ZETA POTENTIAL AND MEMBRANOLYTIC EFFECTS OF CRYSTALLINE MONOSODIUM URATE MONOHYDRATE

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Monosodium urate monohydrate (MSUM) crystals produce the inflammatory reaction seen in acute gouty arthritis. The mechanism of crystal-induced inflammation includes a step where the crystals "bind" or interact with the membrane of the phagolysosome resulting in breaks in membrane continuity or membranolysis and cell death.

Previous studies of the inflammatory potential of MSUM routinely heated the crystals to 200° to destroy pyrogens, before injection. Dieppe et al (1981) believed that electrostatic forces may be important in the crystal-membrane interaction and showed that there was a correlation between electrophoretic mobility of crystals and the degree of inflammation. A comparison of unheated and heated crystals showed that the electrophoretic mobility and inflammation was reduced for heated MSUM. They stated that the lower surface charge of heated crystals was responsible for the reduced inflammation. This work investigates the effect of heating MSUM on the zeta potential of the crystals and their ability to cause hemolysis of erythrocytes which may be used as a measure of membranolytic potential.

MSUM was prepared by precipitation from a solution of uric acid in sodium hydroxide. Samples were heat treated at 200° for 2 hr and 250° for 2 hr and characterised using X-ray diffraction, differential scanning calorimetry (DSC) and scanning electron microscopy. The zeta potentials (Z.P.) of the crystals were measured in saturated solutions of MSUM in water and 0.4% sodium chloride in Tris buffer using a Zeta-Meter. Membranolysis experiments were carried out by tumbling 200 mg unheated and heated MSUM samples (mean surface area 0.47 $m^2.g^{-1}$) in suspensions of red blood cells in pH 7.4 Tris/isotonic saline buffer for 2 hr at 37°. After centrifugation the hemoglobin in the supernatants were analysed at 540 nm in a spectrophotometer. The percent hemolysis was determined relative to 100% lysis (red cells in water). When observed under the scanning electron microscope, ground MSUM had an acicular crystal habit. X-ray diffraction patterns and DSC confirmed that the crystals were MSUM. Heat treatment at 200° for 2 h produced samples containing 25% anhydrous MSU and heating at 250° for 2 h gave 100% anhydrous MSU. After membranolysis, rehydration of heated MSUM to 100% monohydrate was complete for the 200°/2 hr and incomplete for the 250°/2 hr treatments. Percent-hemolysis values and Z.P. of the samples are given in Table 1.

Table 1. Zeta Potential and percent hemolysis values for MSUM.

Sample	Percent-hemolysis Mean ± 1 S.D.	Zeta Potential (mV) Water	0.4% NaCl in Tris
Unheated	68.3 ± 8.7	-60.2	-22.7
200°, 2 h	40.9 ± 11.9	-47.3	-16.9
250°, 2 h	39.5 ± 11.9	-29.4	-10.7

The % hemolysis decreased from 68% for unheated MSUM to 40% for MSUM heated at 250° for 2 h. Unheated MSUM had a high negative surface charge as indicated by a Z.P. of -60mV in water. The Z.P. of the samples decreased with increasing concentration of NaCl due to the counterion shielding effect and quantitative measurements were not possible for MSUM in 0.9% NaCl in Tris buffer. There was a decrease in the Z.P. and % hemolysis values on heat treatment of the crystals.

If the structure of the crystal surface of MSUM is an important factor in membranolysis, then any treatment of the crystal which alters the surface or Z.P. should result in changes in membranolytic potential.

Dieppe, P.A. et al (1981) Arch. Rheum. 24: 975-976

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