THERMAL ANALYSIS OF ANHYDROUS AND HYDRATED CHOLESTEROL
Application to gallstones

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Abstract

Cholesterol constitutes the major component of most gallstones. It was identified and determined, in gallstones, issued from eleven patients, by thermal analysis: differential scanning calorimetry (DSC), with the use of the melting temperature and enthalpy, thermogravimetry (TG), with the mass loss of water. Anhydrous cholesterol (ChA) was characterized by two endothermic peaks (polymorphic, melting) and cholesterol monohydrate (ChH) by two endothermic peaks (dehydration, melting), too. ChA needle and ChH plate crystals were observed under polarizing light microscopy. The numerous stones obtained from nine patients were cholesterol stones: the ChA was higher 45 and lower 96%. ChH was present in stones of three patients.

Keywords: anhydrous cholesterol, DSC, DTA, gallstones, hydrated cholesterol, melting, TG

Introduction

Biliary ‘sludge’ represents an early stage of gallstone formation and gallstones are bile concretions [1]. The categorizing of gallstones into three kinds: cholesterol, brown pigments and black pigment stones, was proposed at the National Institute of Health International Gallstone Workshop at 1982 [2].

Mixed and cholesterol stones are the main gallstones in western countries, their average value in cholesterol is 86.3% (range 41.7 to 100 %), and are characteristically multifaceted, mulberry shaped or ovoid and, on fracture, have varying degrees of pigmented centers, layers or shells. Brown pigment stones have a 11.4% cholesterol content (range 2.8 to 28.3%); they crush easily and have dark and light lamination on cross section. Black pigment stones tend to be small, resist manual crushing and pro-
duce a shiny, glasslike surface on fracture. The average value in cholesterol is 0 to 9.7% [3]. A solitary stone is present in the 86.3% of cases [4].

Cholesterol monohydrate (ChH) constitutes the major component of most gallstones [5–8] and a major crystalline solid of many human atherosclerotic plaques [9]. Anhydrous cholesterol (ChA) can crystallize from model and native biles as filamentous crystals covered by a surface layer of lecithin molecules. During growth, filamentous crystals transformed via metastable intermediates into classical plate-like cholesterol monohydrate crystals [10]. The exact crystalline form of cholesterol which precipitates in the nucleus of forming and growing stone, is not known.

The main components (cholesterol, calcium bilirubinate, calcium carbonate) were characterized by infrared spectrometry and X-ray diffraction, in the 1950’s. The IR spectrum provides a fingerprint for identification and is an accurate test for the determination of molecular structure. FTIR analysis was useful in characterizing cholesterol, and brown and black pigment gallstones [11, 12]. The applications of FTIR in the study of other calculi are very important [13].

Thermogravimetry (TG) can be used as a rapid and accurate method for cholesterol determination, in mixture with calcium oxalate and calcium carbonate in gallstones [14]. But other organic compounds (like bilirubine) can be present in gallstones, and decomposed in the same interval. The monohydrate form of cholesterol was determined by water mass loss [15] and X-ray diffraction [7, 16, 17].

The polymorphic transition phase of ChA in gallstones was analyzed by differential scanning calorimetry (DSC) [18].

In this study the authors present results obtained by thermal analysis, first, on ChA and ChH, and then the level of both cholesterol determined in gallstones from eleven patients.

**Experimental**

**Samples**

ChA was obtained from Sigma – Aldrich Chimie and Prolabo. The solvents: ethyl alcohol and methyl alcohol were ACS grade reagents obtained from Carlo Erba.

ChA was recrystallized in dry methanol, it presented needle-like crystals. ChA recrystallized in the mixture ethanol–water (95–5), led to cholesterol hemiethanolate (Chheth) with plate-like crystals [16]. This sample was immersed in bidistilled water during 48 h, and then, ChH was isolated by filtration and washed repeatedly with bidistilled water. It was slightly and quickly dried under vacuum at room temperature. The wet monohydrate plate-like crystals were placed on a microbalance pan and periodically weighed. When constant mass was observed, it was assumed that all excess water had evaporated from crystal surfaces. The dried sample study was made by thermal analysis. Needlelike ChA and platelike ChH crystals were typified under polarizing light microscopy. Karl Fisher determination of water gave 4.34% for ChH (4.45% theoretical), 1.00% for Chheth and 0% for ChA, (with a standard deviation of 0.11%).

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