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The determination of cholesterol, calcium carbonate and calcium oxalate in gallstones by thermogravimetry

K.S. Alexander [b], D. Dollimore [a], J.G. Dunn [a], X. Gao [a] and D. Patel [b]

[a] *Department of Chemistry, University of Toledo, Toledo, OH (USA)*
[b] *College of Pharmacy, University of Toledo, Toledo, OH (USA)*

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Abstract

It has been demonstrated that thermogravimetry can be used to determine various ratios of cholesterol, calcium carbonate and calcium oxalate in synthetic mixtures. The individual mass losses occur at sufficiently different temperatures for them to be unambiguously assigned to a specific decomposition reaction. The study has been extended to investigate the composition of some 20 natural gallstones, all of which contained cholesterol as the main component. Cholesterol was found to decompose in a single well defined step. Rising temperature experiments were conducted to ascertain the Arrhenius parameters. The kinetics of degradation was unaffected by the presence of the other constituents.

INTRODUCTION

The gall bladder is a distendable outpouching of the extrahepatic biliary duct system which stores, concentrates and discharges micellar bile into the duodenum during periods of digestion. Any prolonged imbalance between cholesterol, phospholipid and conjugated bile acid components can lead to gallstone formation. These calculi become significant when they are forced into the cystic duct by contractions induced by cholecystokinin release following a meal. Patients are not aware that they are building a stone collection until the first attack. In the case of prolonged obstruction, inflammation or acute cholecystitis ensues.

Three major types of calculi are found and include (a) mixed cholesterol stones, (b) cholesterol stones, and (c) pigment stones.

Two of these contain cholesterol as the predominant component. Mixed cholesterol stones are the most common, and contain at least 70% cholesterol. In addition they contain variable amounts of calcium salts, bilirubin, protein, bile salts and debris. Cholesterol stones contain

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Correspondence to: D. Dollimore, Department of Chemistry, University of Toledo, Toledo, OH 43606, USA.

90–100% cholesterol. Pigment stones are comprised of conjugated bilirubin, calcium salts and variable amounts of organic material, but contain no cholesterol.

Thermogravimetry (TG) has been used as a rapid method of determining the composition of stones as a means of identifying the stone type [1, 2]. Many of the components of a stone lose water or decompose, so that the mass loss can be used as a quantitative analytical method. Successful analysis can be performed on stones containing: calcium oxalate; mixtures of oxalates and phosphates; uric acid and ammonium urate; calcium oxalate and urate; cystine. Difficulties were encountered in the determination of $NH_4MgPO_4 \cdot 6H_2O$ because some $Ca(PO_4)_2$ is almost inevitably present, and may also contain $CaHPO_4 \cdot 2H_2O$. The last compound loses water in the same region as the Mg salt, and so high results are obtained. The $Ca(PO_4)_2$ does not decompose until 900°C and so the residue is heavier than would be expected for $NH_4MgPO_4 \cdot 6H_2O$ alone. Studies in the European Community have shown that calcium oxalate is the primary component [2–4], and even when it is not the primary material it is an important secondary one. Appreciable quantities of uric acid are also present.

In this paper we report a TG method that enables calcium carbonate, calcium oxalate and cholesterol to be determined in a single experiment. In addition, information on the kinetic properties of the degradation of cholesterol is presented.

EXPERIMENTAL

Materials

Cholesterol was supplied by Fisher Biotech, calcium oxalate by Baker Phillipsburg NJ and calcium carbonate by Fisher Scientific NJ. All samples were less than 99% pure and were in fine powder form, of particle size $<100\,\mu m$ and were used with no further treatment. Gallstones were obtained from local Toledo hospitals.

Synthetic samples were prepared by mixing together the three components. The cholesterol contents were 20, 40, 50, 70, 80 and 90%, with equal quantities of the other two components making up 100%. The gallstones were washed several times with deionized water to remove the formalin preservative. They were then air dried for a period of three days and ground to have a particle size of less than $100\,\mu m$.

Techniques

TG experiments were carried out using a Du Pont 1090 Thermogravimetric Analyzer. About 10 mg of the sample was weighed into a platinum crucible and a flowing nitrogen atmosphere established. A heating rate of 25°C min^{-1} was used to heat the sample from ambient to 1000°C.

RESULTS AND DISCUSSION

Analytical method

Figure 1 shows the TG/DTG curve of pure cholesterol in nitrogen. The decomposition occurs in a single step in the temperature range 210–370°C. For comparison the TG/DTG curves of calcium oxalate and calcium carbonate obtained under identical experimental conditions are shown in Figs. 2 and 3 respectively. The calcium oxalate monohydrate (Fig. 2) dehydrated at 120–200°C, and further decomposed to calcium carbonate in the range 390–520°C, and formed calcium oxide in the range 580–750°C. The last reaction occurred in a similar temperature range to that of the pure calcium carbonate (Fig. 3). It is evident that the individual reactions of the three components occur at sufficiently different temperatures to make an analytical determination of all three in a mixture possible.

Mixtures of the three compounds were made up in various ratios and their TG/DTG curves obtained. Two examples are given in Figs. 4 and 5. Figure 4 is for a sample containing 20% cholesterol and 40% of each of the other two compounds. The mass loss from just above 200 to 330°C was used to calculate the cholesterol composition, the mass loss from 400 to 520°C the oxalate composition and the mass loss from 580 to 770°C the total carbonate composition. Adjustment of the last value for the

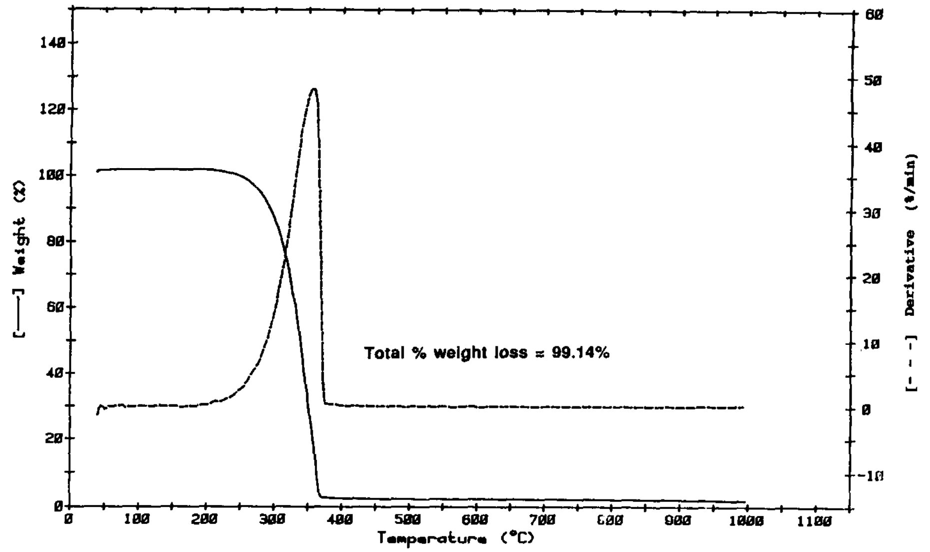

Fig. 1. TG curve of pure cholesterol.

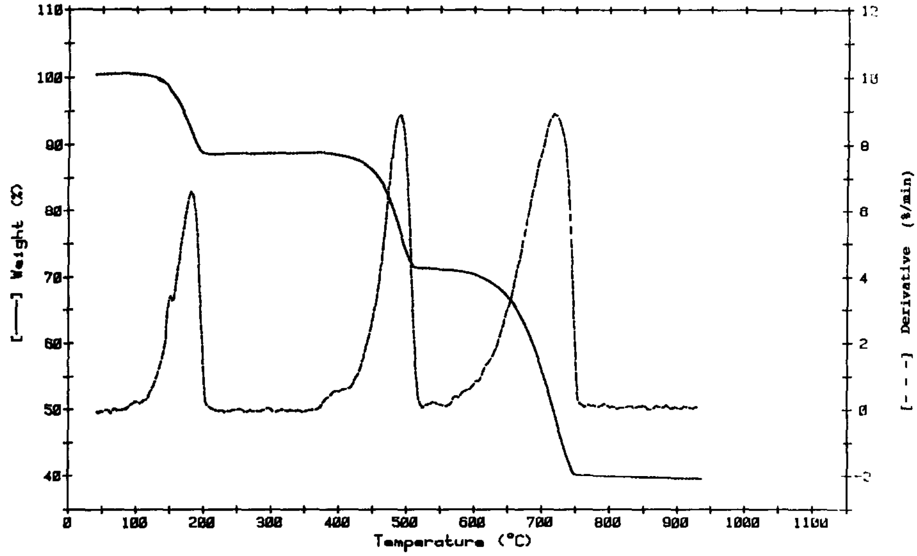

Fig. 2. TG curve of calcium oxalate.

carbonate formed from the oxalate decomposition permitted the calculation of the calcium carbonate content. Figure 5 shows the TG/DTG record for a mixture containing 90% cholesterol and 5% of each of the other two compounds. Although the cholesterol can be readily determined, the mass loss from the oxalate reaction is just visible. However, the calculation of the two minor components is quite accurate. The full set of

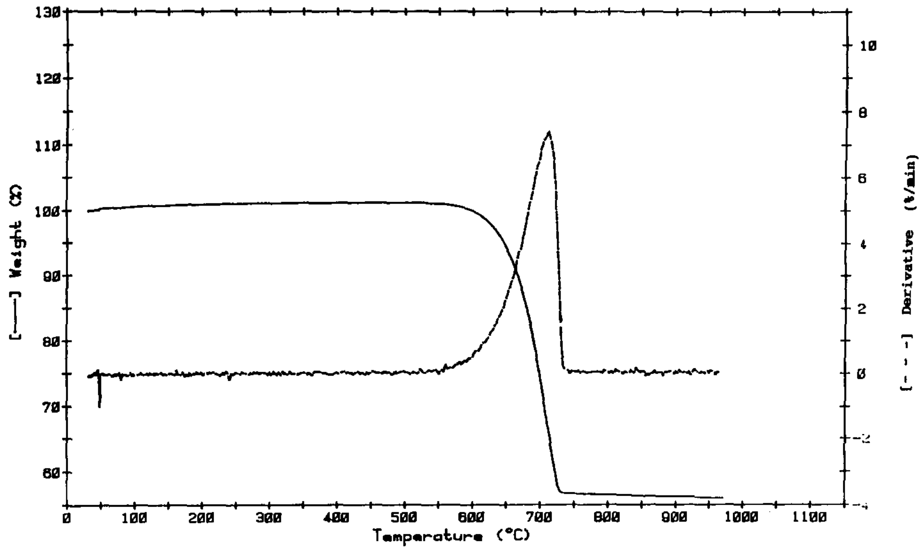

Fig. 3. TG curve of calcium carbonate.

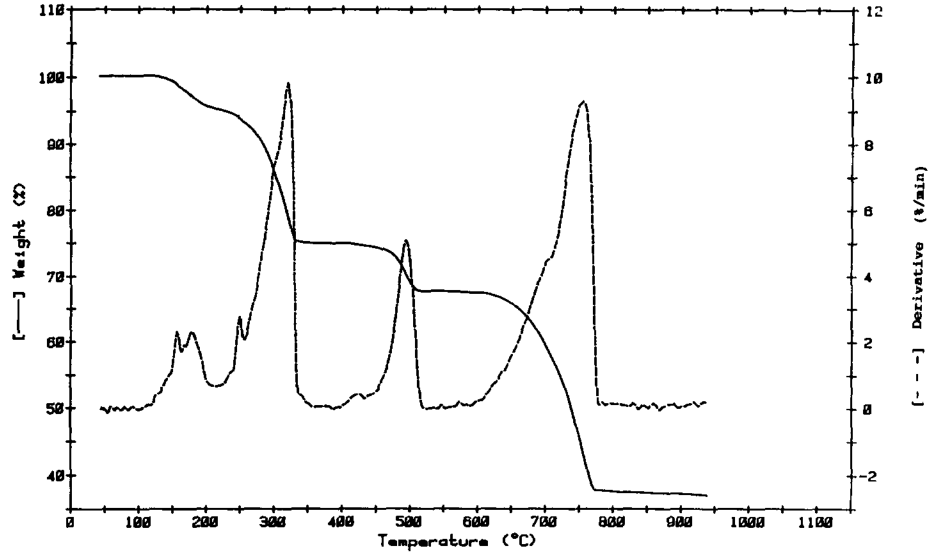

Fig. 4. TG curve of a mixture of cholesterol (20%), calcium oxalate (40%) and calcium carbonate (40%).

results for the mixtures and the values calculated from the TG curves is given in Table 1.

Some 20 gallstones were acquired from local hospitals and subjected to analysis by the TG method. A typical curve is shown in Fig. 6, and indicates that the only detectable compound present is cholesterol. There

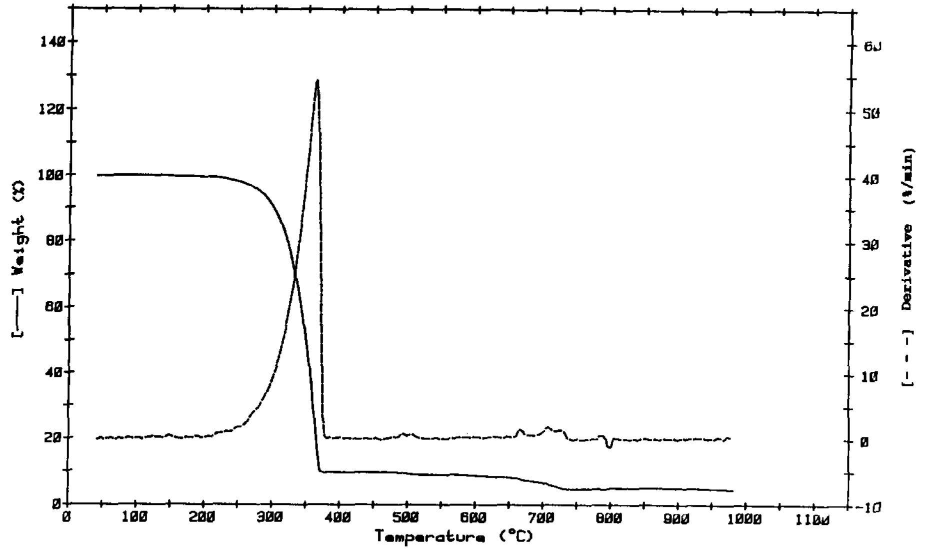

Fig. 5. TG curve of a mixture of cholesterol (90%), calcium oxalate (5%) and calcium carbonate (5%).

TABLE 1

Comparison between composition of mixtures (%) shown by TG curve and actual composition prepared in the laboratory

Sample no.	Composition of mixture prepared in the lab. (%)			Composition determined on TG curve (%)		
	Cholesterol	Calcium oxalate	Calcium carbonate	Cholesterol	Calcium oxalate	Calcium carbonate
1	20	40	40	19.35	40.01	39.65
2	40	30	30	39.85	29.99	30.01
3	50	25	25	49.46	24.98	25.01
4	70	15	15	69.92	14.89	14.92
5	80	10	10	79.77	9.91	9.98
6	90	5	5	89.88	4.98	5.01

was no evidence of either calcium oxalate or calcium carbonate. This was true for all the stones examined, and each contained at least 92% cholesterol. Hence the stones could all be classified as cholesterol stones. This is an interesting contrast to the European situation where the stones consisted primarily of oxalates [2–4].

Kinetic analysis

The TG data were used to determine the kinetic aspects of the pyrolytic decomposition of cholesterol. Both integral and differential methods were

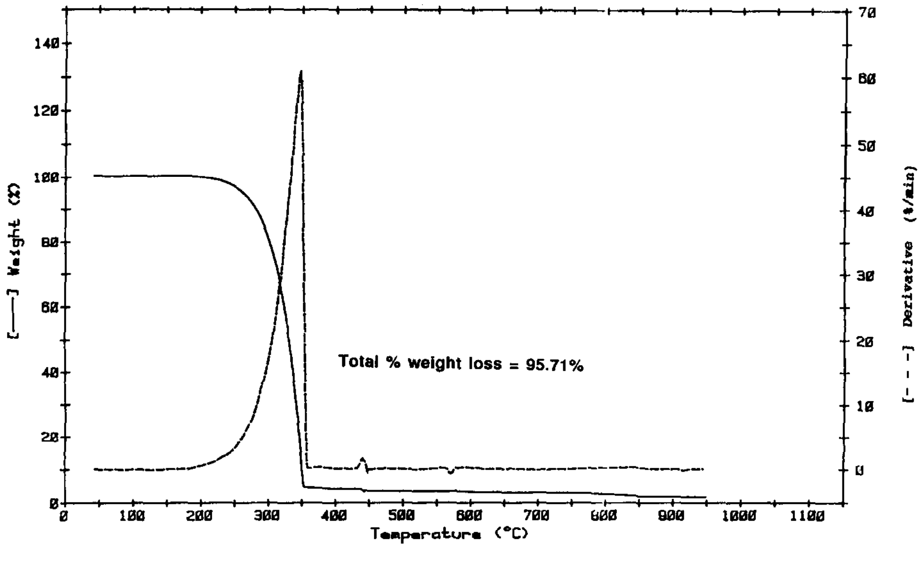

Fig. 6. TG curve of a typical stone from the Toledo region.

TABLE 2

Mechanism and Arrhenius parameters for pure cholesterol

Method	Mechanism function	E (kJ)	A ($\times 10^5$)	R
Integral	R2	95.7	4.14	0.997
	R3	97.7	4.36	0.997
Differential	R2	100.0	10.9	0.991
	R3	101.0	11.1	0.992

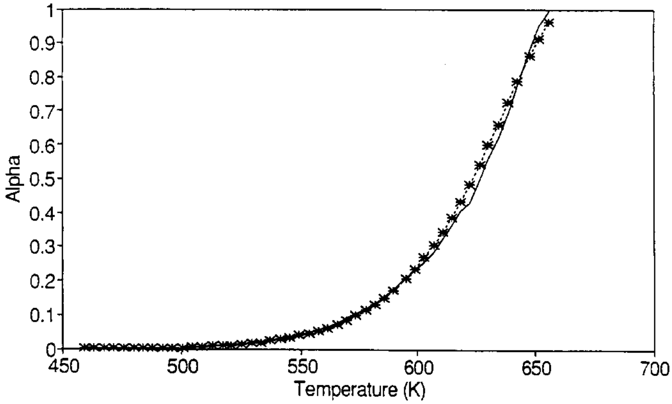

Fig. 7. Comparison of theoretical and experimental $\alpha - T$ curves for the R2 mechanism: (—), experimental curve; (*), theoretical curve.

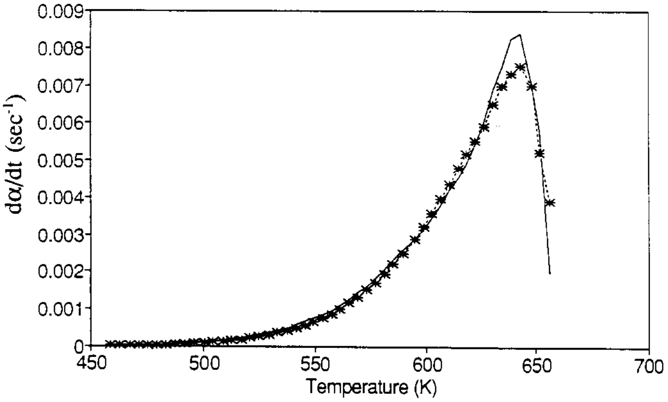

Fig. 8. Comparison of theoretical and experimental $(\mathrm{d}\alpha/\mathrm{d}t) - T$ curves for the R2 mechanism: (—), experimental curve; (*), theoretical curve.

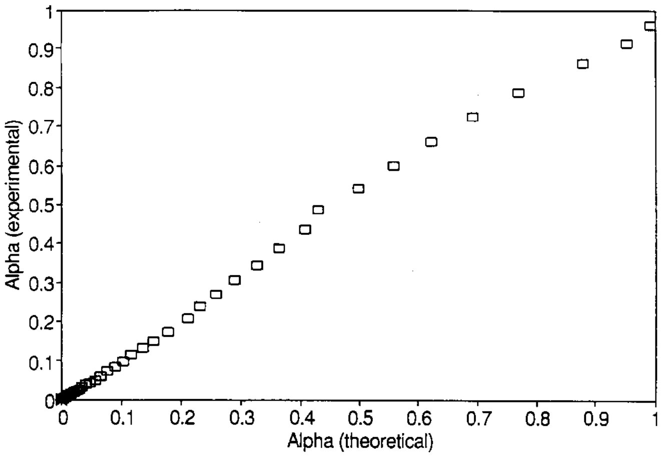

Fig. 9. Comparison of theoretical and experiment α curves for the R2 mechanism.

used. In the latter case, a different expression can be written in the form

$$\ln(\mathrm{d}\alpha/\mathrm{d}t)/f(\alpha) = \ln A - E/RT \quad (1)$$

Alternatively, an integral approach may be used in the approximate form [5]

$$\ln(G(\alpha)/T^{1.9215}) = \ln(AE/BR) + 3.77 - 1.92 \ln E - E/RT \quad (2)$$

where E = activation energy, B = heating rate, A = frequency factor, R = gas constant, α = fraction reacted at a specific temperature T, and $G(\alpha)$ and $f(\alpha)$ are the most probable mechanistic functions.

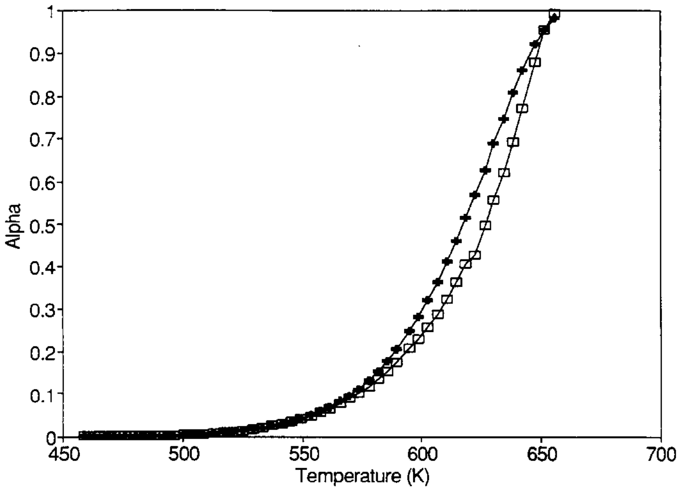

Fig. 10. Comparison of theoretical and experimental α–T curves for the R3 mechanism: (□), experimental results; (+), theoretical results.

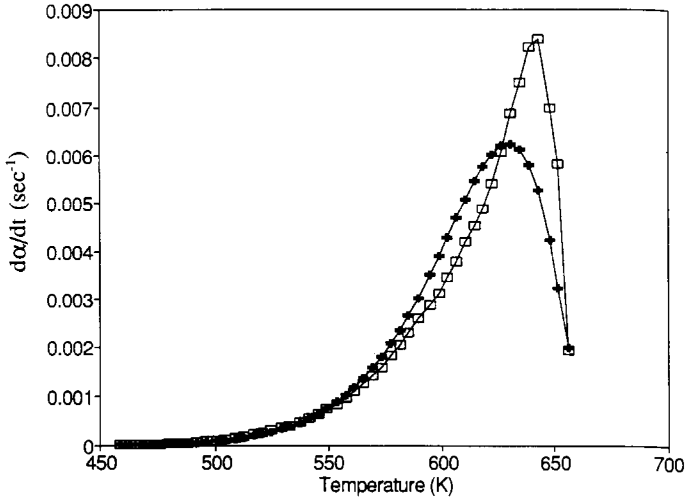

Fig. 11. Comparison of theoretical and experimental $(d\alpha/dt)-T$ curves for the R3 mechanism: (□), experimental results; (+), theoretical results.

To identify the most probable mechanistic functions for the decomposition of cholesterol, the TG data is tested against the usual forms of $G(\alpha)$ and $f(\alpha)$ and the one that gives the best fit between theoretical and experimental data is selected. This process indicated that mechanisms R2 (for a spherical particle the area of the surface decreases with progress of the reaction) or R3 (for a spherical particle the volume of the sphere contracts as the reaction progresses) appeared to be the most likely mechanisms. The computed values of A and E and the regression analysis for the R2 and R3 mechanisms are shown in Table 2. R is the correlation coefficient.

Reconstruction of the TG/DTF curves from the calculated values of A

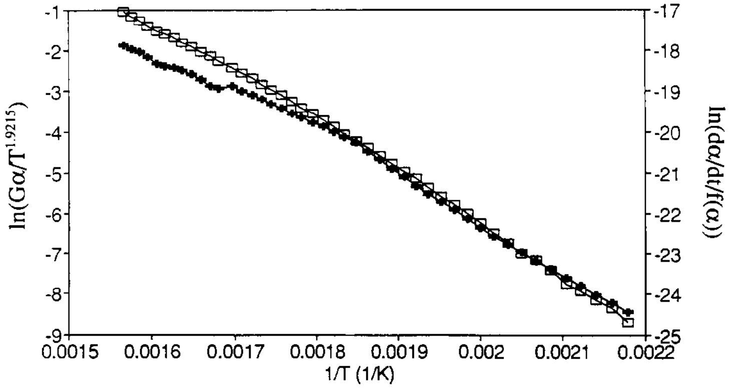

Fig. 12. Arrhenius plot for cholesterol: (□), results from the integral method; (+), results from the differential method.

TABLE 3

Mechanism and Arrhenius parameters for cholesterol gallstones

Kinetic method	Gallstone sample	R2, R3	E (kJ)	A ($\times 10^6$)	R
Integral method	1	R2	108.0	5.82	0.984
		R3	110.0	6.93	0.984
Diff. method	1	R2	103.0	2.24	0.983
		R3	108.0	4.61	0.973
Integral method	2	R2	105.0	5.19	0.991
		R3	102.0	6.21	0.993
Diff. method	2	R2	101.0	2.65	0.992
		R3	102.0	4.43	0.989
Integral method	3	R2	104.0	5.87	0.983
		R3	103.0	6.12	0.979
Diff. method	3	R2	101.0	2.56	0.998
		R3	100.0	5.63	0.987

and E for the R2 (Figs. 7–9) and R3 (Figs. 10 and 11) suggest that R2 exhibits the closest fit between theoretical and experimental data and is therefore chosen as the most likely mechanism.

Both the differential and integral methods gave reasonable results for E (see Table 2) although the values for A show some differences. An Arrhenius plot for the R2 mechanism for differential and integral equations is given in Fig. 12. The closeness of the fit is apparent, especially in the lower temperature range (200–280°C). Above the latter temperature the differential plot tends to deviate from a straight line. A similar analysis was carried out on the cholesterol stones, and the results are presented in Table 3. The values were close enough to those obtained on pure cholesterol to indicate that the same mechanism of degradation occurred. The average value for E was found to be 106 kJ mol^{-1} for an R2 mechanism by the integral method and 102 kJ mol^{-1} by the differential method. This compared with values of 96 and 100 kJ mol^{-1} for the pure cholesterol.

CONCLUSION

TG can be used as a rapid and accurate method for the determination of cholesterol, calcium oxalate and calcium carbonate in synthetic mixtures of the three compounds. Gallstones from the local community were found to be all classifiable as cholesterol stones. Cholesterol decomposed pyrolytically by a mechanism that can be best described as R2, with an activation energy in the range 96–108 kJ mol^{-1} and a frequency factor that lies in the range 4.14×10^5 to 5.87×10^6 s^{-1}.

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