at 1 Hz the concentration of 0.1 %, the value of *logC'* =-4.3848 F, and for 5 % *logC'*= -5.1238 F.

The presented results are novel and show for the first time power of DIS technique to detect a variety of the most common and important monosacharides both aldohesoses and aldopentoses. It displays sensitivity to concentration as low as 0.1 %. The method is fast and effective for detection of various carbohydrates at different concentrations and in different matrices. This would permit easy and fast tracking of changes in the amount of carbohydrate that take place over time.

CONCLUSIONS

The present work demonstrates the ability of the DIS technique to detect several widely used carbohydrates and their highly diluted solutions in different matrices e.g., monosaccharides - aldopentoses xylose and arabinose; aldohexoses - galactose and glucose and oligosaccharaide - rafinose. The proposed extension of the frequency range down to 0.1 Hz is a novel and very valuable approach which allows perceiving of a more complete picture of the ongoing processes by studying the system as a whole. The results provide valuable information about the influence of the polar liquid in the study of the interaction of a non-polar carbohydrate molecule with the polar molecules of the matrix. The present work confirms the high sensitivity (in some cases as low as 0.1 % concentration), accuracy and repeatability of the DIS method for study of carbohydrates. The research technology is quick and easy to manage.

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