Pure & Appl. Chem., Vol. 63, No. 8, pp. 1081–1082, 1991.
Printed in Great Britain.

ADONIS 206922209100094D

Trends in spectroscopic methods for clinical chemistry

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Abstract - Sensitivity and specificity of spectroscopic methods regularly increase and offer new possibilities for clinical chemistry. The main features of these methods are summarized in this paper as an introduction to the Symposium.

INTRODUCTION

Spectroscopic methods have been largely improved during the last years as a result of progress in technology. Energy of lasers is high, which increases sensitivity in absorption or emission spectroscopy. The same result is obtained with photon counting, which detects very low light level. It is possible to record a spectrum in a very short time with diode array or CCD camera. Better information can be obtained from spectroscopic data by computers. At last, use of infrared, near infrared, Raman or NMR spectroscopies is rapidly growing in clinical chemistry. But all the possibilities offered by these technological improvements are far from being extensively used and we will survey now the interest of their application in this field.

For a long time, biochemical parameters were determined by visible spectrophotometry, after a chemical reaction used to make a coloured compound. As the chemical reaction was not specific, it was necessary to eliminate interfering substances (mainly proteins) by a separation step (dialysis, precipitation, extraction ...) which was time consuming. This method is still used now after a chromatographic separation. Use of specific reagents (enzymes, antibodies) allowed to suppress this step and to create a new generation of automatic analysers.

The first problem encountered with these analysers is sensitivity. Immunoassays are a good example of the need of low detection limit. Antibodies can react with very small quantities (femtomoles) of biological substances. But the optical properties of the immun complexes are weak and, to detect them easily, it is necessary to label them with fluorescent or chemiluminescent compounds. Fluorescence and chemiluminescence are indeed very sensitive methods : using a laser, collimated through a microscope on a very small volume, it is possible to detect one molecule of an highly fluorescent product (fluoresceine) in pure dilute solution. This limit is impossible to reach in biological media, because of the background fluorescence, but the detection limit can be very low. The same holds for chemiluminescence. Other methods (surface plasmon resonance, evanescent wave, thermal lensing) are also very sensitive and begin to be used for quantitative analysis in immunoassays.

The second problem is specificity. Reaction between enzymes and substrates, or between antibodies and antigenes are highly specific but the detection step can be disturbed by background interference. Biological samples contain thousands of molecules which can absorb or emit light. For instance, icteric, turbid or hemolysed samples interfere with many colorimetric determinations.

This problem can be solved by chemometry, which uses mathematical and statistical methods to provide maximum informations from analysis of chemical data. For example, different kinds of calculations (bichromatism, Allen correction ...) are used in clinical chemistry analysers to correct - more or less efficiently - interference by hemolysis, turbidity or icterus. But computers can solve more sophisticated problems. Mathematical interpretation of multiwavelength data, recorded in less than one second with a diode array spectrophotometer can enhance selectivity and so, give better resolution of mixtures or eliminate background signals. Derivative spectrophotometry, multicomponent analysis and deconvolution of spectra are different examples of these mathematical treatments of multiwavelength analysis. They are now widely used in clinical chemistry for the determination of hemoglobins, porphyrins etc... Mathematical deconvolution of kinetic measurements has been also used in fluorimetry and absorption photometry.

Absorption and emission spectra in the UV and visible region consist of large bands which strongly overlap in the spectra of mixtures. Another way to eliminate these interferences and specifically determine one compound in biological media, is to obtain narrower bands, easier to discriminate from the background. Several methods have been proposed in the field of fluorescence. Synchronous fluorescence is obtained by simultaneously scanning the excitation and emission wavelengths. The spectra are more simple, the bands are narrower and Rayleigh

diffusion is eliminated. The combination of synchronous and derivative fluorimetry enhances the resolving power of these methods. Different applications have been proposed in the field of biochemistry : determination of phenylalanine tyrosine and tryptophane, of uro and coproporphyrins etc ... Low temperature luminescence (Schopl'skii effect, line narrowing, matrix isolation or supersonic jet fluorimetry) is a powerful method which dramatically reduces the bandwith. Mixtures of up to fifteen compounds can be analysed in this way without separation, even if spectra strongly overlap. But this method is now seldom used in biology although it is very promising for the analysis of carotenes, vitamins etc ... At last some simple technics (fluorescence polarisation, energy transfer, time resolved fluorescence of Europium salts) can also improve the specificity of immunoassays.

CLINICAL CHEMISTRY WITHOUT REAGENTS

The third problem is the price of reagents. For instance, antibodies or chemiluminescence reagents are generally expansive. As the cost of health rises every year, it is necessary to find cheaper ways of analysis. It is now possible to determine biochemical molecules in biological sample without reagents by spectroscopic methods. For this purpose, we need to improve resolution by using spectra with very narrow bands.

NMR spectroscopy has proved to be very efficient in the field of fundamental biology as well as for the analysis fo clinical samples. For instance, determination of metabolites is performed with a small volume of urine, without preparation. Its appplication for the study of organic acidurias is particularly interesting but other pathologies can be diagnosed in this way. Sodium, lithium and ATP have been determined in erythrocytes and metabolism of glucose studied in blood plasma.

Infrared and Raman spectroscopies are powerful tools in biology (for protein or DNA structure determination, or membranes studies) but of little importance in clinical chemistry. Their main application is the analysis of kidney stones. These two methods lack sensitivity but now, surface enhanced and resonance enhanced Raman spectroscopy, and Fourier transform infrared spectrometry have low detection limit and are more adapted for future applications in clinical chemistry.

Near infrared spectroscopy is widely used for the analysis of food and pharmaceutical products, but recently this method has found its first applications for the determination of hemoglobin, glucose, cholesterol or proteins in blood. Although bands are very large and greatly overlap in this spectral region, mathematical treatment of spectral data makes possible to analyse biological samples. We can hope that automatic clinical analysers will use this last method for the determination of biological substances without reagents.

CLINICAL CHEMISTRY WITHOUT SAMPLES

Conventional clinical analysis involves spectrophotometric measurements on body fluids samples. Unfortunately, this procedure suffers some disadvantages. It is sometimes difficult to withdraw such a sample without traumatism. Rapid analysis is difficult as the sample must be transported to the laboratory. Continuous in vivo monitoring in intensive care medicine is impossible. But spectroscopic methods afford solutions for in-vivo analysis.

Once more, NMR is a powerful tool in this field : it provides a non invasive probe of the metabolic status of living tissues as well as a mean for non destructive biochemical assays. Most chemical investigations are done using phosphorus 31, hydrogen-1 and now carbon-13 NMR and are well adapted for diagnosis of metabolic diseases.

Optical biosensors are also availabe for in-vivo analysis and continuous flow monitoring. They are based on fiber-optic technology. A powerful light source (laser for instance) is collimated into the optic fiber and is used for excitation of a fluorophore at the other end of the fiber. Emission radiations are guided back to a detector through the optic fiber. Small diameter fibers can give good spatial resolution and can be contained in small gauged needles, so that their in vivo use is not traumatic. Sometime the intrinsic fluorescence of the analyte is measured. An example is $NADH^+$ which is a good marker of tissue oxygenation. But most of the time, the analyte reacts with a fluorescent dye which is immobilized at the end of the fiber. Such biosensors have been proposed for the determination of pH, glucose, O_2, CO_2 etc... Their use will increase in the future in intensive care medicine and for the feedback control of delivery of drugs, for instance insuline by an artificial pancreas in diabetes.
Spectroscopic methods (mass spectrometry, fluorescence) have also been proposed to detect different metabolites by microdialysis. This recent method seems very promising for pharmacokinetic measurements and metabolic studies.

CONCLUSION

Although spectroscopic methods are in competition with electrochemistry for analytes detection in clinical chemistry, their progress in sensitivity and specificity are so important that clinical chemistry can be completely transformed in the near future. In vivo analysis for metabolic studies and continuous monitoring, use of physical methods to analyse samples without reagents,detection of lower concentrations of biological compounds are some of the possibilities provided by spectroscopy.