AAEC/E311

AAEC/E311

AUSTRALIAN ATOMIC ENERGY COMMISSION

RESEARCH ESTABLISHMENT

LUCAS HEIGHTS

HAEMOLYTIC ACTIVITY OF URANIUM COMPOUNDS: HAEMOLYSIS BY THERMOCHEMICAL DERIVATIVES OF AMMONIUM URANATE

by

W. I. STUART

A. D. TUCKER

R. B. ADAMS

H. E. SMITH

January 1975

ISBN 0 642 99675 X

AUSTRALIAN ATOMIC ENERGY COMMISSION
RESEARCH ESTABLISHMENT
LUCAS HEIGHTS

HAEMOLYTIC ACTIVITY OF URANIUM COMPOUNDS: HAEMOLYSIS BY THERMOCHEMICAL DERIVATIVES OF AMMONIUM URANATE

by

W.I. STUART
R.B. ADAMS
Chemical Technology Division

A.D. TUCKER
H.E. SMITH
Occupational Health Section

ABSTRACT

A study has been made of the haemolytic action on human erythrocytes by ammonium uranate (AU) and various thermochemical products of AU. These products were obtained by heating AU in hydrogen at 5°C min^{-1} to various temperatures.

Haemolysis has been interpreted in terms of a diffusion model which for each product yields a single parameter K_n, the haemolytic activity factor. The magnitude of K_n is a convenient measure of the ability of a powder to damage erythrocytes.

The haemolytic activity of certain thermochemical derivatives indicates

an exceptionally high potential for damage to erythrocytes. Infrared and thermoanalytical measurements have shown that the high activity of these products derives principally from a self-reduction reaction, induced by heating AU to 400-420°C in hydrogen.

National Library of Australia card number and ISBN 0 642 99675 X

The following descriptors have been selected from the INIS Thesaurus to describe the subject content of this report for information retrieval purposes. For further details please refer to IAEA–INIS–12 (INIS: Manual for Indexing) and IAEA–INIS–13 (INIS: Thesaurus) published in Vienna by the International Atomic Energy Agency.

AMMONIUM COMPOUNDS; BIOLOGICAL RADIATION EFFECTS; CELL MEMBRANES; DECOMPOSITION; DIFFUSION; DOSE–RESPONSE RELATIONSHIPS; DUSTS; ERYTHROCYTES; HEMOLYSIS; INHALATION; RADIATION DOSES; RADIATION HAZARDS; TOXICITY; URANATES; URANIUM; URANIUM DIOXIDE

CONTENTS

1. INTRODUCTION

The work described in this report concerns cellular damage induced by certain uranium-bearing dusts. These materials were derived thermochemically by heating ammonium uranate (AU) in hydrogen, and *in vitro* effects of the dusts were investigated using human erythrocytes. The work is part of a wider field of study, within which we propose to examine toxic hazards associated with uranium compounds and selected industrial dusts.

In developing our examination of the AU system we considered the following matters:

(i) It is well recognised that a high risk of occupational lung disease exists near processes involving furnaces, smelting; welding, or thermochemical reactions such as decomposition, calcination or desorption. The high surface activity of dusts, frequently encountered in these circumstances, is undoubtedly an important factor in lung disease; but chemical properties of the particulate surface must also exercise important and specific effects in determining the nature and course of disease.

(ii) Within the nuclear energy industry, uranium dioxide for nuclear fuel is commonly produced by heating AU in hydrogen, through an intricate series of thermochemical reactions which includes dehydration, decomposition and reduction (Price 1971, Price & Stuart 1973). Complex variations in surface activity, particle morphology and chemical behaviour accompany these reactions. It is important therefore to assess the biological activity of the various intermediate materials formed during production of UO_2, and to estimate the toxic hazards associated with these materials.

(iii) Haemolysis of erythrocytes by mineral dusts provides a useful method of quantifying damage to biological membranes. Also, the degree of haemolysis induced by certain minerals can be related to their fibrogenic properties (Stalder & Stöber 1965, Nash, Allison & Harrington 1966), whereas stishovite (the one form of silica which is not fibrogenic) and non-toxic dusts such as corundum and diamond (Nash, Allison & Harrington 1966) show little haemolytic activity.

2. EXPERIMENTAL

2.1 Materials

The ammonium uranate used for haemolysis experiments was obtained as a sample of material prepared on a pilot-plant scale by the AAEC RE's Chemical Engineering Section. The material was precipitated from uranyl nitrate solution and then tray-dried at 80° C. Chemical analysis and i.r. spectrophotometry showed that the dried precipitate was chemically non-uniform.

Chemical homogeneity was achieved by thoroughly grinding the AU in an ethanol slurry. Analysis of the final homogenised powder gave the molar ratios NH_4:U = 0.47 and NO_3:U = 0.05.

2.2 Thermal Analysis

Thermogravimetric (TG) measurements were made with either a Cahn RG Electrobalance and a Cahn RH Electrobalance equipped with a data logging attachment. Differential thermogravimetric (DTG) curves were obtained by analysis of TG data with the aid of a NOVA digital computer. The differential thermal analysis (DTA) apparatus was as used by Price (1971).

2.3 Infrared Spectrophotometry

Infrared spectra were recorded on a Perkin-Elmer Spectrophotometer type 225, with samples in the form of thin films mounted on KRS-5 plates.

2.4 Measurement of Haemolysis

Blood samples were obtained in the course of routine medical examinations. The following standard laboratory estimations were made, using EDTA as anti-coagulant :

(a) Haemoglobin content.
(b) Red cell count.
(c) White cell count.
(d) Erythrocyte sedimentation rate (ESR) at 1 and 2 hour(s).
(e) Haematocrit (packed cell volume).
(f) Platelet count.
(g) Differential white cell count.
(h) Blood urea.
(i) Cholesterol level of serum from clotted blood.

The measurements were carried out using the methods (h)-(i) described by Varley (1969) and the standard haematological procedures (a)-(g).

Haemoglobin content was estimated by the cyanohaemoglobin method using a commercial preparation (ACULITE) of Drabkin's solution as haemolysing agent. Haemoglobin concentration was measured photometrically at 540 nm on a Bausch and Lomb Spectronic 20 photometer. The accuracy of the method was checked at three-monthly intervals against standard haemoglobin samples obtained from the Red Cross Blood Transfusion Service of NSW.

For haemolysis experiments, blood was collected using EDTA as anticoagulant. The cells were washed three times with isotonic saline (0.15 mol NaCl ℓ^{-1}) and then suspended in saline to give a 2 per cent by volume suspension. Two mℓ of the well-stirred suspension was added to a sample of dust, and the mixture was rotated on a Mathum mixer at 37°C for 1 h. The mixture was then

centrifuged. One mℓ of supernatant was diluted to 5 mℓ with isotonic saline. The haemoglobin concentration of the supernatant was then measured photometrically at 540 μm.

3. RESULTS AND DISCUSSION

3.1 Nature and Thermal Analysis of Ammonium Uranate

Before describing results of haemolysis experiments we first discuss briefly the nature and thermochemical decomposition of ammonium uranate.

3.1.1 Composition of ammonium uranate

AU is not a well-defined stoichiometric compound; its composition varies according to the conditions of preparation. Thermoanalytical, X-ray and infrared evidence (Stuart & Whateley 1969) indicates the AU system to be non-stoichiometric and represented by the formula

$$UO_2\ (OH)_{2-x}\ (ONH_4)_x \cdot yH_2O \quad ,$$

where x varies continuously up to the limit x = ~0.7.

AU products used for manufacture of UO_2 are usually obtained by precipitation from uranyl nitrate solution. The precipitates contain occluded nitrate as a major impurity and compositions generally fall within limits corresponding approximately to x = 0.3 and x = 0.5.

3.1.2 Thermochemical decomposition of AU

Thermal analysis of nitrate-free AU Thermochemical decomposition of AU in various atmospheres has been studied in some detail using thermal analysis supported by i.r. spectrophotometry and gas analysis (Price 1971, Price & Stuart 1973). Figure 1 shows typical thermoanalytical data, summarising the various stages of reaction encountered when nitrate-free AU is heated in hydrogen. There are five overlapping stages each involving a loss in weight; these are listed in Table 1 together with differential peak temperatures and the temperature range over which each occurs.

TABLE 1

AU HEATED IN HYDROGEN AT 5°C min^{-1}

Stage	Differential Peak Temperature (°C)	Temperature Range (°C)	Remarks
I	80	20-120	Endothermal
II	160	120-200	"
III	275	200-350	"
IV	400	350-450	Exothermal
V	510	450-520	"

Chemical reactions that occur within the various stages are as follows :

Stages I and II: dehydration and dehydroxylation represented by :

$$UO_2(OH)_{2-x}(ONH_4)_x.yH_2O(c) \rightarrow UO_2(OH)_{2-x-2z}.O.(ONH_4)_x(c) + (z+y)H_2O\ (g) \quad \ldots (1)$$

Stage III: dehydroxylation and ammine formation represented, for example, by the following group reactions:

$$-OH + -ONH_4 = -O- + H_2O(g) + NH_3(g) \quad \ldots (2)$$

$$-OH + -ONH_4 = -O- + H_2O(g) + H_3N \rightarrow \quad \ldots (3)$$

The final product obtained in Stage III is an ammine $UO_3.yNH_3$ where y is usually 0.2 - 0.25 if nitrate impurities are not present.

Stage IV: self-reduction (*i.e.* ammine decomposition), given formally by the equation:

$$3(UO_3.0.22NH_3) = U_3O_8 + 1/3N_2(g) + H_2O(g) \quad \ldots (4)$$

Stage V: reduction of U_3O_8 by hydrogen gas to form UO_2:

$$U_3O_8 + 2H_2(g) \rightarrow 3UO_2 + 2H_2O(g) \quad \ldots (5)$$

Effect of nitrate impurities on thermochemical decomposition of AU

The presence of nitrate impurities markedly affects the self-reduction Stage IV. This is clearly illustrated in Figure 2 which shows DTA traces for samples of AU heated in argon. With increasing NO_3^- content, the self-reduction exotherm at 400°C diminishes. An endothermal peak at 510-570°C then becomes apparent, arising from the reaction

$$3\ UO_3(c) = U_3O_8(c) + \tfrac{1}{2}\ O_2(g)$$

and indicating that self-reduction is incomplete. In addition, NO_3^- impurities initiate an exothermal process between 300°C and 400°C, which we attribute to oxidation of some ammoniate $H_3N \rightarrow$ groups by occluded NO_3^-.

3.2 Haemolytic Activity of AU and Decomposition Products

The DTG curve in Figure 3(a) illustrates the various thermochemical stages for the particular AU used in haemolysis experiments. Because of the level of NO_3^- impurities, self-reduction (Stage IV) is incomplete and an additional decomposition reaction (Stage IVA) represented by the differential

peak at 490°C can be observed. This is due to the reduction of residual UO_3 by hydrogen. Figure 3(b) shows variations in specific surface that accompany these reactions. These data indicate precisely the conditions in which the various powders were prepared for examination of haemolytic activity.

In Figure 4 the percentage haemolysis (P) is plotted as a function of dosage (D) (pg per cell) for AU heated to 465°C in a 3 vol. per cent H_2-N_2 mixture. Figure 5 shows similar plots of P *vs* D for AU heated to different temperatures (for clarity, individual points are not included in Figure 5).

In general, with increasing dosage, P rises to a maximum of about 60 per cent and then decreases with still higher doses. The diminishing value of P at higher dosage is paralleled by increasing cellular flocculation, clumping and spherocytosis. Clearly, cellular damage by these dusts is a complex affair of which haemolysis is only one consequence.

On heating AU through Stages I and II up to about 260°C, the haemolysis curve does not alter. This is as one might expect: only H_2O vapour is released in these stages; reabsorption of H_2O on subsequent exposure to air gives a product chemically indistinguishable from the original AU; and, as shown in Figure 3(b), specific surface remains unchanged.

Further heating to temperatures above 260°C induces substantial and complex alterations in haemolytic activity. To illustrate the effect of reaction temperature on haemolysis, it is convenient to analyse the initial part of the haemolysis curve (represented by increasing values of P with increasing dosage, where we assume P to be a predominant measure of cellular damage). We then adopt a diffusion model relating degree of haemolysis to cellular membrane damage, and assume the following:

(i) Cellular damage occurs rapidly and is complete after a time which is very much less than the incubation time t.

(ii) After exposure to dust, the cellular membrane remains as a coherent diffusion barrier with enhanced permeability.

(iii) Haemolysis observed at time t arises as a consequence of Fickian diffusion of haemoglobin through the cell membrane.

We define the following quantities:

A = total membrane area in a sample,

V_s = volume of saline solution,

N_c = total number of cells present,

V_c = volume of a cell,

C = concentration of haemoglobin in cell,

C_1 = concentration of haemoglobin in saline,

for $t = o$, $C = C_o$, $C_l = 0$,

for $t = t$, $C = C_c$, $C_l = C_s$,

j = flux density of haemoglobin, and

a = permeability constant.

The following relationships are then valid:

$$j = -a\,(C_c - C_s) \qquad \ldots (1)$$

$$j = \frac{-N_c V_c}{A}\frac{dC_c}{dt} = \frac{V_s}{A}\frac{dC_s}{dt} \qquad \ldots (2)$$

$$\frac{d(C_c - C_s)}{dt} = -a\left(\frac{A}{N_c V_c} + \frac{A}{V_s}\right)(C_c - C_s) \qquad \ldots (3)$$

Integration of (3) then gives

$$\frac{C_c - C_s}{C_o} = \exp\left[-a\left(\frac{A}{N_c V_c} + \frac{A}{V_s}\right)t\right] \qquad \ldots (4)$$

and since $V_s >> N_c V_c$,

$$\frac{C_c - C_s}{C_o} = \frac{C_g}{C_o} \approx \exp\frac{-a\,A\,t}{N_c\,V_c}$$

and

$$\ell n\,\frac{C_o}{C_g} \approx \frac{a\,A\,t}{N_c\,V_c} \qquad \ldots (5)$$

Figure 6 shows how the parameter $\ell n\,(C_o/C_g)$ varies with dosage, as given by the empirical expression

$$\frac{1}{t}\,\ell n\,\frac{C_o}{C_g} = K_h\,(D)^{3/2} \ . \qquad \ldots (6)$$

where t = 1 hour.

The empirical factor K_h then provides a characteristic measure of the haemolytic activity exhibited by a particular powder. We define K_h as the *haemolytic activity factor*.

Figure 7 demonstrates the variation of K_h with temperature for AU heated between 25°C and 630°C. A substantial increase in haemolytic activity is induced by heating AU through Stage III between 260°C and 400°C. No change in specific surface occurs in this temperature range, so that enhanced

haemolysis is due entirely to chemical changes in AU arising from release of NH_3.

On heating AU to temperatures above 400°C, K_h increases still further to a maximum value at about 420°C, and between 420°C and 620°C, K_h diminishes by about two orders of magnitude. The maximum value of K_h at 420°C coincides with the differential peak corresponding to self-reduction Stage IV (Figure 3a); it does not coincide with the maximum in specific surface (Figure 3b). Thus chemical change occurring through self-reduction is a most important factor in determining haemolytic activity, although the increase in specific surface above 400°C must also contribute to increased haemolysis.

3.3 Haemolytic Activity of Some Mineral Dusts - Comparison with Activity of AU Derivatives

A further series of haemolysis experiments was carried out using various minerals, the toxic properties of which are well known.

Figure 8 shows plots of percentage haemolysis *vs* dose (mg dust) for samples of γ-alumina (BET surface area 180 m^2g^{-1}), CABOSIL grade silica (BET surface area 120 m^2g^{-1}) and chrysotile asbestos. These results support the commonly-held view propounded for example by Allison (1971) that the capacity of a mineral dust to react with membranes, as shown by haemolysis, is related to its toxic properties:

- The fibrogenicity of silica is well established;
- inhalation of asbestos induces serious biological effects such as asbestosis and lung cancer; and
- γ-alumina is considered to be much less harmful, exhibiting only slight haemolytic activity.

It can be seen (Figures 5 & 8) that the biological activity (as indicated by percentage haemolysis at low doses) of certain thermochemical derivatives of AU is substantially greater than that of both chrysotile and CABOSIL silica.

4. CONCLUSIONS

(i) Membrane damage by uranium dusts is a complex process, and measurement of percentage haemolysis for a single dose of dust can give an inaccurate and often misleading impression of haemolytic activity and potential toxicity.

(ii) By measuring haemolysis as a function of dose, and by interpreting haemolysis data in terms of a diffusion model, a reproducible semi-empirical measure of membrane damage for low dosage is attained.

(iii) We have compared haemolytic activity of AU and its thermochemical derivatives with the activity of various other minerals. In our opinion,

these results provide circumstantial evidence that the inhalation of certain dusts, derived by heating AU in hydrogen, may represent a serious occupational hazard in terms of chemical reaction within lung tissue. This conclusion refers particularly to products of the self-reduction reaction.

5. REFERENCES

Allison, T.C. (1971) - Inhaled Particles, vol. 3 (ed W.H. Walton) Unwin, Surrey, p.437.

Nash, T., Allison, A.C. & Harrington, J.S. (1966) - Nature, 210 : 259.

Price, G.H. (1971) - J. Inorg. Nucl. Chem., 33 : 4085.

Price, G.H. & Stuart, W.I. (1973) - AAEC/E276.

Stalder, K. & Stober, W. (1965) - Nature, 207 : 874.

Stuart, W.I. & Whateley, T.L. (1969) - J. Inorg. Nucl. Chem., 31 : 1639.

Varley, H. (1969) - Practical Clinical Biochemistry, Interscience Books, New York.

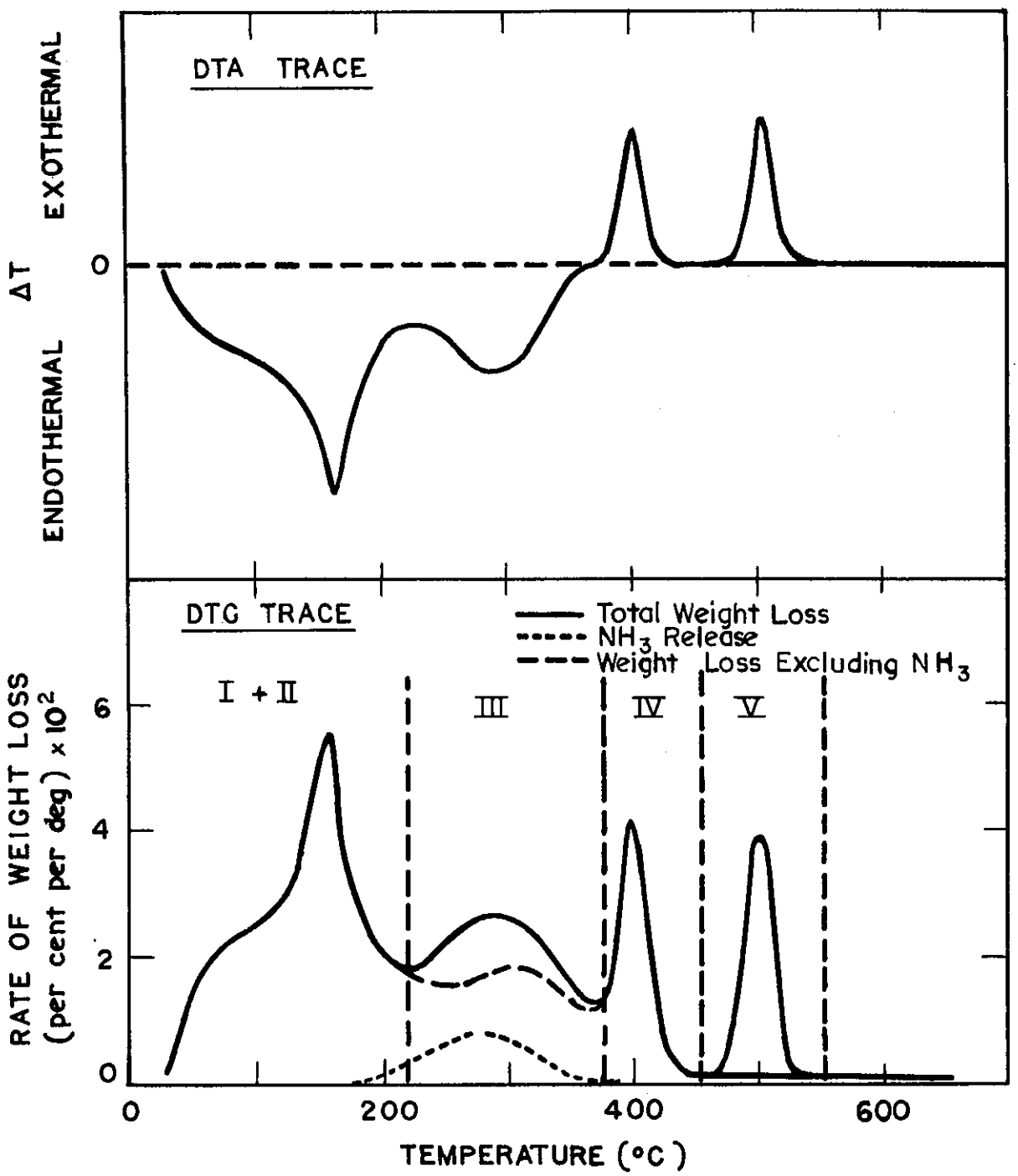

FIGURE 1. THERMOANALYTICAL DATA FOR AMMONIUM URANATE (NH_4^+: U = 0.4) HEATED IN HYDROGEN AT 5°C min^{-1}

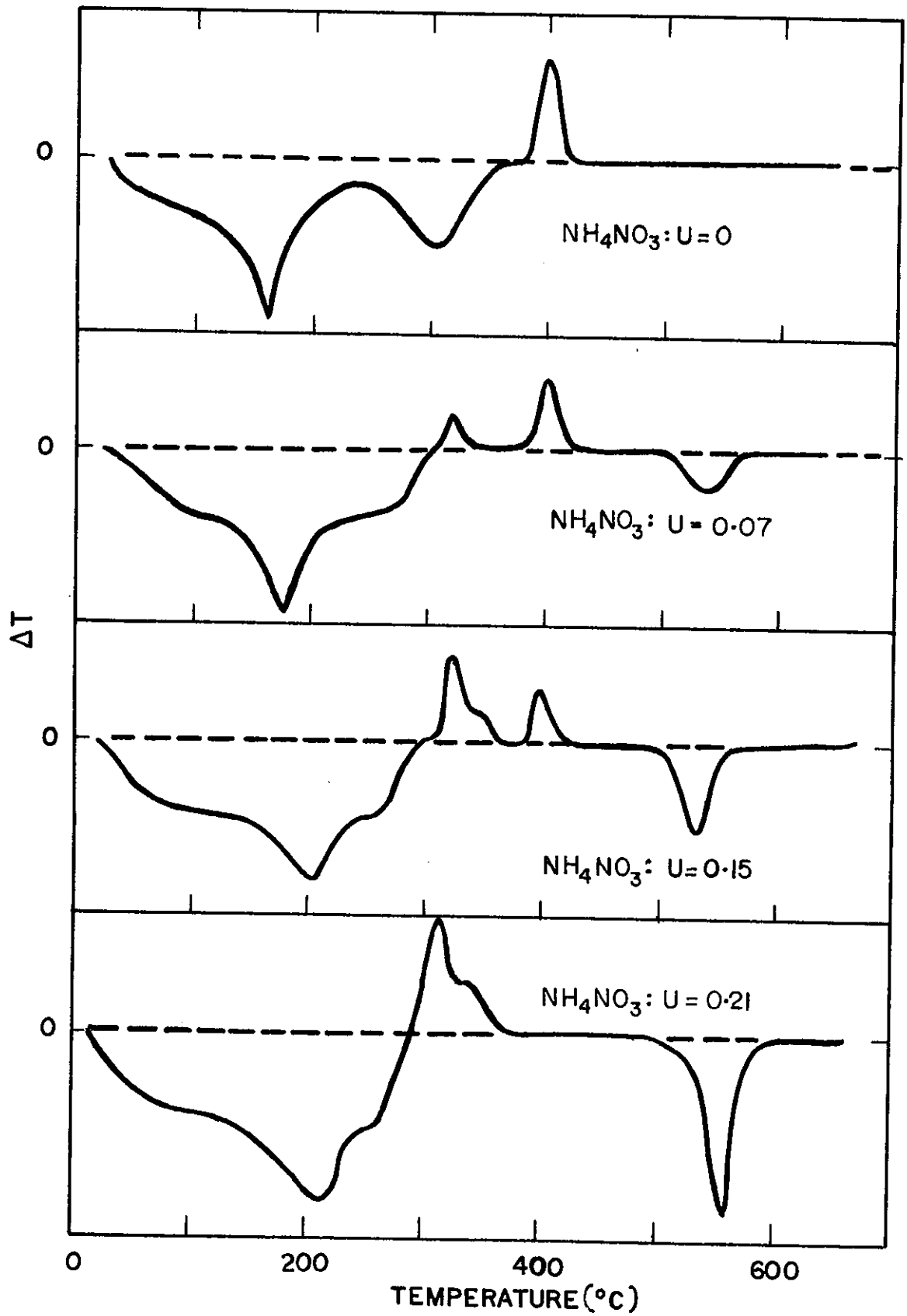

FIGURE 2. DTA FOR AMMONIUM URANATE (NH_4^+: U = 0.4) HEATED IN ARGON AT 5 °C min^{-1}: EFFECT OF NH_4NO_3 IMPURITY

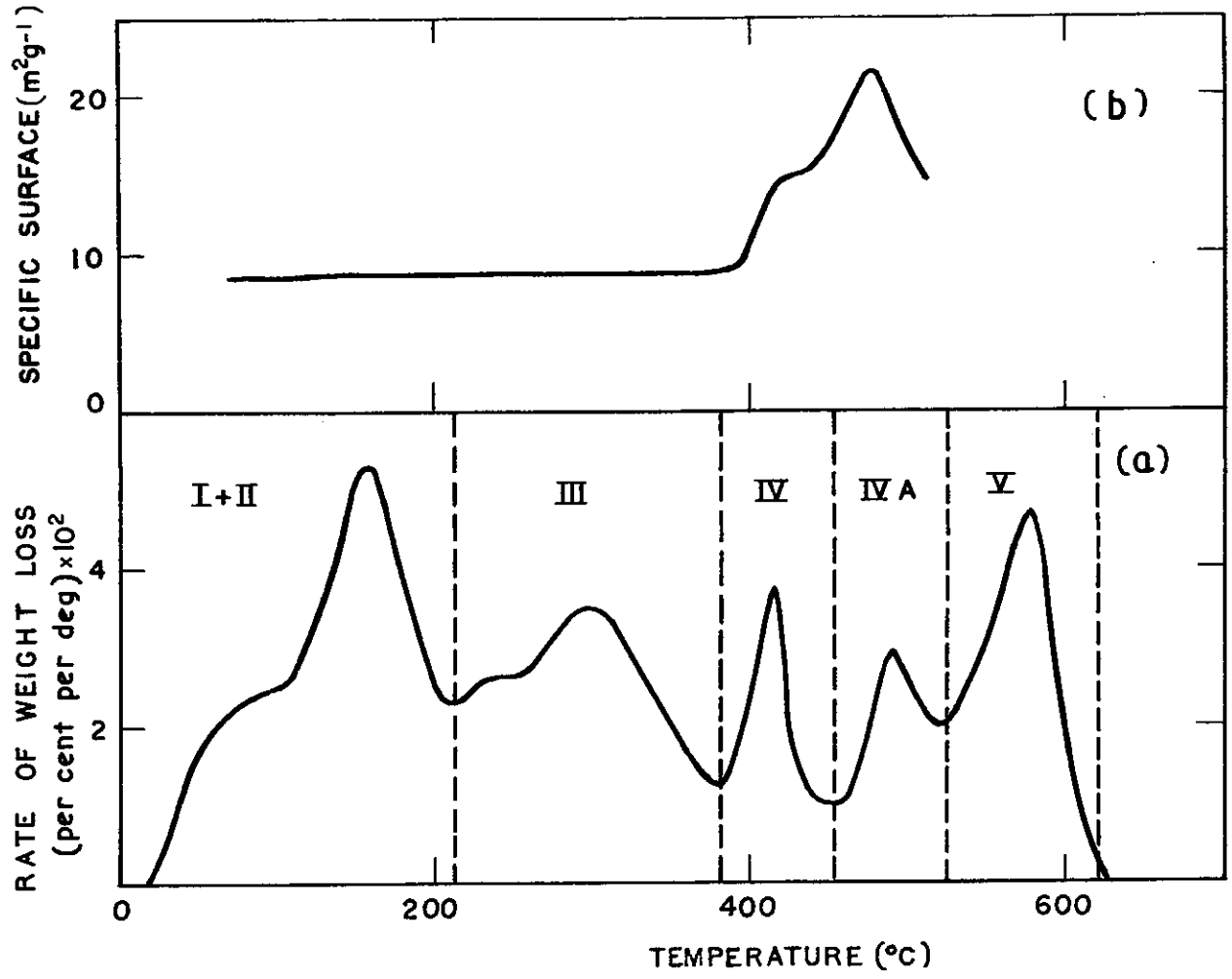

FIGURE 3 (a) DTG AND (b) SURFACE AREA VARIATION FOR AMMONIUM URANATE (NH_4 : U = 0.4, NO_3 : U = 0.07) HEATED IN 3 VOL. PER CENT H_2-N_2 AT 5 °C min

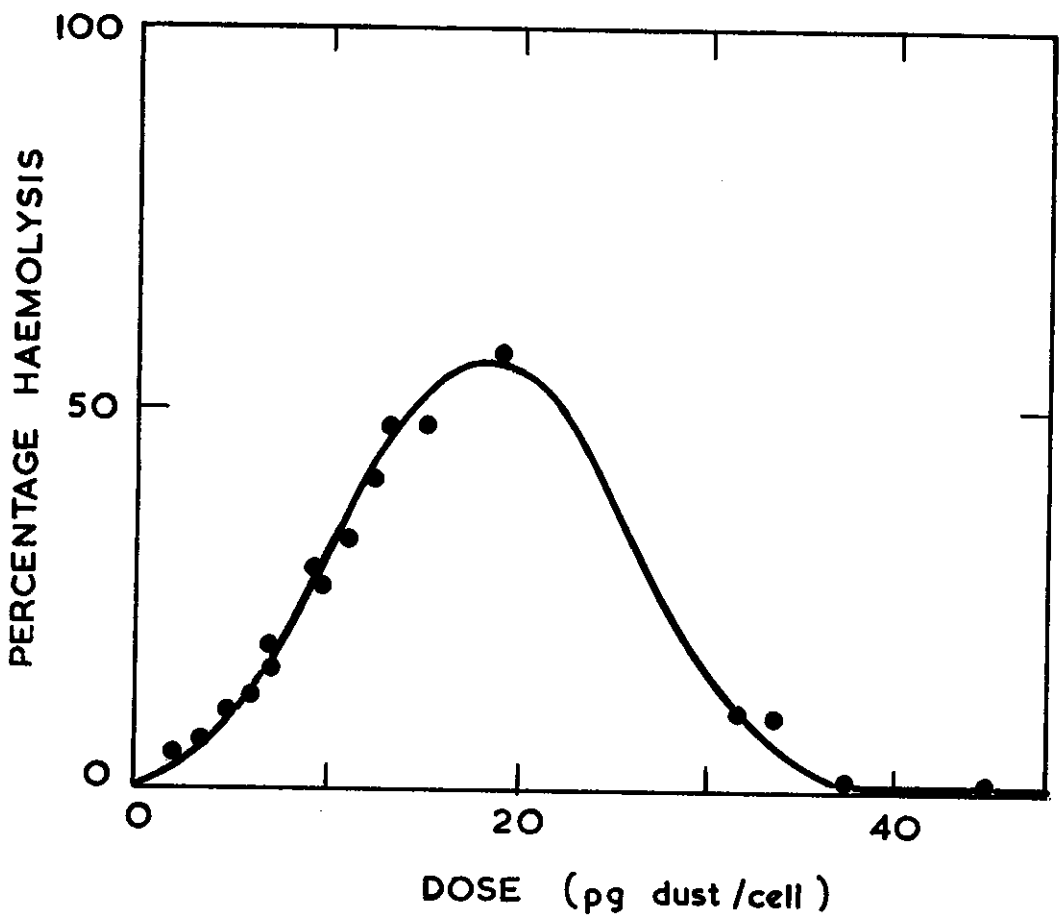

FIGURE 4. HAEMOLYSIS BY POWDER DERIVED BY HEATING AU IN 3 VOL. PER CENT H_2-N_2 TO 465°C AT 5°C min^{-1}

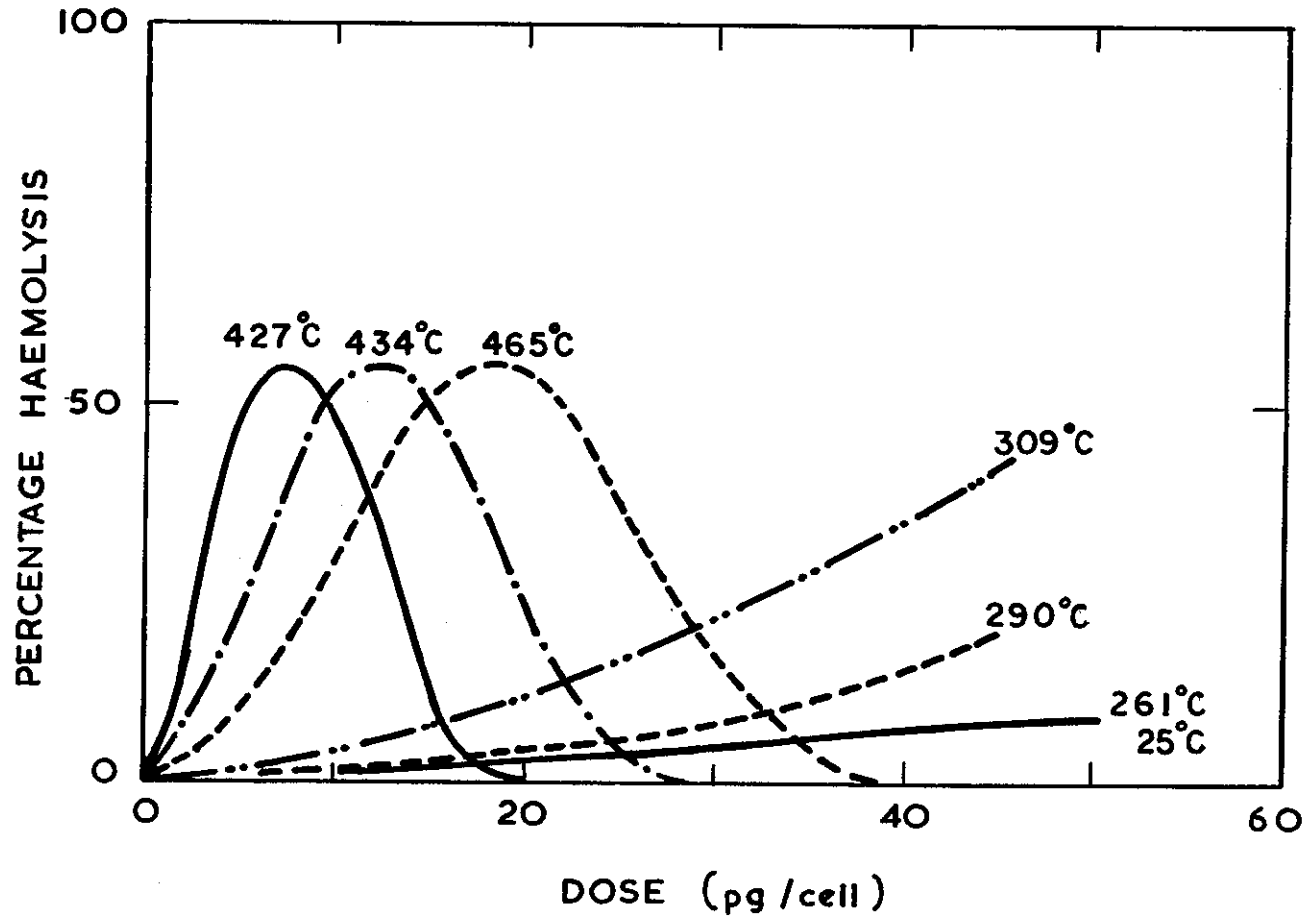

FIGURE 5. HAEMOLYTIC ACTIVITY OF POWDERS DERIVED BY HEATING AMMONIUM URANATE IN 3 VOL. PER CENT H_2-N_2 AT 5 °C min^{-1} TO VARIOUS TEMPERATURES

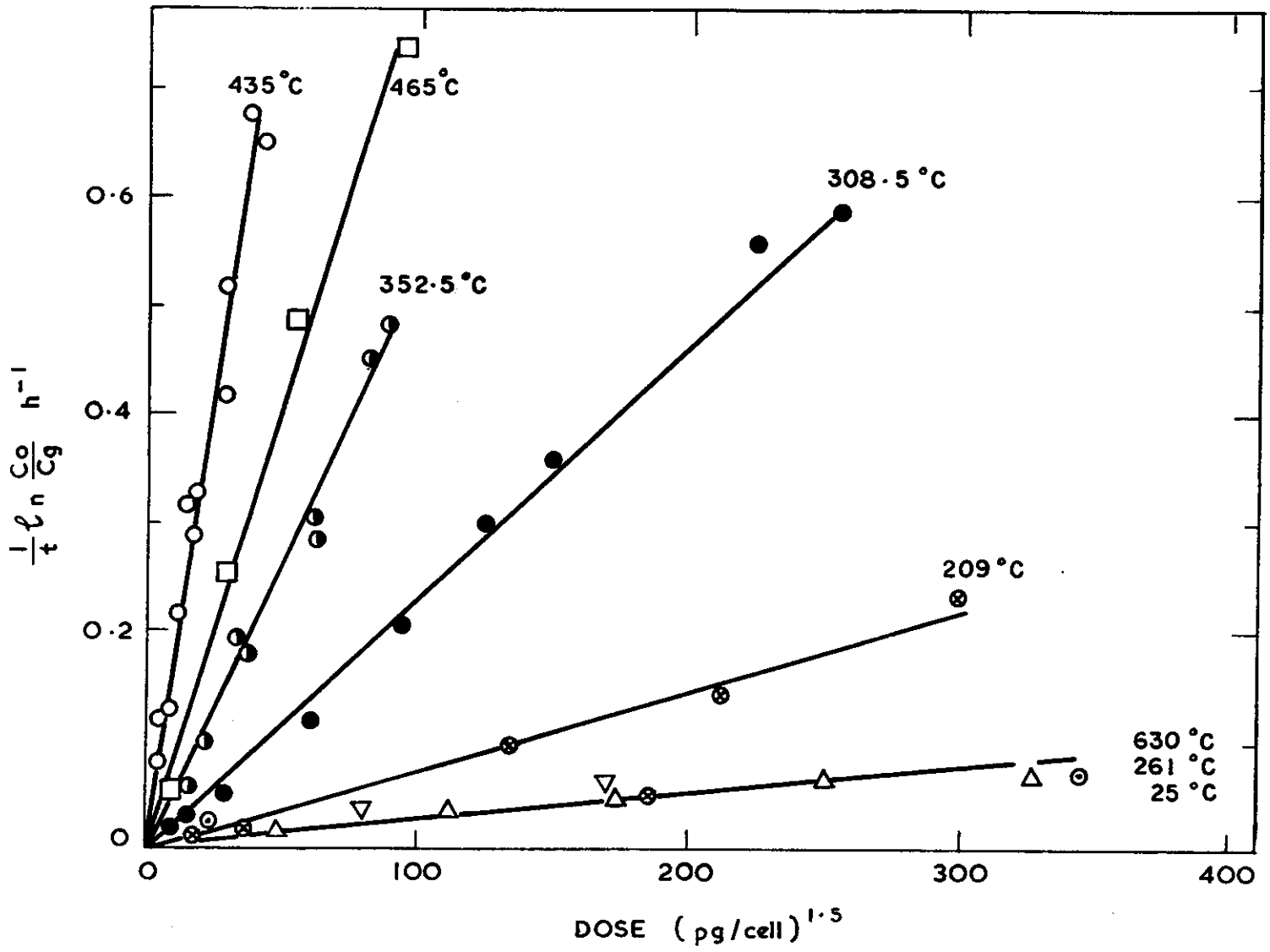

FIGURE 6. HAEMOLYSIS AT LOW DOSAGE BY THERMOCHEMICAL DERIVATIVES OF AU

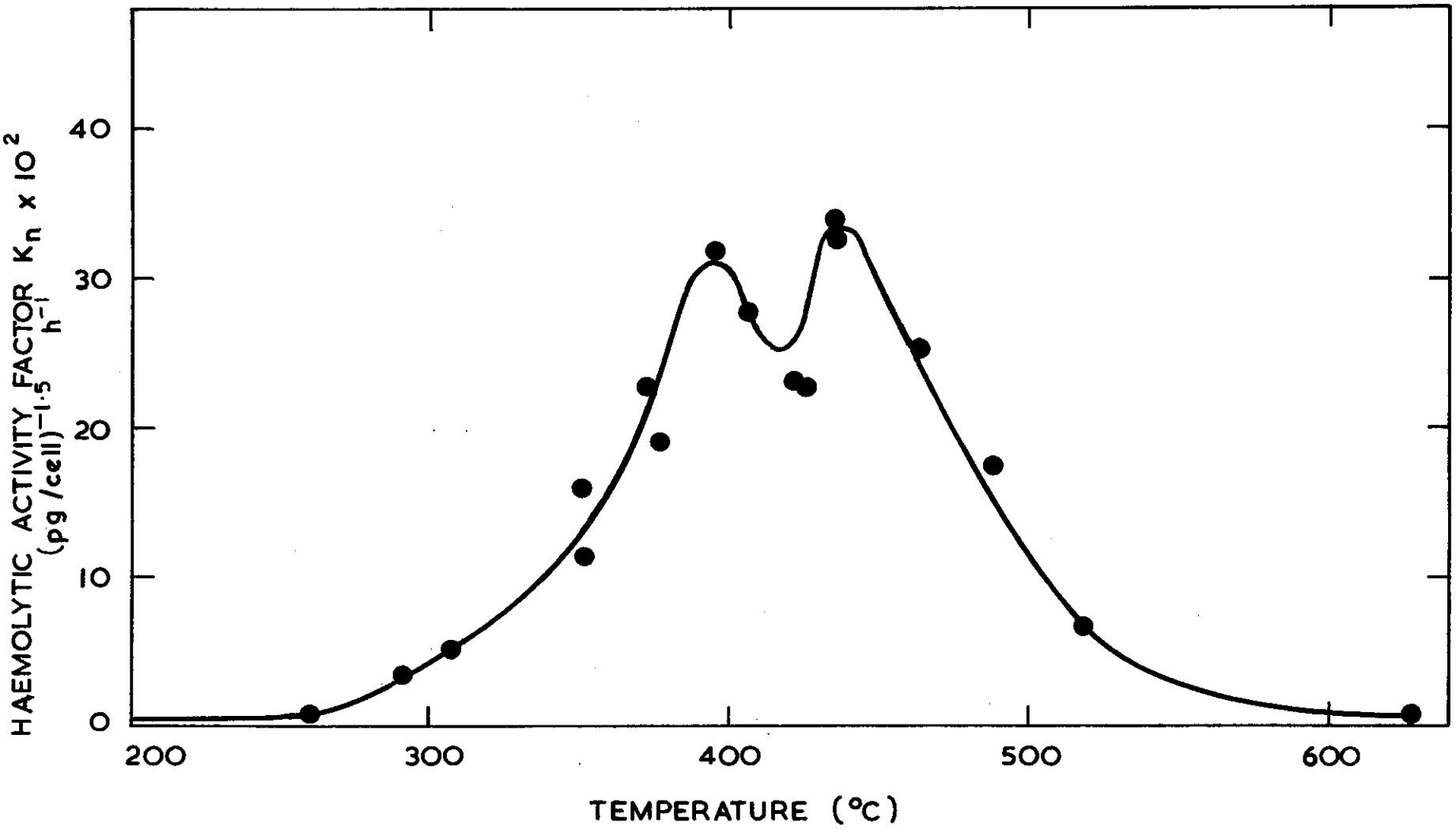

FIGURE 7. HAEMOLYTIC ACTIVITY OF POWDERS OBTAINED BY HEATING AMMONIUM URANATE IN 3 VOL. PER CENT H_2-N_2 AT 5 °C min^{-1} TO VARIOUS TEMPERATURES

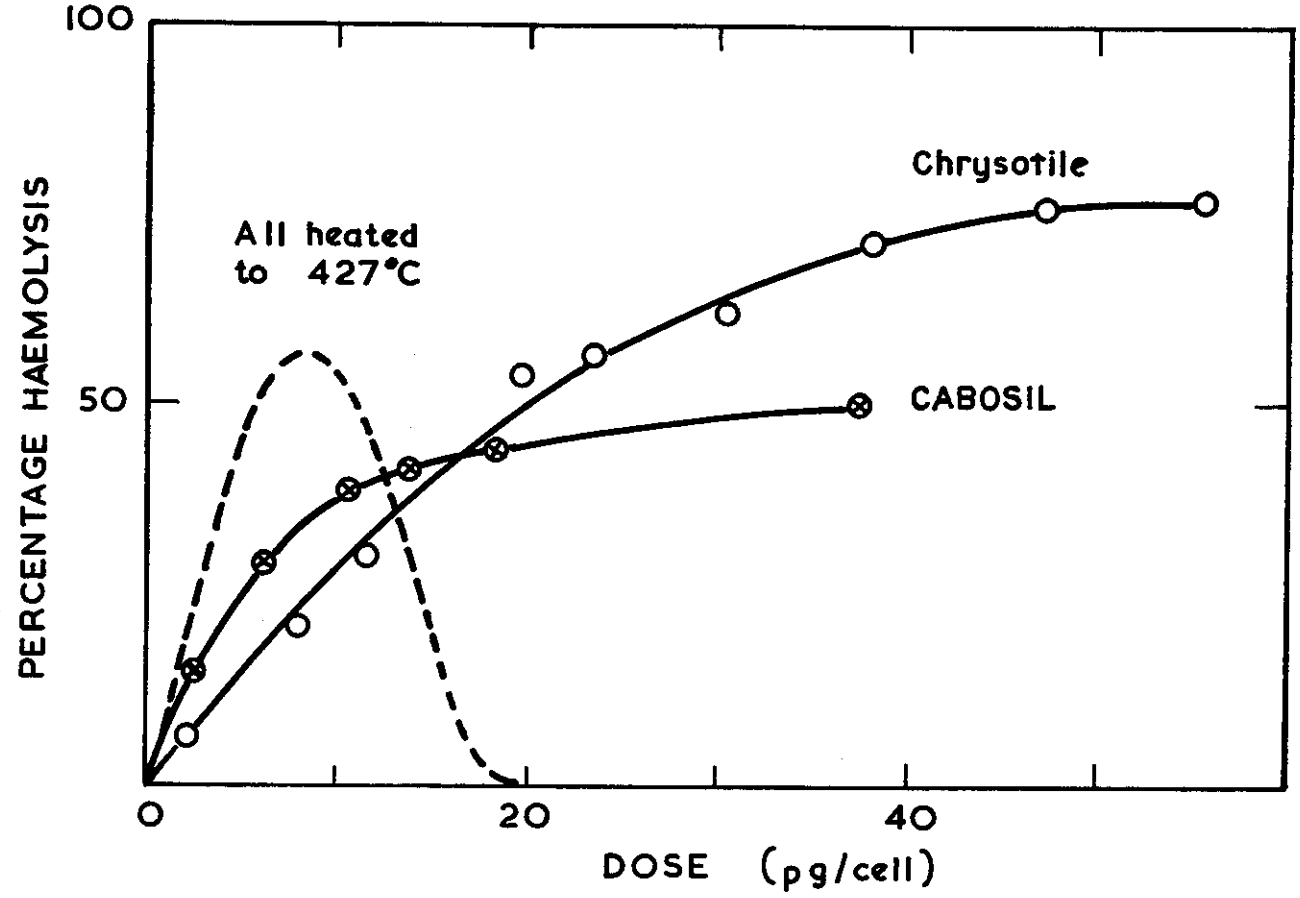

FIGURE 8. HAEMOLYTIC ACTIVITY OF SOME MINERAL DUSTS