Infrared spectroscopy applied in biochemistry: Characteristic spectra of some substances present in foods and uroconcrements

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Abstract

This paper presents an overview on some constituents that are decelated in the nutrients involved in the biogenesis of lithiasic concrements. There is focused on the infrared (IR) spectra of some constituents of uroconcrements. Thus, are evidenced the wavelengths and the specific spectral molecular vibrations for calculi' constituents, e.g. uric acid, oxalic acid, oxalates, phosphates, carbonates. Results on chemical pure substances are compiled in a "data base" that is useful for the evaluation of calculi' composition. One can distinguish the following types of lithiasis: uric, oxalic, phosphatic, cystinic. We can mention that neither the foods, nor the constituents contain a limited composition of nutrients. Currently appear mixt (binary, ternary) or simple lithiasis.

Keywords: infrared spectroscopy in foods implied in lithogenesis and in uroconcrements

Introduction

Spectroscopy as a physico-chemical method, is currently used in the investigation of the bioconstituents of living matter concerning biochemistry and molecular biology, but also nutrition, pharmacology and the material science.

Most frequently used spectroscopical methods are from the domaines of optical spectroscopy; X-ray spectroscopy; mass spectrometry (often in tandem mass spectroscopy – chromatography); Raman spectroscopy.

The present paper presents general data regarding the spectroscopic methods, some applications of IR spectroscopy as an instrumental method, predilectly used in the investigation of food compounds that are involved in lithogenesis, in general, and in urolithogenesis, in special.

1.Spectroscopy in the physico-chemical analysis

Spectroscopic investigations belong to the domaine of physico-chemical methods currently used in laboratories of analysis. Infrared spectroscopy measures the vibrations of molecules. Each functional group, or structural characteristic, of a molecule has a unique vibrational frequency that can be used to determine what functional groups are in a sample.

When the effects of all the different functional groups are taken together, the result is a unique molecular "fingerprint" that can be used to confirm the identity of a sample. (Price, 1979; Atkins, 1982; Simmons, 1999; Günzler and Gremlich, 2002; Ellis and Goodacre, 2006) short presentation is given below.

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1.1. Types of spectroscopy

When a beam of electromagnetic radiation of intensity I_o and energy E passes a substance, it can be either absorbed or transmitted, depending upon its frequency, $E = h\upsilon$, and the structure of the molecules it encounters. The type of absorption spectroscopy depends on the wavelength and frequency range of the electromagnetic radiation absorbed (fig. 1).

Thus: a) microwave spectroscopy involves a transition from one molecular rotational energy level to another; b) vibrational spectroscopy (or infrared spectroscopy) measures transitions from one molecular vibrational energy level to another, and requires radiation from the infrared portion of the electromagnetic spectrum; c) ultraviolet (UV)-visible(VIS) spectroscopy (also called electronic absorption spectroscopy) involves transitions among electron energy levels in the molecule, which require radiation from the UV-visible portion of the electromagnetic spectrum.

Such transitions alter the configuration of the valence electrons in the infrared region of the electromagnetic spectrum is divided into three regions; the near-, mid- and far-infrared, named for their relation to the visible spectrum. The far-infrared, approximately 400-10 cm-1 (1000–30 μm), has low energy and may be used for rotational spectroscopy. The mid-infrared, approximately 4000-400 cm-1 (30–1.4 μm) may be used to study the fundamental vibrations and associated rotational-vibrational structure (Pecsok and Shields, 1968; Pogany and Banciu, 1972; Avacovici et al., 2003).

The type of spectroscopy depends on the measured intensity, or energy absorbed or produced. One can distinguish: 1) *electromagnetic spectroscopy* – involves interactions of matter with electromagnetic radiation, such as light; 2) *electron spectroscopy* – is based on interactions with electron beams. Auger spectroscopy involves inducing the Auger effect with an electron beam.
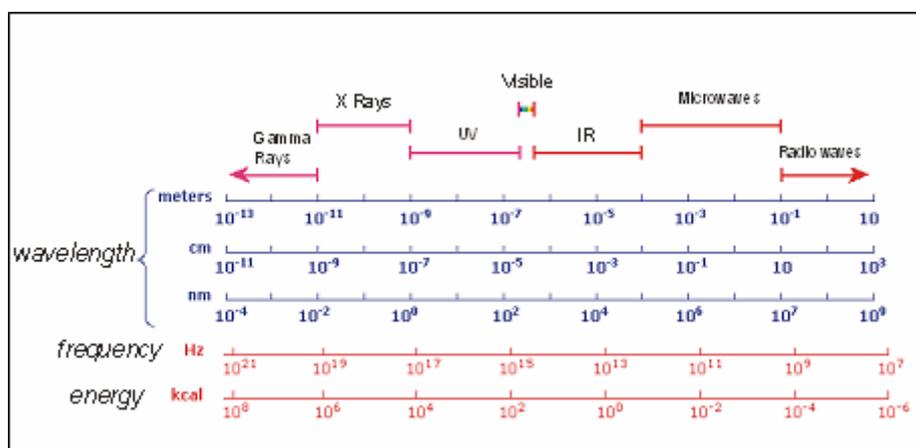

Fig. 1. The electromagnetic spectrum

In this case the measurement typically involves the kinetic energy of the electron as variable; 3) *mass spectrometry* - occurs at interaction of charged species with magnetic and/or electric fields.

This spectrum has the mass m as variable, but the measurement is essentially one of the kinetic energy of the particle; 4) *acoustic spectroscopy* - refers to the frequency of sound; 5) *dielectric spectroscopy* - implies the frequency of an external electrical field .

The X-ray diffraction and infrared spectroscopy (IRS) are the current analytical methods. The method of X-ray diffraction is suitable for quantification of mineral containing samples, such as urinary calculi. Infrared (IR) spectroscopy has been applied in clinical chemistry for analyses of biofluids and solid biosamples. This technique often produces complex spectra. Analyses of these complex spectra is facilitated by the use of chemometrics, which is a generic term for the application of neural networks and other mathematical and statistical methods (Que, 2000; Hollas, 2004).

In order to express the position of an IR absorption are used either the wavelength λ (μm) or the wavenumber λ^{-1} (cm^{-1}). The absorption bands for most organic and inorganic compounds are found in the infrared region. Any absorption band can be characterized by two parameters: the *wavelength* at which maximum absorption occurs and the *intensity of absorption* at this wavelength. This principle consequently leads to quantitative analysis. The general regions of the infrared spectrum in which various kinds of vibrational bands are observed are outlined in the following chart (fig. 2).
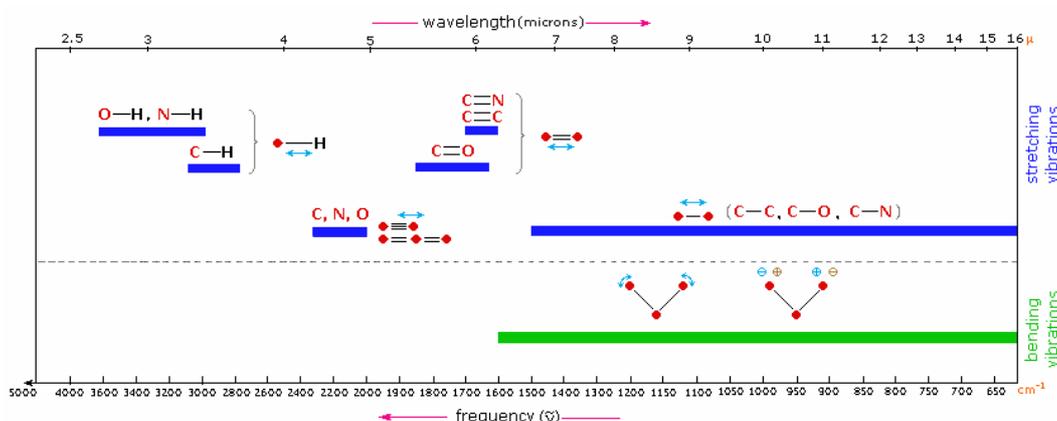

Fig. 2. Types of vibrations evidenced in IR spectra

Infrared spectroscopy is based on the fact that molecules have specific frequencies at which they rotate or vibrate corresponding to discrete energy levels. These resonant frequencies are determined by the shape of the molecular potential energy surfaces, the masses of the atoms. In order for a vibrational mode in a molecule to be IR active, it must be associated with changes in the permanent dipole.

1.2. Study of inorganic and organic compounds

In the infrared region of the spectrum, the resonance frequencies of a molecule are due to the presence of molecular functional groups specific to the molecule.

Although absorption bands are characteristic of the molecule as a whole, it is a useful approximation to consider that molecular vibrations are localized in particular functional groups. This allows one to relate absorption band position with a particular functional .The infrared spectra of relatively simple, purely *inorganic compounds* containing only a few atoms and of inorganic salts containing complex ions, are quite distinctive and can be used to rapidly identify the ions.

In case of *organic compounds* the studied functional groups differ both in the strengths of the bond(s) and in the mass of the atoms involved. For instance, the O-H and C=O functional groups each contain atoms of different masses connected by bonds of different strengths (Pecsok and Shiels, 1968; Smith, 1979; Silverstein et al., 1981; Ning, 2005).

2. Some applications in biochemistry generalities

Infrared spectroscopy offers the possibility to measure different types of inter atomic bond vibrations at different frequencies. Especially in organic chemistry the analysis of IR absorption spectra shows what type of bonds are present in the sample. Applications are found in medical, food and pharmacologic biochemistry (Smith, 1979; Osborne and Fearn, 1993; Que, 2000; Luypaert, 2003).

The steps used to find information about molecular structure from the IR spectrum are as follows: 1. Obtain a spectrum of the material on an IR spectrophotometer; 2. Using information from correlation tables and absorbances from the functional group region of the spectrum, identify the functional groups that are present or sometimes more importantly absent; 3. Compare this spectrum with those of known compounds or obtain a known sample of a suspected material and run its spectrum for comparison.

Many of the group frequencies vary over a wide range because the bands arise from complex interacting vibrations within the molecule. Absorption bands may, however, represent predominantly a single vibrational mode. Certain absorption bands, for example, those arising from C-H, O-H, and C=O stretching modes, remain within fairly narrow regions of the spectrum. Most organic and inorganic solids have absorption patterns that include several absorption maxima and wavelengths characteristic to particular functional groups that make up the molecules.

3. Some special applications of infrared spectroscopy

3.1. Applications in the study of foods

Infrared spectroscopy offers an accurate analysis of ingredients and constituents used in the manufacture of foods. It plays a role in maximizing food potential which is one of the priorities of the food industry.

Near IRS facilitates quality measurements made early in the production when fresh products are still edible, helping to determine whether the product goes to fresh market or to processing and thereby minimizing waste.

The method is used in the domains: foodstuffs and processed foods (i.e. flours, breads, cereal foods, livestock, dairy products); agricultural and marine products (i.e. grains, seeds, fruits, vegetables, meat and fish products); food safety and disease diagnosis (on line analysis in food engineering, fermentation engineering); food additives characterization.

Future uses of near IRS will include grading and classification of materials and organoleptic-type categorization of materials and foods.

3.2. Applications in the study of uroconcrements

Urinary calculi formation is mostly related to the living modes of the nations and their geographic distribution. It is influenced by diet (composition of protein nutrients, minerals a.o.). Also, its appearance is related to tap water - considered as food, too. Urinary calculi are concretions composed by crystalline components and organic matrix. The major role of cations in the formation, consolidation and further development of calculi is assesed during the crystallization process. From the initial nuclei evolve the uroconcrements during the bioinorganic process of lithogenesis (Garban et al., 1989).

Acidic urine is a prerequisite for uric acid stone formation and growth. Uric acid is the end product of purine degradation in humans. Oxalates result from carbohydrate and protein metabolism while phosphates from various material and energetic metabolism.

Cystine is a non-essential aminoacid concentrated in various protein structures, that appears in exces in blood and organs and can cause lithiasis with diverse localizations, implicit renal ones. Nearly all foods contain sufficient amount of cystine. The IRS method is efficiently used in evaluation.

3.2.1. Studies on uroconcrements' constituents

Infrared spectroscopy of urinary calculi can be used for the qualitative determination of the major constituents in mixed stones; the method is simple and consists in area-measurements of specific absorption peaks of the spectrum of each compound. It is suitable for routine use in a clinical laboratory.

The importance of kidney stone analysis by IR spectroscopy is growing. Infrared microscopy is a valuable method, because it combines optical microscopy and infrared spec-troscopy.

Depending on the chemical composition, the urolithiasis are classified in two groups: a) inorganic: with phosphates (calcium phosphates and ammonium-magnesium phosphates), carbonates; b) organic: with purine (uric acid, urates, xanthine, 2,8-di-hydroxyadenine), oxalic acid, cystine (Drăgan et al., 1981; 1986; Campbell and Dwek, 1984; Hesse et al., 1989; Gârban et al, 2003).

3.2.2. Types of urolithiasis defined by infrared spectra

In order to complete our information on the chemical compounds present in uroconcrements, called in some cases "standard" molecules, we proceeded to the identification of spectrum from catalogues (Hesse and Sanders, 1988; Dao and Daudon, 1997).

IR absorption information is generally presented in the form of a spectrum with wavelength or wavenumber as the x-axis and absorption intensity or percent transmittance as the y-axis.

Transmittance (*T)* is the ratio of radiant power transmitted by the sample (*I*) to the radiant power incident (*I_0*) on the sample.

$$T = \frac{I}{I_0} x100\%$$

Absorbance (*A*) is the logarithm to the base 10 of the reciprocal of the transmittance:

$$A = -\log T$$

The analyst should be aware that the same sample will give quite different profiles for the IR spectrum, which is linear in wavenumber, and the IR plot, which is linear in wavelength. It will appear as if some IR bands have been contracted or expanded.

IR spectra are obtained by detecting changes in transmittance (or absorption) intensity as a function of frequency. Most commercial instruments separate and measure IR radiation using dispersive spectrometers or Fourier transform spectrometers.

From the catalogue elaborated by Hesse and Sanders (1988) we exemplify the IR spectra for uric acid (Fig.3).
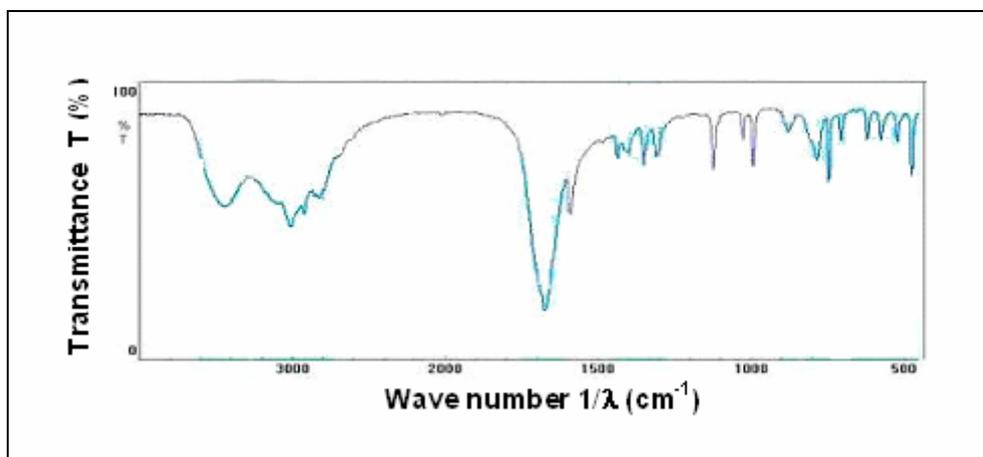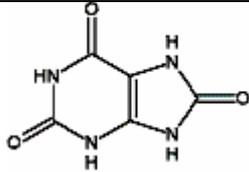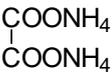

Fig. 3. Infrared spectra of uric acid

Using these data completed with those from catalogues we made up the Table 1 which contains information on the spectral characteristics of „standard molecules", e.g. vibration types (ν); specific wabvenumber ($1/\lambda$). Electronic transitions that appear when an energy quantum is absorbed, differ upon the nature of the initial and final orbitals of the transition (σ, π etc.).

As a consequence, simultaneous modifications of the vibration and rotation state appear. Variations of vibration energy lead to the fine structure of spectral lines.

Having these information on „standard spectra" we can evaluate the recorded spectra of each studied calculi.

Table 1. Characteristic spectral vibration of molecule of standard substance in urolithiasis

No.	Compound	Wave number (cm^{-1})	Type of vibration
1.	Uric acid	3020 1670 1590	ν_{N-H} $\nu_{C=O}$ $\nu_{C=O} + \nu_{C=N}$
2.	COOH | COOH Oxalic acid	3423 3267 1726 1262 723	ν_{OH} ν_{OH} $\nu_{C=O}$ ν_{C-O} ν_{C-C}
3.	COONH$_4$ | COONH$_4$ Oxalat de amoniu	3402 3207 2993 1654	ν_{N-H} $\nu_{N-H} + \nu_{OH}$ $\delta_{(NH4^+)}$
4.	$Ca_3(PO_4)_2$ Calcium phosphate	1150 635 600	Group vibration of phosphate
5.	$CaCO_3$ Calcium carbonate	1417 875 712 1417 875 712	vibration of carbonate ion $\delta_{asimCO3}$ $\pi_{o.p (CO3)}$ $\delta_{i.p(CO3)}$

Preventive measures for avoiding each type of renal calculus formation involve specific dietary considerations (Hesse et al., 1993; Osborne and Fearn, 1993; Ciurczak, Drennen, 2002).

Urine composition is directly related to diet. Each type of urolithiasis is related to different dietary factors (liquid intake, pH, foods containing calcium, phosphate, oxalate, citrate, phytate, urate and vitamins). By controlling urinary pH, uric acid stone disease can be prevented, Maintaining a moderate consumption of animal protein, seafood and alcohol is also important.

Conclusions

1. Infrared spectroscopy is very useful for qualitative analysis (identification) of organic compounds because a unique spectrum is produced by every organic substance with peaks corresponding to distinct structural features.
2. Types of urolithiasis can be detected using the spectra of their compounds. Each functional group absorbs infrared light at a unique frequency.
3. IRS is mainly used for detection of functional groups and identification of compounds in mixtures.

4. Near IRS has become an indispensable tool of analysis for certain research areas and applications, ranging from material science, via chemistry to life sciences, because it is fast and effective.

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