Analytica Chimica Acta 896 (2015) 52–62

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Water revealed as molecular mirror when measuring low concentrations of sugar with near infrared light

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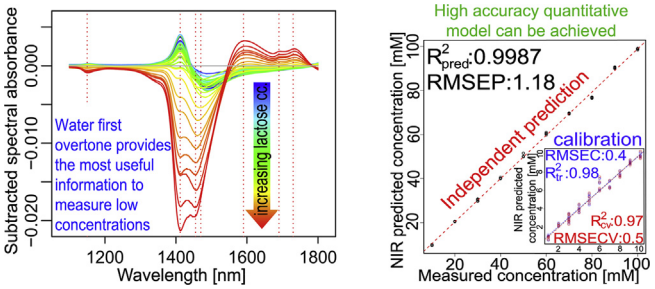
HIGHLIGHTS

- Quick and accurate measurement of saccharides at millimolar concentration level.
- NIR technique to identify & quantify saccharide solutes at millimolar concentration.
- Water molecular rearrangement caused by changes of solute can be used for modeling.
- Quantification of identical sugars in mixtures with NIR at millimolar level.
- Water absorption region gives the most information in highly diluted solutions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form
25 August 2015
Accepted 6 September 2015
Available online 28 September 2015

Keywords:
Near infrared spectroscopy
Aquaphotomics
Low concentrations of sugar
Molecular vibrations at water bands

ABSTRACT

Near infrared spectroscopy is an overtone spectroscopy regarded as a quick and non-destructive method that provides analytical solutions for components that represent approximately 1% or more of the total mass of the investigated composite samples. Aquaphotomics offers the possibility for disentanglement of information remaining hidden in the spectra when conventional data evaluation methods are used, since this concept utilizes changes of the water structure induced by the measured solute as specific molecular vibrations at water bands. Here, near infrared technique and aquaphotomics are applied for non-destructive identification and quantification of mono- and di-saccharide solutes at 100–0.02 mM concentration that is accepted as unachievable with near infrared spectroscopy. The results presented in this study support the aquaphotomics' water molecular mirror concept that explores spectral changes related to water molecular rearrangements caused by minute changes of the solutes in the aqueous systems. The method provides quick and accurate alternative for classical analytical measurements of saccharides even at millimolar concentration levels.

1. Introduction

Near infrared (NIR) spectroscopy is considered as a non-destructive, quick, user friendly, highly accurate analytical method

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http://dx.doi.org/10.1016/j.aca.2015.09.014
0003-2670/

being able to measure simultaneously qualitative and quantitative parameters of various products through their single scans. Moreover, this technique is also ideal for monitoring and functional studies where it is more important having a general fingerprint of a sample as a system than knowing the exact concentration of certain components one by one [1]. It has been demonstrated within the past few decades that NIR technique can be applied both in laboratory environment and in practical applications of agriculture or industry [2–4]. NIR as an overtone spectroscopy has been recognized as a very precise method for moisture measurement and can provide analytical solutions for other absorber components that represent at least 1% of the total mass of the investigated composite samples [5]. This determination is based on the fact that the absorption signals of various fundamental vibrational bands decrease drastically, with orders of magnitude, in the first, second and third overtone regions (400–2500 nm) where NIR technique is applied. This phenomenon is beneficial in case of water that has immense absorbance in mid infrared region, but can be measured in NIR, on the other hand, signals of a low concentration compound which are detectable in the fundamental region are, generally, difficult to measure in the overtone regions if using their respective bands. However, it is necessary to find a method to measure water content together with low concentration compounds, even indirectly, within the NIR region, because instrumentations and measurements are more cost-effective than that of the fundamental infrared region, resulting practical benefits and understanding all the elements of a system. Water bands appearing in spectra of samples having high moisture have been generally regarded as disturbing factors in data analysis targeting components other from water. However, since water –OH bonds are altered easily by other molecules, there is a possibility for investigation of induced by the solute changes detected in water molecular system itself.

Aquaphotomics offers the possibility for disentanglement of information remaining hidden in the NIR spectra when conventional data evaluation methods are used. In the frame of this concept, the well pronounced and measurable NIR absorption of large amount of water molecules in aqueous solutions allows describing structural changes, interactions and conformations, within liquid water through the evaluation of different absorption bands related to the overtones and combinations of stretching and bending vibrations of –OH. Thus, induced by the solute changes of water structure in aqueous solutions are described as molecular vibrations at specific water wavelength bands [1]. Since different species and concentrations of solutes structure the water solvent differently, the aquaphotomics concept is a rational alternative to investigate not the characteristic absorption bands of the solute in question, but the vibrational bands of water's –OH bands that have been altered by the solute. Low concentrations of solutes can result in considerable changes of the molecular structure of water, thus, looking at the water as a mirror extended in the NIR range of the spectrum can amplify the information on solutes and even very low concentrations of solutes can be measured and characterized [6]. As water's H-bonds are present in most natural samples, this analytical approach, using multi-dimensional NIR spectra of perturbed water in different environments as a mirror for the rest of the molecules in the sample, has been effectively applied to various fields [1,6–9]. NIR spectroscopy does not require derivatization or other labor-intensive time consuming sample preparations which are common in classical analytical methods of measuring micro-compounds, like thin-layer chromatography, HPLC, GC, GC–MS [10–12]. The ability to be used in non-destructive sample monitoring provide a big advantage, especially in biology, thus, NIR scanning means a good alternative in many cases. The determination of carbohydrate content such as glucose, fructose, sucrose, etc. has been an emerging need for a very long time. Numerous publications have shown applications of standard analytical methods and various alternative measurement techniques for sugar evaluation. Common applications are for instance the determination of sugar content in soft drinks [13], fresh fruits and vegetables [14] and their juices [15,16] and monitoring the changes of sugar composition during grape ripening and winemaking [17]. In diagnoses in particular the determination of monosaccharides and disaccharides has an increasing application in research and clinical practice [18,19].

The objective of this work was to demonstrate the use of water as molecular mirror through applying NIR technique and aquaphotomics for identification and quantification of solutes (mono- and di-saccharides) at millimolar concentration in aqueous solutions that is applicable for chromatography methods but accepted as unachievable in NIR spectroscopy.

2. Materials and methods

D(+)-glucose (GLU, $C_6H_{12}O_6$ = 180.16 g/mol), D(−)-fructose (FRU, $C_6H_{12}O_6$ = 180.16 g/mol), sucrose (SUC, $C_{12}H_{22}O_{11}$ = 342.30 g/mol) and lactose monohydrate (LAC, $C_{12}H_{22}O_{11}\cdot H_2O$ = 360.32 g/mol) (all of analytical grade) were obtained from Hirose Chemicals Co., Ltd. (Kobe, Japan), and water (MQ) was produced by a Milli-Q apparatus (resistivity 18 MΩcm, Direct-Q, Millipore, Molsheim, France). Three series of experiments were conducted. In the 1st experiment (Exp-1) LAC was diluted in MQ for definite concentrations between 0.02 and 100 mM. During the sample preparation 0.01 mM step was applied in 0.02–0.1 mM range (range-4), 0.1 mM step was applied in 0.1–1 mM range (range-3), 1 mM step was applied in 1–10 mM range (range-2) and 10 mM step was applied in 10–100 mM range (range-1). In the 2nd experiment (Exp-2) aqueous solutions of GLU, FRU, SUC and LAC were prepared in 1 mM concentration, respectively. Mixed aqueous solutions of three sugars, GLU, FRU and LAC, were analyzed in 3rd experiment (Exp-3).

2.1. Quantitative analysis: sugar measurement at low concentrations

Four independent replicates were prepared for the 0.02–100 mM dilution line of lactose (LAC) in Exp-1. Two of the replicates were made with direct dilutions while the other two replicates were prepared with serial dilutions. Each of the four replicates was NIR scanned once, while three consecutive spectral measurements were recorded of each sample. Concentrations and replicates were presented to scanning randomly. The total number of spectra in Exp-1 was 432 (4 concentration ranges × 9 dilutions × 4 replicates × 3 consecutive spectra = 432). In order to test model stability, solutions were prepared and measured on three different days. Samples of the four replicates and concentration levels were distributed among days randomly. MQ was also scanned every day and spectra of solutions were synchronized by subtracting the MQ spectra of the concerning days to eliminate the effect of scanning date.

2.2. Qualitative analysis: sugar type discrimination at low concentrations

Three independent replicates of 1 mM solutions of each sugar (GLU, FRU, SUC, LAC) were prepared in Exp-2 with direct dilution, and also three MQ samples were added to the sample set. Each of the three replicates was scanned repeatedly, three times. The samples were scanned in random order, and each sample was scanned five times, consecutively. Total number of spectra in Exp-2 was 225 ((4 sugars + 1 MQ) × 3 replicates × 3 repeats × 5 consecutives = 225).