Chapter 8
Aquaphotomics: Near Infrared Spectroscopy and Water States in Biological Systems

Roumiana Tsenkova, Zoltan Kovacs, and Yosuke Kubota

Abstract Aquaphotomics is a new discipline that provides a framework for understanding changes in water molecular system presented as a water spectral pattern, to mirror the rest of the solution and to give a holistic description related to system functionality. One of its main purposes is to identify water bands as main coordinates of future absorbance patterns to be used as a system biomarker. This chapter presents the Aquaphotomics methodology and illustrates a way to identify specific water bands using temperature change and addition of solutions of different ionic strength as perturbations. Rapid and precise measurement of low concentration solutes has been given as a strong evidence of the vast information that "the water spectral pattern as molecular mirror" approach provides. Few applications using near infrared spectroscopy and multivariate analysis as main tools of Aquaphotomics have been presented.

Keywords Water states • Near infrared spectroscopy • Water structure • Effect of salts

8.1 Introduction

Visible light reflected from the surface of the water and the surroundings explains the « water mirror » effect. Next to the visible light in the electromagnetic spectrum between 680 and 2500 nm is the near infrared (NIR) light, which penetrates deep into the water, but does not get fully absorbed. Thus, it makes it possible to measure the spectrum of the light that comes back out of the water. It contains immense

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information about the hydrogen bonding in the whole system of water molecules related to the water structure and perturbed not only by the environment, but by the rest of the molecules in the solution (Tsenkova 2009, 2010).

Since the mid '90s, in contemporary biology and life sciences, an explosion of the use of -omes and -omics terms is observed. All the new -omics disciplines consider specific constituents and follow the reduction "analysis" pathway. For further understanding of bio functionality, holistic analysis is required. It would help the next stage of investigation aiming at the "synthesis" pathway. Unfortunately, all of the existing -omics disciplines exclude water despite the fact that it is the matrix of a biological system, its scaffold. In these regards, Aquaphotomics has been proposed (Tsenkova 2009) to fill in this gap and to present a new approach to study complex living and aqueous systems as a whole and as a complimentary to the rest of the -omics disciplines, Fig. 8.1. Aquaphotomics is based on the presumption that all the components of the system shape up the water molecular matrix. Therefore, jumping over the individual constituents and analyzing only the relation between changes of the water matrix (the system "envelope") caused by changes in system functionalities, could explain new phenomena and the "structure-function" relationship. Aquaphotomics means "all about water-light interaction". It is based on the fact that water absorbs energy over the whole electromagnetic spectrum, Fig. 8.2, and water-light interaction can be used as a probe to study changes of water molecular system perturbed by energy of various frequencies. Very informative spectral window to study water-light interaction is the near infrared range. Being located between the visible and infrared range, NIR has proven to be very informative when it comes to studies of water in bio- or aqueous systems as the light is not fully reflected (like in the visible range) or fully absorbed (like in the infrared range). In the near infrared range, water has specific spectral pattern which changes under perturbations. The main concept of Aquaphotomics is to establish a database of all water absorbance bands, called aquaphotome, and to use the spectral patterns based on these bands as biomarkers related to system functionality.

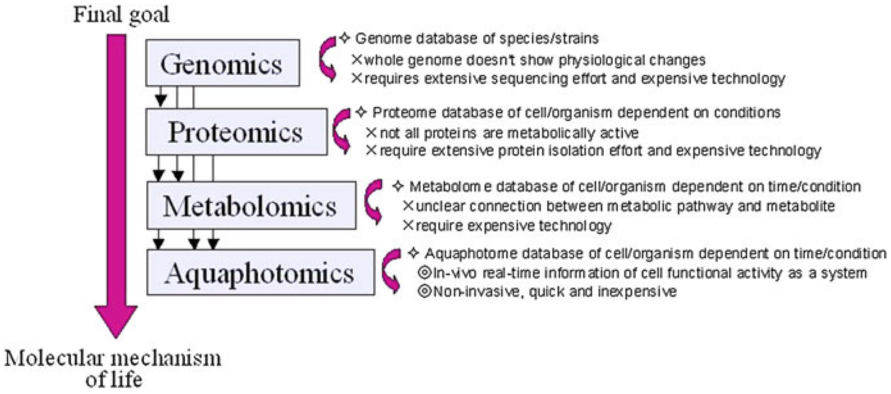

Fig. 8.1 Aquaphotomics in relation with other -omics disciplines

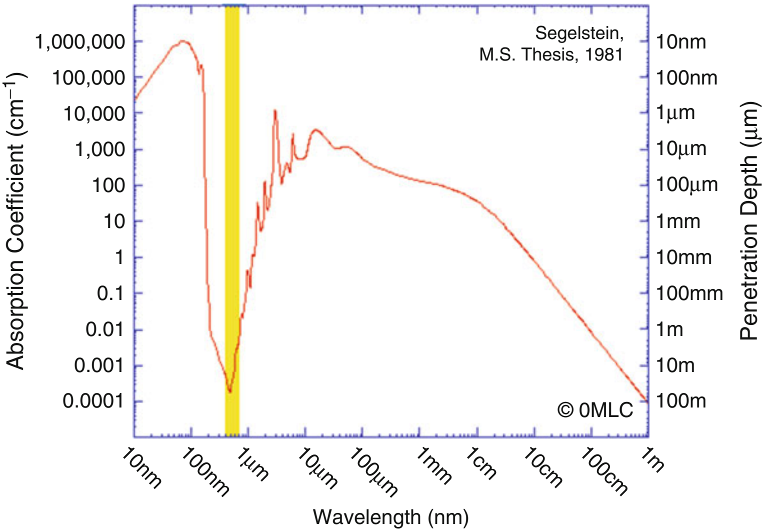

Fig. 8.2 Water absorbance pattern over the whole electromagnetic spectrum (Segelstein 1981)

8.2 Aquaphotomics: Water Spectral Pattern as Holistic Biomarker of System Functionality

8.2.1 Spectroscopy of Aqueous and Biological Systems

Water molecular conformations, for example water dimers, trimers, solvation shells, etc., are known to contribute very specifically to its spectrum (Smith et al. 2005; Robertson et al. 2003; Weber et al. 2000; Franks 1973). They are very sensitive to configuration, hydrogen bonding and charges of the solvated and solvent molecules or clusters. NIR spectrum of the solvent (i.e., water) has been found to contain significant information about its solutes (Tsenkova 2009). In previous research, protein solutions (Murayama et al. 2000), biomolecular water solutions (Murayama et al. 2000; Tsenkova et al. 2004), suspensions of small particles in water and various biological systems such as cells, plants, animal body fluids and tissues have been analyzed with NIR (Tsenkova 2009, 2010; Tsenkova et al. 2001, 2004; Jinendra 2010; Jinendra et al. 2010; Kinoshita et al. 2012; Matija et al. 2012; Nakakimura et al. 2012). In these studies, well known water absorbance bands have been observed under various perturbations (i.e., concentrations of solutes (Nakakimura et al. 2012), nano particles (Matija et al. 2012; Tsenkova et al. 2007a), molecules which don't absorb (Tsenkova et al. 2007a; Gowen et al. 2013) in the NIR range, temperature (Segtnan et al. 2001; Maeda et al. 1995), light illumination, etc.). Dynamic spectra have been acquired and analyzed with multivariate methods. As a result, numerous water absorbance bands have been identified. Many of these bands are in a good agreement with previously reported or calculated overtones of already published water bands in the IR region (Smith et al. 2005; Weber et al. 2000). Some of them have been newly discovered (Tsenkova 2009).

In research carried out at Biomeasurement Technology Laboratory at Kobe University, Japan, water has been perturbed with lactose, human serum albumin (HSA) (Murayama et al. 2000), different isomers of prion protein (PrP) (Tsenkova et al. 2004), NaCl (Gowen et al. 2013), metals (Putra et al. 2010; Tsenkova et al. 2007b) and other solutes at various concentrations. The most prominent wavelengths with highest variations caused by the used perturbations were identified in the spectra through multivariate analysis. It has been found that they are predominantly water absorbance bands. Contrary to the common understanding of overtone spectroscopy (100 to 1000 times lower absorbance than in the mid-IR range), it has been shown that even very small concentrations (at ppb level) (Tsenkova et al. 2004; Gowen et al. 2013) of the solute could be measured with NIR. Measuring low concentrations of polystyrene particles in water is a good example. When the first overtone of water absorbance region at 1300–1600 nm has been used to develop a model for low concentration (1 %–0.0001 %) measurement of polystyrene particles (Tsenkova et al. 2007a), spectral measurements have achieved high accuracy over a wide region of low concentrations. In contrary, with decreasing the concentration, accuracy has decreased substantially when only the polystyrene overtone band at 1680 nm was used. Similar findings have been reported for proteins in solution (Murayama et al. 2000), metals in solution (Putra et al. 2010), etc. Another study has even shown that NIR spectral models for metal concentration measurement (ppb range) in diluted samples are influenced by the method of dilution (serial or direct dilution) (Putra et al. 2010; Tsenkova et al. 2007b). These findings led to the conclusion that the NIR water spectral pattern describes the water matrix in relation to the rest of the molecules in solution in great details. For the first time (Tsenkova 2009), water has been discussed as the common spectral denominator of aqueous and biological systems. In order to systemize the already abundant information concerning the interactions of water in biological and aqueous systems with light at various frequencies, predominantly in the NIR region, a new scientific area called "Aquaphotomics" has been proposed by Prof. Roumiana Tsenkova at the Biomeasurement Technology Laboratory at Kobe University, Japan.

8.2.2 Aquaphotomics: Main Concept and Terms

The idea of Aquaphotomics originates from near infrared studies of raw milk as an output product of the mammary gland. It has been found, using NIR spectroscopy, that milk quality is highly related to the physiological status of the respective mammary gland and highly dependent upon milk constituents and water structure (Putra et al. 2010). The NIR light penetrates deep into the water, but does not get fully absorbed. Thus, it makes it possible to measure the spectrum of the light that comes back out of the water after interacting with all the molecules in the solution.

It contains immense information about the covalent O-H bonds and hydrogen bonding in the whole system of water molecules related to the water structure and perturbed not only by the environment, but by the rest of the molecules in the system. Therefore, all water absorbance bands called **Water Matrix Coordinates (WAMACS)** become « informational hubs ». The combination of the « activated » water bands, at which the light absorbance gets influenced by the perturbations, depicts a characteristic spectral pattern called **Water Spectral Pattern (WASP)**, which reflects the condition of the whole water molecular system. It contains huge amount of physical and chemical information for the solution because the water hydrogen bonding network is easily influenced by any kind of even subtle perturbations (Matija et al. 2012) including the solutes. Graphically, WASP is presented as an **aquagram**, which is a radial graphic of the normalized absorbance at characteristic water bands. Figure 8.3b presents an aquagram depicting the spectral patterns of water and water vapor in the area of the first overtone of water (Fig. 8.3a), 1300–1600 nm when using the main water absorbance bands found in the first overtone of the water. The database of characteristic water bands in the whole electromagnetic spectrum of water is called **aquaphotome**. Each particular system has its own aquaphotome comprised of the activated water absorbance bands under respective perturbation.

8.2.3 Methodology

The main goal of Aquaphotomics is to identify the « activated » water bands, i.e. to build up the aquaphotome of the system of interest and to use further the specific WASP as biomarkers. For that purpose, NIR spectra of the system of interest are acquired under respective perturbations. Subtracted spectra, spectra derivatives, loadings of Principal Component Analysis (PCA), regression vectors of Partial Least Squares (PLS) Regression, discriminating power of Soft Independent Modeling of Class Analogy (SIMCA) extract the bands where spectral variations under controlled and uncontrolled perturbations could be observed, i.e. the variables of interest (Fig. 8.5). The obtained bands define the aquaphotome of the respective system.

In order to build up a database consisting of the « activated » water bands i.e. the WAMACS of various bio- and aqueous systems various experiments have been conducted in the Biomeasurement Technology Laboratory at Kobe University, Japan. Dynamic spectra of various biological systems have been acquired and analyzed using the above described methods to determine the aquaphotome of these systems under different perturbations. Table 8.1 demonstrates the collection of WAMACS of some of the examined systems. In the process of specific water band extraction from the spectral data of various biological and aqueous systems, it has been found that, always, the same twelve wavelengths regions: C1 to C12 were repeatedly activated (in different combination and strength depending on the perturbation and the system) in the first overtone of water, 1300–1600 nm. These

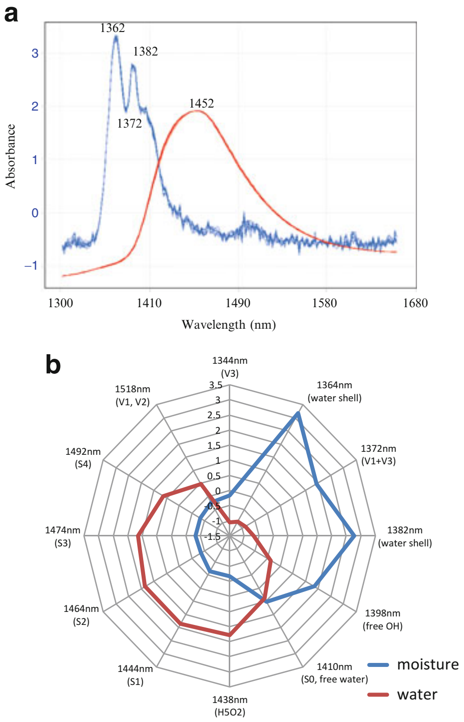

Fig. 8.3 Spectra (**a**) and aquagram (**b**) depicting the spectral patterns of water and water vapor in the area of the first overtone of water, 1300 nm–1600 nm * (Tsenkova 2009)

$$A'_{\lambda} = \frac{A_{\lambda} - \mu_{\lambda}}{\sigma_{\lambda}} \tag{8.1}$$

A'_{λ} : Value of Aquagram

A_{λ} : Absorbance after MSC applied on 1st overtone region

μ_{λ} : Mean of all spectra

σ_{λ} : SD of all spectra

λ : 12 Wavelengths$^{(*)}$

*<1342, 1364, 1372, 1382, 1398, 1410, 1438, 1444, 1464, 1474, 1492, 1518 nm>

bands are in a very good agreement with the calculated first overtone of the water bands found by various experiments and molecular dynamic calculations in the IR range.

NIR spectroscopy is a non-destructive, non-invasive, fast method to study biological and aqueous systems as the NIR light is not fully absorbed by water. NIR spectra are characterized by broad and overlapped bands (predominantly water bands). This feature makes the assignment and interpretation of NIR spectra not straight-forward. Therefore, advanced multivariate data analysis techniques are necessary to fully understand the effect of solutes on NIR spectra. Protocol and a number of chemometrics methods have been proposed (Tsenkova 2009, 2010; Seasholtz and Kowalski 1990) for identification of water absorbance bands (Fig. 8.5). Examples include principal component analysis loadings, multivariate curve resolution-alternative least squares (MCR-ALS) (Abdollahi and Tauler 2011), two dimensional correlation spectroscopy, 2DCOS, partial least square regression vectors etc.. PCA is an unsupervised technique in which the major variations in a spectral dataset are compressed into the first few principal components, which are

Table 8.1 WAter MAtrix CoordinateS (WAMACS) of various bio- and aqueous systems (Tsenkova 2009)

C1: 1336~1348	C2: 1360~1366	C3: 1370~1376	C4: 1380~1388	C5: 1398~1418	C6: 1421~1428	C7: 1436~1444	C8: 1448~1454	C9: 1462~1468	C10: 1472~1482	C11: 1482~1495	C12: 1506~1516	
	■		■	■		■						Quarter Milk(Mastitis)
			■	■	■							Quarter milk(Healthy)
■		■	■	■			■				■	Milk-Cell count
		■	■	■							■	Milk-Protein(Mastitis)
			■	■			■				■	Milk-Protein(Healthy)
■	■	■	■	■			■		■			Water
■	■		■	■			■		■			PrP(Mn)
■	■	■		■			■		■			PrP(Cu)
■			■	■			■		■			PrP
■	■			■			■	■		■		Globulin
	■		■									HSA-Low
■				■		■	■			■		HAS-High
■	■	■	■			■		■	■			CHO cells
		■	■	■							■	CHO cells with Aquaporin
				■		■	■	■		■		DNA in water (High cc.)
						■	■	■	■	■		DNA in water (Low cc.)
				■	■	■	■	■	■			DNA irradiated with UV
				■	■	■	■	■	■		■	Lactose(High cc.)
■	■		■	■	■	■		■		■		Lactose(Low cc.)
■	■		■	■	■			■	■		■	KCl(High cc.)
■	■		■	■		■	■		■	■		KCl((Low cc.)
			■	■	■	■			■	■	■	NaCl(High cc.)
■			■	■		■	■		■	■		NaCl(Low cc.)
			■	■	■		■	■		■	■	Acetic acid(High cc.)
	■		■	■	■	■	■	■	■	■	■	Acetic acid (Low cc.)
■	■		■	■	■	■					■	Stored raw milk
	■	■		■	■				■	■	■	Stored defatted milk
	■		■	■			■	■		■		Electrolyzed(acidic)Water
■	■		■	■						■		Amyloid+NaCl solution
				■	■		■	■	■	■	■	Water(HighMineralCont)
	■	■		■	■	■		■				Water(LowMineralCont)
■	■	■		■	■	■	■	■				Bacteria(Probiotic)
	■					■	■	■			■	Bacteria(NonProbiotic)

orthogonal to each other. PCA loadings are useful for interpretation, especially in cases where the perturbation in question is well characterized.

The spectral database of water absorbance bands that have been discovered experimentally and calculated as overtones of already found bands in the IR range has more than 500 bands in the area of the first, second and third overtones of water, i.e. in the near infrared, 700–2500 nm, wavelength region.

Water spectral monitoring and spectral data analysis applied to a time series spectral data of pure water under consecutive illuminations has revealed the existence of a numerous of water absorbance bands underneath the first overtone of water spectrum, 1300 nm–1600 nm, Fig. 8.4. All of the characteristic bands found in this experiment belong to the 12 characteristic water regions (C1–C12) found under the first overtone of water (Tsenkova 2009).

The methodology of Aquaphotomics will be further illustrated by the results of spectral data acquisition and analysis of aqueous system where the only perturbation is temperature (Segtnan et al. 2001). Water near infrared spectra have been acquired at various temperatures in the region from 30 to 65 °C, Fig. 8.6. Broadly speaking, there are two main categories of models for describing water structure: mixture and continuum models. Mixture models consider water to be a multi-component mixture of species with different numbers of hydrogen bonds, which break as temperature is increased (Segtnan et al. 2001; Maeda et al. 1995). Continuum models regard water as a system which is almost completely hydrogen bonded but these bonds weaken with increasing temperature (Smith et al. 2005). A number of studies on the effect of temperature on vibrational spectra of water have been shown to support the mixture model of water structure (Segtnan et al. 2001; Maeda et al. 1995), while the continuum model has also been shown to be consistent with observed data (Smith et al. 2005). Nevertheless, it is agreed that temperature changes result in alterations in hydrogen bonding configurations in water.

The main feature of the NIR spectrum of water is a broad peak around 1450 nm, which is comprised of many overlapped bands, described mainly as overtones of OH stretching and combinations of OH stretching and bending vibrations. The existence of an isosbestic point in the NIR spectra of water measured at different temperatures has been offered as evidence that water can be considered as a two component mixture, comprising of two different types of water: strongly and weakly hydrogen bonded, with the proportion of weakly bonded species increasing as temperature increases (Segtnan et al. 2001). In the middle infrared wavelength range, researchers employing fiber optic evanescent wave spectroscopy detected two isosbestic points: one during the ice-water phase transition at 3280 cm^{-1} (3048.78 nm) and another in the 20–100 °C temperature range located at 3530 cm^{-1} (2832.86 nm) (Raichlin and Katzir 2008). This led them to propose a four component mixture model for liquid water structure: molecules with 4 bonds (ice-like); two 3-coordinate species (either with a broken OH bond or broken lone pair electron bond) and one 2-coordinate species (with two broken bonds). However, studies using Raman spectroscopy and

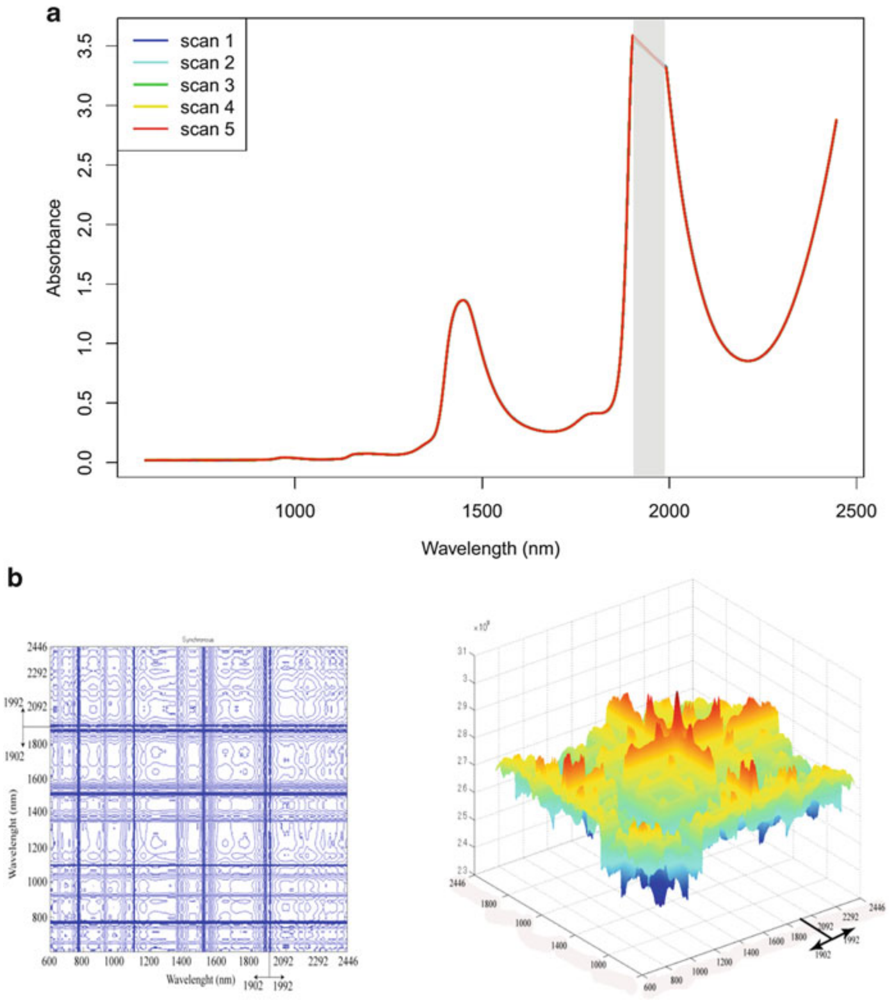

Fig. 8.4 Water spectral monitoring in the spectral range between 600 and 2446 nm (1902–1992 nm was excluded due to undetectable high absorption). (**a**) Raw spectra of water, 1 mm light pathlength. (**b**) Two dimensional correlation spectroscopy plots

Monte Carlo simulations have refuted this hypothesis, by showing that thermal variations of a single species line shape can also generate isosbestic points (Smith et al. 2005). Less hydrogen bonded water is predominant at higher temperature while low temperature spectra are depicted mostly in the area of the hydrogen bonded water where water molecules formed species with one (S1), two (S2), three (S3) and four (S4) hydrogen bonds (Franks 1973).

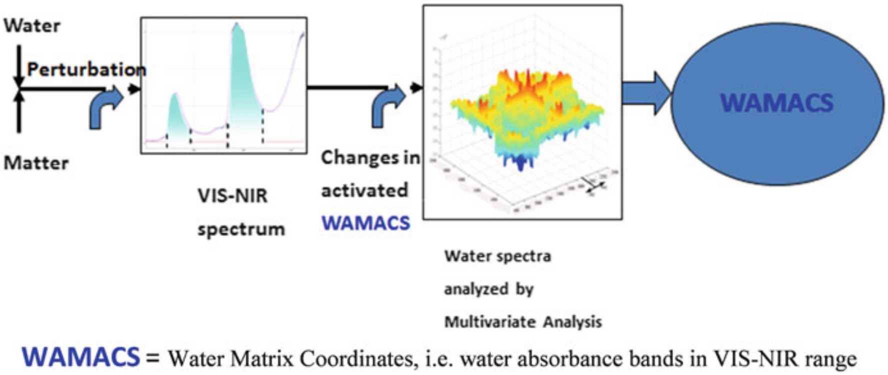

Fig. 8.5 Spectral data analysis flow chart towards finding specific water bands

8.3 Applications

8.3.1 Characterization of WAMACS and WASP for Water Molecular System Using Perturbations, Spectroscopy and Multivariate Analysis as Main Tools in Aquaphotomics

Temperature change and addition of solutions of different ionic strength as water molecular system perturbations were applied to Milli-Q water and compared in terms of activated water molecular structures, i.e. water absorbance bands. Rapid and precise measurement of low concentration solutes were employed to show that it is possible to quantify very low concentrations of contaminants in water and further on, to identify the main absorbance bands of water activated by contaminants in the presence even of another perturbation like temperature.

8.3.1.1 Investigation of the Effects of Temperature on Water Structure

Aquaphotomics aims to relate water spectral pattern as a biomarker to the structure related functions of water in biological systems using spectroscopy. A number of multivariate spectral analysis methods have been proposed for identification of water absorbance bands (i.e. wave bands related to water structure) from near infrared spectra, in order to characterize the so-called "water spectral pattern, WASP". Examples include principal component analysis loadings and partial least square regression vectors. Interpretation of these spectral patterns has to be made very carefully and in an educated manner because of the following reasons:

1. the profile of a PLS regression vector is sensitive to model parameters (e.g. number of latent variables selected, spectral data pretreatment methods etc.);

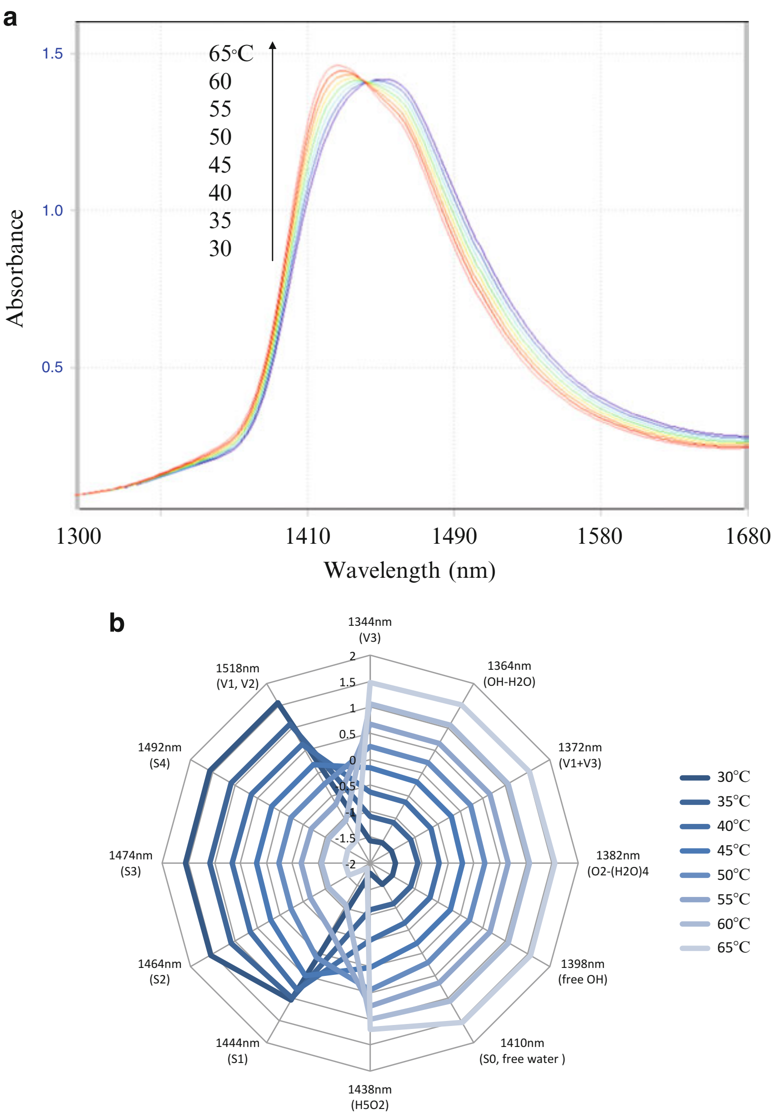

Fig. 8.6 Water spectra at various temperature from 30 to 65 °C, (**a**) in the range of the water first overtone, 1300–1600 nm and (**b**) their respective aquagrams

2. due to the overlapping nature of vibrational overtone and combination bands in the NIR wavelength range, peaks in regression vectors cannot be directly related to absorbance peaks (Seasholtz and Kowalski 1990);
3. interfering factors, such as the strong influence of ambient temperature of NIR spectra on water, may confound the elucidation of spectral responses related to the perturbation of interest.

PCA is more appealing, since it is an unsupervised technique in which the major variations in a spectral dataset are compressed into the first few principal components, which are orthogonal to each other. PCA loadings are useful for interpretation, especially in cases where the perturbation in question is well characterized. One well-known example is aqueous system where the only perturbation is temperature (Segtnan et al. 2001; Maeda et al. 1995). However, in cases where multiple simultaneous (typically unknown) perturbations are present, it may be difficult to interpret the resultant PC loadings. We will concentrate on three different perturbations that might occur simultaneously: temperature, salt type and each salt concentration (Gowen et al. 2013). NIR transmission spectra of deionized water from a Milli-Q water purification system (Millipore, Molsheim, France, resistance = 18 mΩ) at different temperatures, ranging from 30 to 45 °C and its salt solutions at various low concentrations and types of salt were obtained using two types of spectrometers: (1) dispersive grating based spectrophotometer, NIR System 6500 (Foss NIR-System, Laurel, USA), fitted with a quartz cuvette with 1 mm optical path length, wavelength region of 400–2500 nm with 2 nm step acquisition and (2) interferometer based MPA spectrophotometer (Brucker Inc. Billerica, MA) fitted with a quartz cuvette with 1 mm optical path length.

In order to observe water bands activated in the presence of two or more simultaneous perturbations, four different types of salt solutions were analyzed. All salts ($NaCl$, KCl, $MgCl_2$, $AlCl_3$) solutions had concentrations ranging from 0.2 to 1 $mol.L^{-1}$ in steps of 0.2 $mol.L^{-1}$. These experiments were carried out at three different temperatures: approximately 28, 38 and 46 °C.

Principle component analysis (PCA) and Multivariate curve resolution (MCR) (Gowen et al. 2013; Abdollahi and Tauler 2011) were applied to the spectral and reference data using the toolbox developed by Jaumot et al. (2005) for MATLAB (The MathWorks, Inc., Natick, MA). The wavelength range of the acquired spectra was trimmed to 1300–1600 nm in order to examine the 1st overtone of the OH stretch vibration in water.

Spectra of pure water at temperatures varying from 30 to 45 °C, measured on the two different spectrometers employed, are shown in Fig. 8.7a, b. Overall, the trends shown by each instrument were very similar, slight variations such as maximum value of log(1/T) and spectral shape, which arise due to the differences in the effective path length and optical configuration of the different spectrometers were observed. As it has been demonstrated by previous research (Segtnan et al. 2001; Maeda et al. 1995), the peak of the first overtone spectra experiences a shift towards lower wavelengths ("blue shift") as temperature is increased, with an apparent isosbestic point around 1440–1442 nm.

Principal component (PC) scores and loadings for the data obtained on each spectrometer are shown in Figs. 8.8 and 8.9, respectively. For each spectrometer, the first two principal components described more than 99 % of the spectral variations in the datasets and the second components varied linearly with temperature. Third component with nonlinear temperature dependence and a structured PC loading was evident. The existence of a third, spectrally distinct component in the evolution of NIR spectra of water with changing temperature has been previously observed

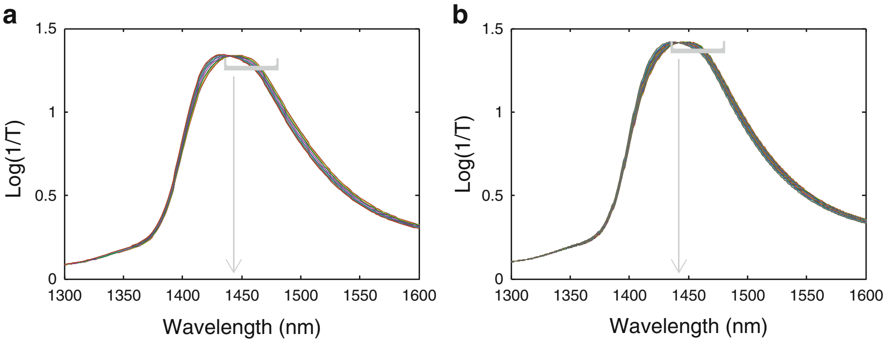

Fig. 8.7 First overtone NIR spectra of water in the temperature range 30–46 °C, obtained using dispersive grating based (**a**) and interferometer based (**b**) spectrometers (first experimental replicate). Region of isosbestic point is marked

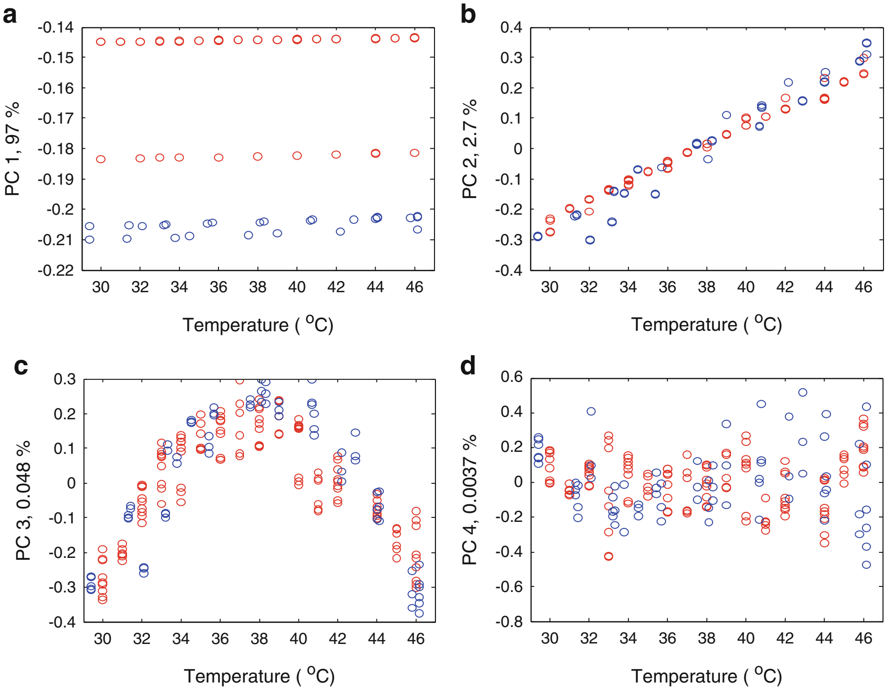

Fig. 8.8 Principal component scores (PC1 (**a**), PC2 (**b**), PC3 (**c**) and PC4 (**d**)) plotted as a function of temperature for first overtone spectra of water in the range 30–46 °C obtained using dispersive grating based spectrometer (*shown in blue*) and interferometer based spectrometer (*shown in red*)

(Segtnan et al. 2001). These experiments indicated an increase in the third PC score with temperature, to a peak at around 37 °C, followed by a decrease to 45 °C, indicating that this represents an intermediate state of water.

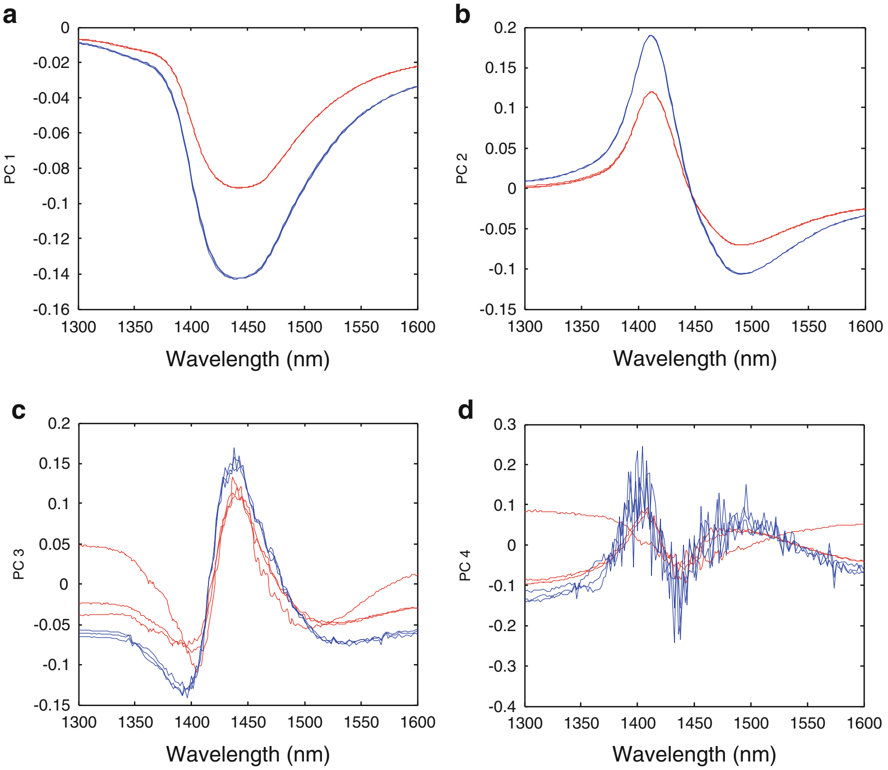

Fig. 8.9 Principal component loadings (PC1 (**a**), PC2 (**b**), PC3 (**c**) and PC4 (**d**)) plotted as a function of wavelength for first overtone spectra of water in the range 30–46 °C obtained using dispersive grating based spectrometer (*shown in blue*) and interferometer based spectrometer (*shown in red*)

The locations of the main turning points in each of the first three PC loadings are summarised in Table 8.2. These can be regarded as the characteristic water matrix coordinates, WAMACS, for the perturbation of changing temperature applied to the water matrix (Gowen et al. 2013). The table contains the main WAMACS for salt solutions, too, which will be discussed further.

The first PC exhibited a single broad peak at 1440–1443 nm, representing a combination of the first overtone of the OH bending and fundamental OH asymmetric stretching vibration ($2v_2 + v_3$) (Lin and Brown 1993). The second PC loading exhibited a positive peak at 1412 nm and a negative one at 1492 nm. These peaks have been previously assigned by various authors. The feature at 1412 nm has been attributed to a form of water with relatively weak hydrogen bonding (Segtnan et al. 2001), a combination mode of the fundamental symmetric and asymmetric OH stretching vibrations ($v_1 + v_3$) for water molecules with no hydrogen bonds (S_0) (Franks 1973; Maeda et al. 1995) or the first overtone of the

Table 8.2 Locations of peaks in principal component (PC) loadings for temperature and salts applied as perturbations to water

Samples	PC1 loading bands	PC2 loading bands	PC3 loading bands
Water	1440-3 (−)	1412 (+), 1492 (−)	1393-1400(−), 1438 (+)
NaCl 28 °C	1448-50 (+)	1396 (+), 1430(−), 1526(+)	1406(+), 1466(−)
NaCl 36 °C	1442 (+)	1396 (+), 1432(−), 1524(+)	1406(+), 1468(−)
NaCl 46 °C	1436-8 (+)	1396 (+), 1434(−), 1528(+)	1406(+), 1470(−)
KCl 28 °C	1448-50 (+)	1396 (+), 1430(−), 1524–6(+)	1404(+), 1468(−)
KCl 36 °C	1440-2 (+)	1398 (+), 1430(−), 1524(+)	1406(+), 1464-72(−)
KCl 46 °C	1436-8 (+)	1398 (+), 1434(−), 1530(+)	1406(+), 1478(−)
MgCl2 28 °C	1450-2 (+)	1404(−), 1492(+)	1364(+), 1400(−), 1428(+)
MgCl2 36 °C	1444-6 (+)	1404(−), 1496(+)	1368(+), 1400(−), 1428(+)
MgCl2 46 °C	1440 (+)	1406(−), 1494(+)	1368(+), 1400(−), 1428(+)
AlCl3 28 °C	1452 (+)	1408(−)	1404-22 (+), 1470-1494 (−)
AlCl3 36 °C	1446-8 (+)	1408(−)	1404-22 (+), 1470-1494 (−)
AlCl3 46 °C	1442-4 (+)	1408(−)	1404-22 (+), 1470-1494 (−)

antisymmetric stretch for free OH in the free water molecule (Czarnik-Matusewicz and Pilorz 2006). The feature at 1492 nm has been attributed to a water species with stronger hydrogen bonds (Franks 1973), a combination mode of the fundamental symmetric and asymmetric OH stretching vibrations ($v_1 + v_3$) for water molecules with four hydrogen bonds (S_4) (Franks 1973; Maeda et al. 1995). The feature at 1412 nm relates to weaker hydrogen bonding and that at 1492 nm relates to stronger hydrogen bonding. The opposing signs of these features in the second loading indicate that as one species increases in concentration, the other decreases. In other words, as the temperature increases, the proportion of weakly bonded water increases and that of strongly bonded water decreases. As for the third PC loading, a small negative feature was observed at 1393–1400 nm and a relatively larger, positive feature at 1438 nm. The feature at 1393–1400 nm is probably due to the changing concentration of weakly hydrogen bonded water in the system (Franks 1973; Segtnan et al. 2001; Maeda et al. 1995), while that at 1438 nm has been previously assigned as a third species of water (Segtnan et al. 2001) or a protonated water dimer in gas phase (Tsenkova 2009). The evidence for a nonlinear system dynamics regarding temperature as water perturbation and the existance of more than two water matrix coordinates suggests the existance of water molecules in transitional state that maintain the balance of the two populations of strongly bonded and weakly bonded water molecules. With this investigation, it was possible to establish the aquaphotome of the Milli-Q water in the 30–45 °C temperature range. Three characteristic water bands were found to be related to temperature perturbation, in this temperature range: C5, C7 and C11. One additional bands was found, too: 1393 nm. In the C7 range, there 2 bands related to temperature: 1438 nm and 1440 nm. This is how the study of this aqueous system brought a contribution to Aquaphotomics in terms of a new specific water absorbance band and finding two specific bands in one region. All together, five WAMACS were identified and all the loadings depict the specific WASP, i.e. the weights of each of those bands with the change of the temperature in the 30–45 °C range. The simultaneous activation of the found WAMACS would be always identified with temperature changes in water.

8.3.1.2 Investigation of the Effects of Multiple Perturbations on Water Structure; Low Concentrations of Salts and Temperature

Another perturbation that illustrates changes in the OH bonding of the water molecular system is the effect of low concentrations of salts diluted in water (Gowen et al. 2013; Inoue et al. 1984; Molt et al. 1997; Frost and Molt 1997). However, we are aware that unavoidable, though minor, fluctuations in ambient laboratory conditions (mainly temperature) also have an influence on water structure, manifest as a shift in the peak position of the OH first overtone peak (located at around 1450 nm). It can be seen, Fig. 8.10, that upon the addition of salt, the so-called isosbestic point of water spectrum becomes even more blurred than was the case for pure water. It can also be seen that changing temperature by 20 °C had a far greater effect on the water matrix than did adding salts up to 1 Mol concentration. In addition, the spread among spectra at each temperature is informative: it can be seen that $AlCl_3$ had the greatest effect on the spectrum, as compared to the other salts. This is due to its relatively higher Lewis acidity. The trend of the shift in the peak position due to temperature was similar to that observed in the temperature perturbed pure water (i.e. the peak moves towards lower wavelengths as temperature is increased), but the actual peak locations were different to those for the pure water. In order to diminish the temperature effects, spectra of control samples of pure water were measured during the course of each experiment. The principal variations in these spectra were obtained by applying PCA and extracting the 1st PC loading as an "interferent spectrum". Then extended multiplicative scatter correction (EMSC) (Afseth and Kohler 2012) was applied to the spectra of salt solutions, to correct the data for the interferent spectrum. This effectively diminished the influence of ambient conditions on the spectra of salt solutions. Consequently, when applying PCA to the EMSC-corrected data, the 1st PCA loading could be related to the perturbation of interest (i.e. addition of salt), and would thus present us with a reasonable estimate of the WAMACS and WASP related to salts, i.e. the aquaphotome of salts could be established.

8.3.1.3 Evaluate the Performance of NIRS Combined with Chemometrics Modelling for Prediction of Low Concentrations of Salt in Water

In order to investigate the influence of the environmental when concentrating on the extraction of WAMACS related to salts, spectral data acquired at four different test locations (Gowen et al. 2015) were used to construct the respective WASP of different salts. As salts do not absorb NIR light, accurate measurement of even

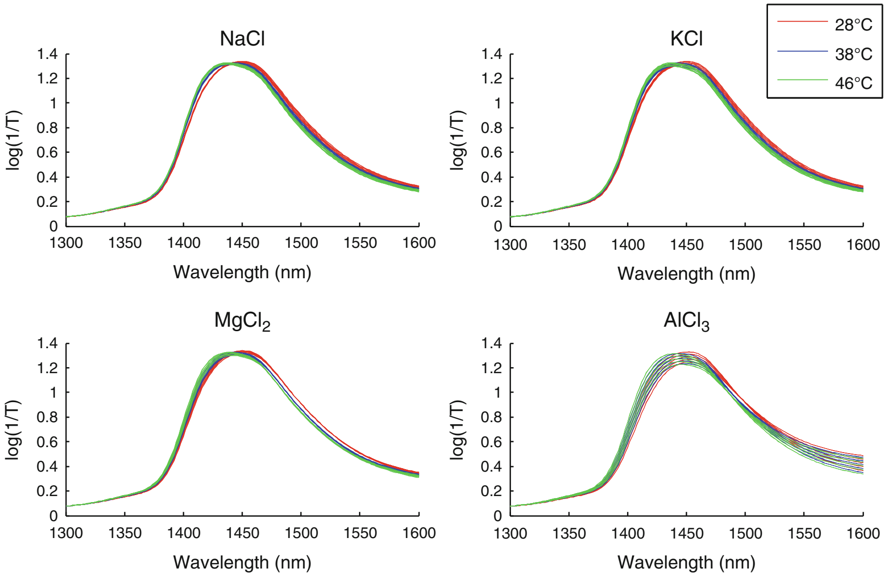

Fig. 8.10 Absorbance (Log(1/T)) spectra of salt solutions of different concentrations, grouped by temperature

low concentration of salts means that salts change the surrounding water molecular structure according to the number of the solvent molecules in the solution. High prediction of salt concentration when using the respective solution spectra means that specific WASP has been found for each salt. In this process, firstly, after spectra for each location studied and each experimental day were pooled, spectral data in the 1300–1600 nm wavelength region (the so-called “first overtone region of water”) were extracted. EMSC (Afseth and Kohler 2012) was applied to this data, using the 1st principal component loading of the control (Milli-Q water) spectra as an interferent spectrum. Finally, principal component analysis was applied to the SNV + EMSC pre-treated data, and the 1st principal component loading was extracted. In order to avoid sign and scale ambiguities, this loading was squared and scaled to the range [0,1]. The resultant scaled loading was employed as a water spectral pattern (WASP) for each salt/location, Fig. 8.12. The combination of investigated spectral pre-treatment, wavelength range, salt and test day resulted in a rather large number of competing models (432) for each test location. In order to compare the effects of spectral pre-treatments and wavelength ranges on model performance, the Root Mean Squared Error of Prediction (RMSEP) values corresponding to models for each test set and location were averaged. The average RMSEPs obtained are plotted for each salt as a function of wavelength range and spectral pre-treatment in Fig. 8.11. It is evident from this figure that in most cases the first overtone of water, 1300–1600 nm wavelength range, resulted in the lowest prediction error, while the most effective spectral pre-treatment was EMSC.

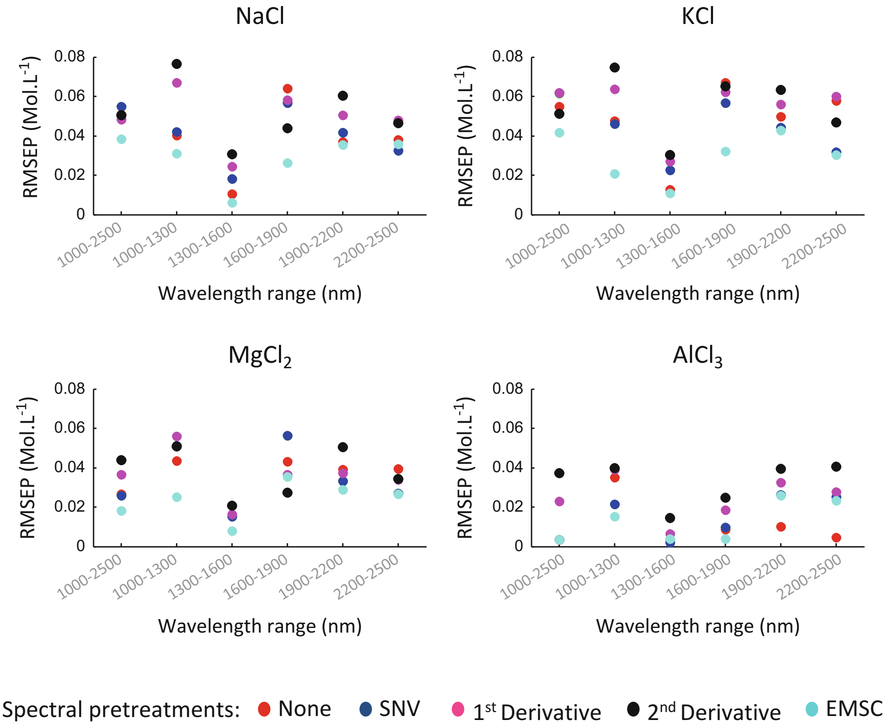

Fig. 8.11 Root mean squared error of prediction (RMSEP) averaged over permuted experimental test days and test locations for each salt, pre-treatment and wavelength range studied (*SNV* standard normal variate, *EMSC* extended multiplicative signal correction)

When RMSEP values from the individual test locations were evaluated separately, similar findings were obtained. Calibration models built in this wavelength range and employing the EMSC pre-treatment resulted in highly accurate prediction with RMSEP values ranging from 0.004 (for $AlCl_3$) to 0.01 (for KCl) Mol.L^{-1}. Water absorbance spectra first PC loadings for each salt/experimental location are plotted in Fig. 8.12. Each of the salts' WASP exhibited peaks around 1400, 1430–1450 and 1500–1520 nm, which can be related to different hydrogen bonding strengths in the water molecules (bond strength decreasing with decreasing wavelength) perturbed by salts. Similar to the previous results for water perturbed by temperature, mainly three ranges underneath the first overtone of water were activated, but their location was different. Although the peak locations, the WAMACS, in the WASP were similar for each salt studied, the shapes of the profiles varied markedly with the number of Chloride ions present.

The presence of ions in solution affects hydrogen bonding in water, i.e. different ion concentration works as a perturbation that can be observed directly by NIR spectroscopy (NIRS) (Weber et al. 2000). Consequently, NIRS has been used to estimate the concentration of OH^- and H^+ ions in solution, i.e. pH (Molt et al.

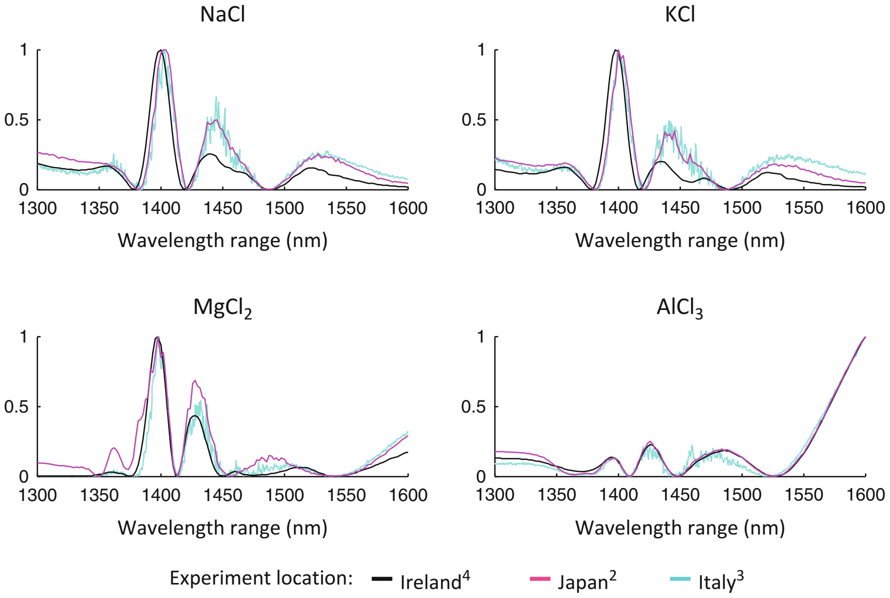

Fig. 8.12 Water absorbance pattern (1st principal component loading of SNV followed by EMSC pre-treated data) for each salt and test location

1997). It was demonstrated that binary mixtures of inorganic ionic salts (e.g. NaCl, KCl, $MgCl_2$ and $AlCl_3$) can be quantitatively analyzed with NIR due to the specific effects of cation/anion OH interactions (Frost and Molt 1997), also known as the Lewis acidity effect (McNaught and Wilkinson 1997). Therefore, identification of water absorbance bands related to respective perturbations would lead to further use of these bands as coordinates of a specific spectral patterns that can be exploit as biomarkers. The action of salts during dissociation in water is a complex phenomenon. Structure induced shifts are highly dependent on several parameters, such as the valence and charge distribution of ions in solution, the electron affinity and the size of the ions. This dependence can be observed by the changes in the pH observed in different solutions of the same ionic salt at different concentration levels.

Shifts according to the salt employed have been observed: for NaCl or KCl the peak locations ranged from 1436 to 1450 nm, for $MgCl_2$, they ranged from 1440 to 1452 nm and for $AlCl_3$ they ranged from 1442 to 1452 nm. The appearance of the principal components PC2 and PC3 loadings differed according to the salt used and also differed substantially in profile from those obtained for the temperature perturbation, indicating that the addition of salts to water perturbed the hydrogen bond network in a manner distinct from that of changing only temperature. Considering the second PC, both NaCl and KCl exhibited positive features at 1396 nm and 1526 nm and negative features at 1430–1434 nm; $MgCl_2$ and $AlCl_3$ exhibited negative features at around 1404–1408 nm, and $MgCl_2$ exhibited a positive feature at 1492 nm. As for the third PC, both NaCl and KCl exhibited positive features at 1404–1406 nm and negative ones at around 1468–1478 nm, while $MgCl_2$ exhibited positive features at 1364–1368 nm and 1428 nm and a negative one at 1400 nm. $AlCl_3$ exhibited a positive feature at 1404–10,422 nm and negative ones around 1470–1494 nm.

It has been shown that multivariate analysis can be applied to characterize the effects of different perturbations separately and simultaneously (i.e. temperature and addition of salts) to the water matrix in terms of principal spectral components and concentration profiles. Based on the temperature perturbation experiments, it has been found that three distinct components with varying temperature dependence are present in water in the temperature range 30–45 °C, a range which is significant for biological systems. The salt solutions studied exhibited similar trends with respect to the temperature perturbation, while the locations of WAMACS varied according to the salt used. This gives insight into the differing characteristics of these salts and their respective effects on the hydrogen bonded network in water. Each salt and concentration level affected water in a different way, according to its acidity. The results indicate that water structure can be reasonably interpreted as a multi-state system, and are in line with the traditional definition of salts as structure makers/breakers.

8.3.2 Other Applications

The first publications where water bands have been used to understand and present new phenomena have been on diagnosis of mammary gland inflammation (Tsenkova et al. 2001), prion protein isoforms identification (Tsenkova et al. 2004) and water functional analysis based on real time monitoring of the water bands in the spectra of prion protein solution (Tsenkova 2010). Recent publications have shown at various levels of system organization that WAMACS are real « informational hubs ». Research on soybean species identification related to their cold resistance abilities have proved that genetically modified soybean plants with different resistance can be characterized by different respective water absorbance pattern. It has been found that there are specific WASP for different level of cold resistance (Jinendra 2010; Jinendra et al. 2010). Further on, soybean mosaic virus diagnosed with near infrared spectroscopy using the Aquaphotomics concept showed that for the sick plants, there was an inhibition of water absorbance at the high energy bands, i.e. less hydrogen bonded water (Jinendra 2010). Another interesting application is the use of Aquagrams to illustrate the specific WASP of urine when Giant Panda is in estrus (Kinoshita et al. 2012). Aquaphotomics has been proposed in the research of nanomaterials where Aquagrams showed water spectral pattern as characterization of hydrogenated nanomaterial (Matija et al. 2012). With Aquaphotomics, we have found that successful bacteria identification with NIRS is highly accurate not because of the difference in bacteria cells, but because of the metabolites that change the conformation of the water molecular system (Nakakimura et al. 2012) surrounding them.

8.4 New Frontiers

Water in biology is still underestimated, underdeveloped subject. On the contrary, for the last few years, water has been « noticed » and like an avalanche, the number of papers on water are increasing very fast. Near Infrared Spectroscopy is a powerful tool to study water: rapid and noninvasive. Acquisition of immense number of spectra of various systems in real time as time series and under various possible perturbations became an important research subjects of other laboratories, too. We, together, are building up the Aquaphotome of water and other aqueous systems in order to « see » the « hubs » where we can harvest information from the « water computers ». Without chemometrics, multivariate analysis and other new methods for spectral data analysis (to be developed), it would be impossible to utilize all the spectral data that we can acquire. New, super computers will help meeting the experimental data with the results of computer simulation and modeling, which will show expected consistency and will open new horizons in understanding unknown phenomena in various fields. There are so many areas related to water, but especially life science will gain most. Non-invasive diagnosis, identification and understanding of DNA, cells, tissue, whole organisms is not a dream anymore. Abnormality in other molecular pathways is “reflected” by the water molecular system as a mirror. We can even foresee that having information from the water molecular system in the body, in real time, gives the chance to think about how can we influence and change it in order to maintain its balance and get it back to the normal condition. It would open a completely new field of water molecular network manipulation. The definition of « normal », « healthy » from the point of view of Aquaphotomics is the ever standing challenge.

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