s8.m27.p14 **X-Ray diffraction study of gallstones.** [1,2]Nikitina E., [2]Kuz'micheva G., [1]Orlova S., [2]Efimova Yu., *[1]Department of Clinical Nutritiology of Russian University of Nation's Friendship, Moscow, Russia, [2]Department of Solid State Physics and Chemistry of Lomonosov State Academy of Fine Chemical Technology, Moscow, Russia. E-mail: nully@rol.ru*

Keywords: Gallstones; X-Ray study; Chemical analysis

Gallstone disease is very actual and important problem in medicine. The most widespread method of treatment is a radical laparoscopic cholecystectomy, but the perspective one is litholytic medical treatment. A study of phase and element composition, crystal structure, and physicomechanical properties of gallstones is necessary for a choice of the treatment and estimation of prognosis. Medical influence can reduce a dimensions or change a properties of gallstones and consequently facilitate stone disintegration process.

According to literature data cholesterol, bilirubin, bile and fatty acids and their calcium salts, and inorganic compounds (mainly calcium carbonates) are the general components of all gallstones. In accordance with the prevalence of cholesterol or bilirubin all stones divided into three groups: black pigment stones, brown pigment stones (rare in Europe) and yellow cholesterol stones. The ratio of cholesterol, phospholipids and bile acids determinates the process of lithogenesis in bile.

Of the 27 components of the gallstones, at least six types of cholesterol (cholesterol I, II, III, IV, V, VI) with various crystal structures, numerous variants of fatty and bile acids have been found and studied by scientists. However, there is not any reliable information about kinds of cholesterol and another compounds presented in gallstones.

The main purpose of our study was to systematize the data on the composition and the crystal structure of gall bladder stones and to study the composition of 20 gallstones removed operationally from residents of Moscow and its region. The phase composition and crystal structure of gallstones were examined by powder X-ray diffraction method (a single counter powder diffractometer HZG-4; CuK_α radiation; graphite monochromater). The element composition was determined by the standard chemical analysis. The study of gallstones was carried out from periphery to center ones.

First of all the interplanar spacings (d,A) together with the corresponding relative intensities (I,%) of all components of bile stones and their density were adopted from well-known experimental data or calculated. These results afford to identify of the composition of stones using X-ray diffraction method.

All examined bile stones are divided into the two groups: black bilirubin stones and many-coloured cholesterol stones. In turn, cholesterol stones are divided into two groups: monophase stones and multiphase ones. The first group of gallstones contained cholesterol I only. The second group of ones contained cholesterol I, an intermediate crystal form from cholesterol II to monohydrate cholesterol and possibly a small amount of deoxycholic acid II. It was found that the composition of the some analysed stones changes from periphery to center and besides, as a rule, the calcium concentration is higher in the center as compared with periphery. In all stones has been found the albumin.

For accuracy assay we analysed a commercial cholesterol test. The X-ray diffraction pattern of this sample revealed the presence of the main phase cholesterol I as well as three impurity phases: cholesterol II, VI, and a small amount of monohydrate cholesterol.

s8.m27.p15 **Characterisation of insulin microcrystals with x-ray powder diffraction.** Mathias Norrman and Gerd Schluckebier, *Insulin engineering, Novo Nordisk A/S, Denmark. E-mail: gesc@novonordisk.com*

Keywords: Powder diffraction; Insulin; Microcrystals

Insulin preparations with an extended action profile consist of microcrystalline suspensions that slowly dissolve and release the insulin into the blood stream. The action profiles are partly dependent on the crystal form and composition of crystals and soluble insulin. Intermediate acting insulin (NovoMix30) is a mixture of soluble- and crystallised insulin in the ratio of 30/70. The crystals consist of hexameric insulin in complex with two zinc atoms on the three-fold axis and two phenolic derivatives in the dimer-dimer interface and protamine; they crystallise in space group $P4_32_12$ with cell constants 62 62 86 90 90 90. Ultralente is another product with a long acting profile, consisting of 100% crystalline insulin suspension of rhombohedral crystals (cell constants 78 78 40 90 90 120), also in complex with two zinc atoms on the three-fold axis and one phenol derivative per insulin monomer. Careful chemical- and physical characterisation of the crystallinity of these suspensions is important, not only for regulatory and patent related reasons, but also due to the fact that different polymorphs may adversely affect the stability, bioavailability and the therapeutic properties of the insulin. The use of single crystal x-ray diffraction studies has been limited, due to the microcrystalline nature of the insulin suspensions. However, in this study, x-ray powder diffraction has been used to characterise and classify different polymorphs of insulin microcrystals. Powder data from insulin products were collected both in house, using a rotating anode generator (Cu-Kα radiation) with a Mar345 imaging plate, and at synchrotron (Maxlab, Lund, Sweden). The powder patterns could clearly be distinguished into separate crystal groups with high internal similarity. The powder rings were reduced to 2D diagram using the Datasqueeze software [1]. For further confirmation, powder patterns of some of the products were simulated from coordinate data using the Winprep software [2]. The result shows that the technique can be used to fingerprint crystallographic material and could be useful in validation and quality control of insulin microcrystals.

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