ΣΥΝΕΔΡΙΑ ΤΗΣ 13ΗΣ ΝΟΕΜΒΡΙΟΥ 1980

ΠΡΟΕΔΡΙΑ ΓΕΩΡΓΙΟΥ Ε. ΜΥΛΩΝΑ

ΑΝΑΛΥΤΙΚΗ ΧΗΜΕΙΑ.— **Qualitative and quantitative analysis of gallstones by infrared spectroscopy,** *by John K. Logios - Theodore F. Zafiropoulos - John K. Kouinis* *. Ἀνεκοινώθη ὑπὸ τοῦ Ἀκαδημαϊκοῦ κ. Γ. Τσατσᾶ.

INTRODUCTION

In the past, various studies in U.S.A. [**1**], Japan [**2, 3**], France [**4**] and Greece [**5**] were concerned mainly with the qualitative analysis of gallstones. In the three cases quantitative analysis was done by microanalysis [**1, 6, 7**]. J. D. Edwards and his coworkers [**1**] analysed thirty (30) gallstones for calcium bilirubinate, calcium carbonate and cholesterol, using infrared spectroscopy.

Similar analyses were carried out previously by B. Halpert [**8**] and D. E. Beischer [**9**]. In 1968, F. Nakayama reported the analysis of gallstones by microanalysis and chromatography [**2**] and some years later P. Hraban [**10**] reported the analysis of cholic acids by visible spectrophotometry. Very recently, M. Armahamian et al [**4**] reported a morphological, X-ray and crystallographic analysis of gallstones.

The procedure, which is discribed in this paper, combines infrared spectrophotometry, the Beer - Lambert law [**11**] and the base-line technique [**12**], and offers a relatively simple method of qualitative and

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quantitative analysis of gallstones. The samples need not be dissolved in organic solvents or modified (crystallization), as required in visible spectrophotometry, microanalysis or crystallography [**2, 13**].

Mathematically, the Beer-Lampert law is expressed by the following equation :

$$\log (P_o / P) = abC$$

where P_o denotes the rate at which energy is transported in a beam of radiant energy for the incident beam ; P the quantity remaning unabsorbed after passage through a sample or container ; a is the absorptivity ; b is the thickness of the absorbing medium ; C is the concentration of the absorbing material (g per liter).

It is also known that the absorbance $A = \log (P_o / P) = abC$.

The base-line method involves selection of an absorption band of the component analyzed which does not fall too close to the bands of other matrix components. The value of the incident radiant energy P_o is obtained by drawing a straight line tangent to the spectral absorption curve at the position of the absorption band of the component being analyzed. The value of P is measured at the point of maximum absorption. The value of $\log (P_o / P)$ or A is then plotted against concentration in the usual manner. Pellets from the KBr disk technique can be employed in quantitative measurements. Uniform pellets of similar weight are essential for quantitative analysis. Known weights of KBr are taken, plus known quantities of the component or analyte ; absorption data are thus obtained and a calibration curve can be constructed. From these curves, the concentration of the component being analyzed can be read off, using the experimental values of the ratio long (P_o / P) or A from the spectrum of the sample.

EXPERIMENTAL

The infrared spectra were recorded with a Perkin-Elmer, model 577, infrared spectrophotometer. An electric balance, type Sauter, was used for all weighings. The potassium bromide (KBr) used as window material, was of spectroscopic quality. Pure calcium carbonate, bilirubin and cholesterol, commercially available, were employed. Calcium bili-

rubinate was prepared according to the method described by Edwards and coworkers [1]. All chemicals were dried at 110°C, and then stored over phosphorus pentoxide (P_2O_5), to avoid moisture absorption from the air.

Prior to the sample analysis, spectra of the pure substances were obtained, and the best absorption bands were selected for the measurements of log (P_o / P). Two of these spectra and the bands chosen are shown in Fig. 1.

As a sample holder or window material potassium bromide (KBr), spectroscopic quality, was employed for all spectra and in quantity of 100 mg.

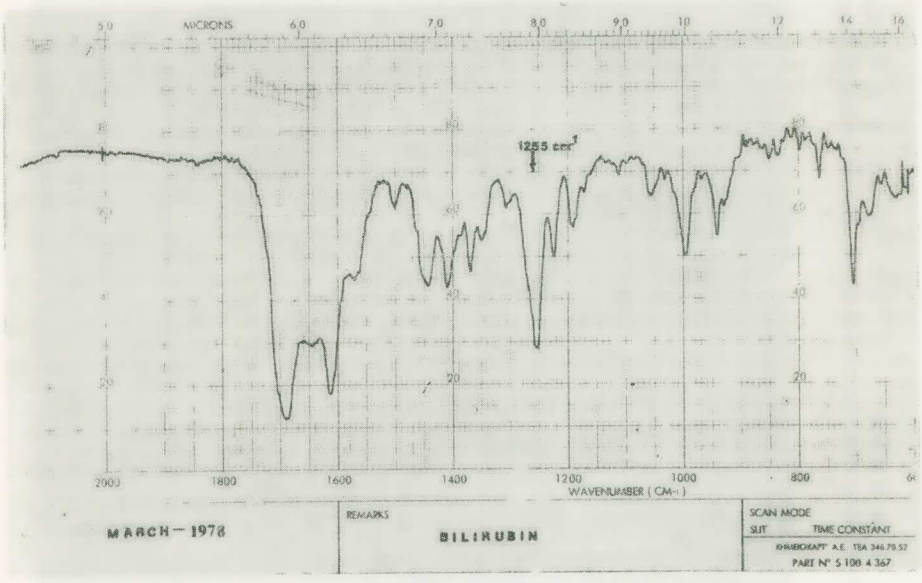
Then calibration curves were constructed for each pure substance, that is, bilirubin, cholesterol, calcium carbonate and calcium bilirubinate. Thus, spectra of these substances were obtained and in quantities 0.2, 0.4, 0.6 and 0.8 mg for each one of them. The values of log (P_o / P) were, then, calculated and these were plotted against the corresponding concentrations (mg of substance / 100 mg KBr) in a semilogarithmic paper as shown in Figs. 2, 3.

For comparison and possible shifting of bands due to interaction, the spectrum of a mixture of all four substances was also obtained. In this spectrum, it was observed that the bilirubin band at 1690 cm^{-1} shifts to 1660 cm^{-1} and a band interposed at 1625 cm^{-1} (see spectra of bilirubin and calcium bilirubinate). Repeated recordings with various combinations of substances showed, that the shifting and the presence of the band at 1625 cm^{-1} between the two bands of bilirubin at 1690 and 1615 cm^{-1} is due to the presence of calcium bilirubinate.

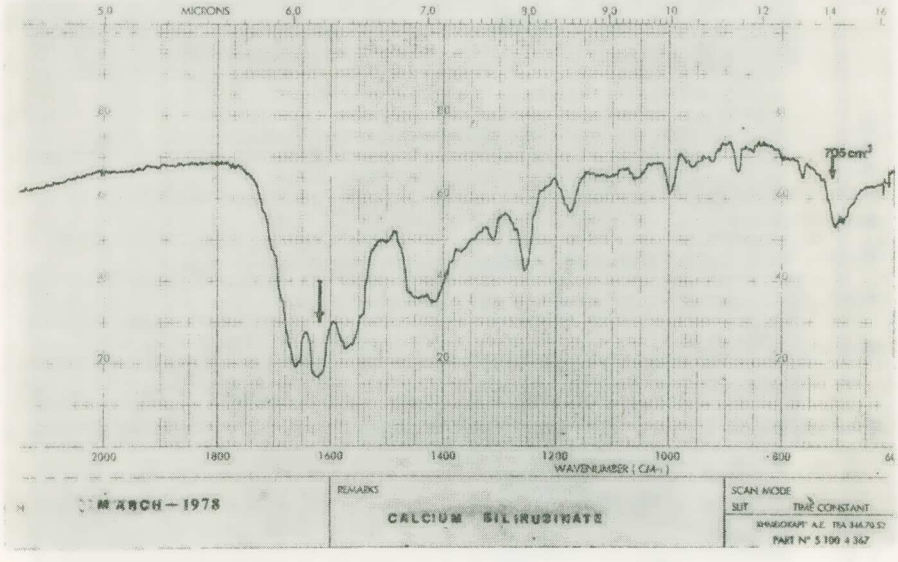
Finally, the following frequencies for the measurements were chosen :

Bilirubin	1255 cm^{-1}
Cholesterol	1055 cm^{-1}
Calcium carbonate	875 cm^{-1}
Calcium bilirubinate	705 cm^{-1}

The samples to be analyzed were obtained from patients, who had undergone cholocystectomy. These patients, prior to the operation, were studied with regards to the factors favoring cholelithiasis (gallstone

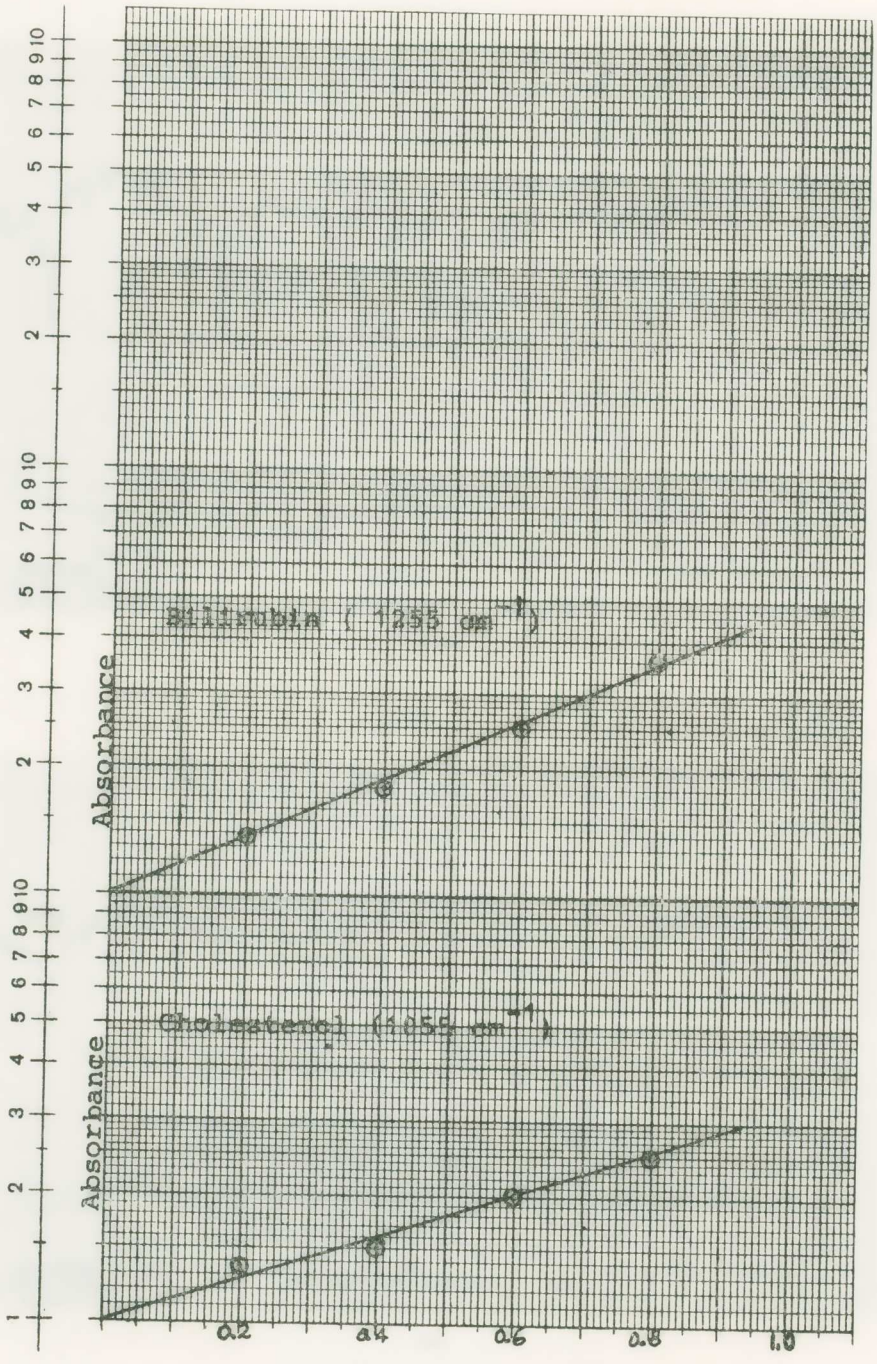


(a)



(b)

Fig. 1. Infrared spectra of :
(a) Bilirubin, (b) Calcium bilirubinate.



mg of substance / 100 mg KBr

Fig. 2. Calibration Curves.

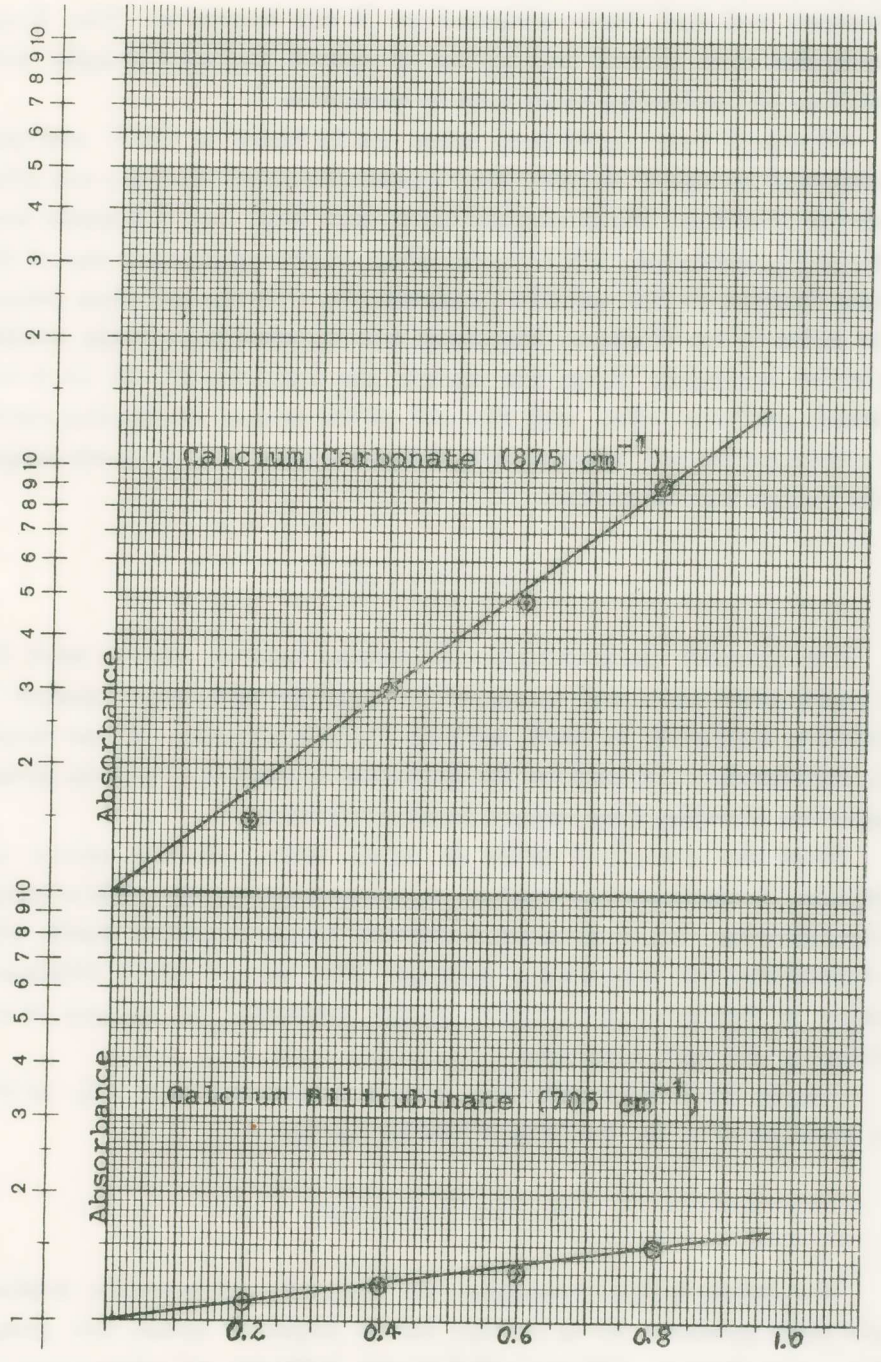


Fig. 3. Calibration Curves.

formation) and had been subjected to X-ray treatment. The X-ray examination was carried out either by simple cholecystography with biloptin or by cholangiography with Billigraphin.

All stones were dried in an oven for 24 hours at 110^0C and then powdered in an agate mortar. Part of the powdered samples was dried again for 24 hours. These samples were then kept in a desiccator over phosphorus pentoxide. For the recording of the spectrum 1 mg of the sample mixed with 100 mg KBr in an electric vibrator and then pressed into a pellet in an oil press. The pellet was weighed prior to the recording of the spectrum. From the spectra the ratio log (P_o / P) or A was obtained and from this, with the aid of the proper calibration curve, the concentration and, subsequently, the per cent of the substance sought in the sample was calculated.

DISCUSSION

The infrared spectroscopy, the authors believe, can be used for the rapid qualitative and quantitative analysis of a large number of gallstones, sufficient to yield reliable statistical results. These results may, subsequently, be used for the medicinal treatment of certain groups of patients, avoiding thus surgery (cholocystectomy).

From the analytical point of view, many possible errors are eliminated by the base-line method. All measurements are made at points of the spectrum which are sharply defined by the spectrum itself, with no dependence on wavelength settings. The use of ratios eliminates changes in instrument sensitivity, source intensity, or changes in adjustment of the optical system.

Finally the method described herein, is accurate to $\pm$ 5%, as this was established by the analysis of known samples.

ΠΕΡΙΛΗΨΙΣ

Ἡ παροῦσα ἐργασία περιγράφει τὴν ποιοτικὴν καὶ ποσοτικὴν ἀνάλυσιν ἑκατὸν (100) χολολίθων διὰ τὰ τέσσαρα βασικὰ συστατικὰ αὐτῶν, ἤτοι χολερυθρίνην, χολερυθρινικὸν ἀσβέστιον, ἀνθρακικὸν ἀσβέστιον καὶ χοληστερίνην, δι' ὑπερύθρου φασματοσκοπίας.

Ἡ περιγραφομένη μέθοδος συνδυάζει ὑπέρυθρον φασματοσκοπίαν, τὸν νόμον Beer-Lambert τὴν τεχνικὴν τῆς γραμμῆς-βάσεως καὶ προσφέρει μίαν ἁπλῆν διαδικασίαν διὰ τὴν ποιοτικὴν καὶ ποσοτικὴν ἀνάλυσιν χολολίθων. Ἡ διαλυτοποίησις τῶν δειγμάτων εἰς ὀργανικοὺς διαλύτας, ἀπαραίτητος εἰς τὴν δι' ὁρατῆς φασματοσκοπίας ἀνάλυσιν, ἢ ἡ ἀνακρυστάλλωσις διὰ τὴν μικροανάλυσιν ἢ κρυσταλλογραφίαν ἀποφεύγονται.

Ἡ ἀκρίβεια τῆς ἀναφερομένης μεθόδου, ὡς αὕτη προσδιωρίσθη διὰ τῆς ἀναλύσεως γνωστῶν δειγμάτων, εἶναι $\pm$ 5%.

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