

## CYANATE FORMATION IN SOLUTIONS OF UREA

### I. CALCULATION OF CYANATE CONCENTRATIONS AT DIFFERENT TEMPERATURE AND pH

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#### SUMMARY

Data from the literature on the equilibrium between urea and cyanate which were difficult to combine, have been correlated by computer calculation. Accumulation of cyanate in urea solutions was quantitatively studied at different temperature and pH.

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#### INTRODUCTION

The interaction of urea with proteins and polypeptides has been studied by many workers<sup>1-6</sup>. High concentrations of this compound destroy non-covalent interaction in proteins. This structure is assumed to result from hydrogen bonds, coulombic forces and hydrophobic interaction. Although the individual forces are very weak as compared with covalent bonds, their sum causes the very stable configuration of various proteins.

The use of buffers containing urea in gel electrophoretic techniques has become a very useful tool in the study of subunit structure of proteins. In connection here-with the relevance of multiples zones frequently observed in gel electrophoresis performed in urea-containing buffers has to be envisaged. That the dissociation of proteins into subunits by the action of urea may be accompanied by the formation of cyanate has been stressed earlier<sup>7-9</sup>.

In the present paper we demonstrate that taking into consideration the kinetic data from the literature a calculation of the cyanate content in aqueous solutions of urea is possible. The accumulation of cyanate in aqueous solutions of urea is the net result of the reversible decomposition of cyanate yielding ammonium and carbonate ions<sup>10-14</sup>. The kinetics of these reactions have been reviewed by FROST AND PEARSON<sup>15</sup>.

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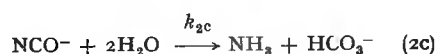
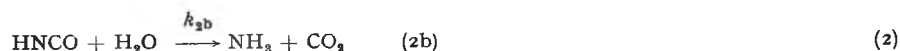
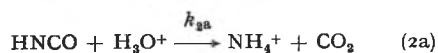
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In the ideal case carbamylation of the free amino groups of a protein in urea solution is prevented. The solution used should then be completely free of cyanate ions<sup>16-18</sup>. This means that in practice the cyanate concentration has to be kept as low as possible.

## METHODS AND CALCULATIONS

The following reactions occur in an aqueous solution of urea:

*Reaction (+1)*

KEMP AND KOHNSTAM<sup>19</sup> have measured the rate of decomposition of urea in aqueous solutions at 60°. SHAW AND BORDEAUX<sup>20</sup> have studied this reaction at temperatures between 60 and 100° in neutral and in acid aqueous solutions. From the experimental values given in their articles we were able to calculate rate constants at different temperatures by applying the Arrhenius equation. The obtained values are summarized in Table I.

TABLE I

CALCULATED RATE CONSTANTS  $k_{+1}$  OF THE DECOMPOSITION OF UREA IN AQUEOUS SOLUTIONS

$H = 31.8$  kcal/mol.

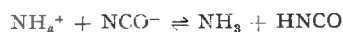
Temp.	$k_{+1}$ (sec <sup>-1</sup> )	$k_{+1}$ (min <sup>-1</sup> )
0°	$5.90 \cdot 10^{-12}$	$3.54 \cdot 10^{-10}$
18°	$2.30 \cdot 10^{-10}$	$1.38 \cdot 10^{-8}$
25°	$8.40 \cdot 10^{-10}$	$5.04 \cdot 10^{-8}$
60°	$2.37 \cdot 10^{-7}$	$1.42 \cdot 10^{-5}$
70°	$8.80 \cdot 10^{-7}$	$5.28 \cdot 10^{-5}$
80°	$3.60 \cdot 10^{-7}$	$2.16 \cdot 10^{-4}$
100°	$4.00 \cdot 10^{-6}$	$2.40 \cdot 10^{-3}$

*Reaction (-1)*

The conversion of ammonium cyanate into urea has extensively been studied by kinetic methods. WALKER AND HAMBLY<sup>21</sup> found the reaction to be second order whereas the rate expression is given by:

$$\text{Rate} = k_{-1} [\text{NH}_4^+] [\text{NCO}^-].$$

It has been pointed out<sup>22</sup> that due to the equilibrium between the possible pairs of reactants:



the ionic and molecular mechanism are kinetically indistinguishable. However, at present the non-ionic mechanism is accepted as the more probable one. This has been concluded from the analogy between this reaction and the formation of substituted ureas from ammonia and organic isocyanates<sup>18</sup>.

The reaction of cyanic acid with several amines has been studied by JENSEN<sup>23</sup> and in the special case of amino acids and peptides by STARK<sup>24</sup>. For our calculations we used the values of the rate constants of the formation of urea from ammonia and cyanic acid obtained by JENSEN<sup>23</sup> at 18° at an ionic strength of  $I = 0.20$  and by KEMP AND KOHNSTAM<sup>19</sup> at 60° and 80° at an ionic strength  $I = 0.25$ . As the rate constants are dependent on the ionic strength we had to transform the results of JENSEN to an ionic strength  $I = 0.25$  using activity corrections obtained from the Debye-Hückel expression<sup>25</sup>:

$$-\log f = \frac{A \cdot z^2 \sqrt{I}}{1 + aB\sqrt{I}} \quad (a = 5 \text{ \AA})$$

The rate constants of the conversion of ammonium cyanate into urea at different temperatures were obtained by applying the Arrhenius equation. The results are summarized in Table II.

TABLE II

CALCULATED RATE CONSTANTS  $k_{-1}$  OF THE CONVERSION OF AMMONIUM CYANATE INTO UREA IN AQUEOUS SOLUTIONS AT AN IONIC STRENGTH  $I = 0.25$

$H = 22.7 \text{ kcal/mol.}$

Temp.	$k_{-1} (M^{-1} \cdot \text{sec}^{-1})$	$k_{-1} (M^{-1} \cdot \text{min}^{-1})$
0°	$7.70 \cdot 10^{-7}$	$4.62 \cdot 10^{-5}$
18°	$9.30 \cdot 10^{-6}$	$5.58 \cdot 10^{-4}$
25°	$2.30 \cdot 10^{-5}$	$1.38 \cdot 10^{-3}$
60°	$1.25 \cdot 10^{-3}$	$1.50 \cdot 10^{-2}$
70°	$3.40 \cdot 10^{-3}$	$2.04 \cdot 10^{-1}$
80°	$9.22 \cdot 10^{-3}$	$5.53 \cdot 10^{-1}$
100°	$5.00 \cdot 10^{-2}$	3.00

### Reaction (1)

The equilibrium constant of the urea-ammonium cyanate equilibrium:

$$K = \frac{k_{+1}}{k_{-1}} = \frac{[\text{NH}_4^+][\text{NCO}^-]}{[(\text{NH}_4)_2\text{CO}]}$$

has been calculated at different temperatures and at an ionic strength  $I = 0.25$  from the results of Table I and Table II (Table III).

The calculated value of the equilibrium constant at 25° agrees reasonably with the value obtained earlier by LEWIS AND RANDALL<sup>17</sup>:  $3.02 \cdot 10^{-5}$ .

From Table III it can be seen that a raise of the temperature from 0 to 100° results in an increase of the equilibrium constant with a factor 100 and consequently of the relative amount of cyanate by a factor 10. Furthermore it can be derived from Table I and Table II that the time required for reaching the equilibrium state is reduced by a factor  $10^5$ . Hence it is clear that the temperature should be low when the formation of cyanate has to be kept at a low level.

TABLE III

CALCULATED EQUILIBRIUM CONSTANTS OF THE UREA-AMMONIUM CYANATE EQUILIBRIUM IN AQUEOUS SOLUTIONS AT AN IONIC STRENGTH  $I = 0.25$  $H = -9.1$  kcal/mol.

Temp.	Equilibrium constant, $K \times 10^5 (M)$
0°	0.76
18°	2.48
25°	3.65
60°	19.0
70°	25.9
80°	39.0
100°	80.0

TABLE IV

THE DECOMPOSITION CONSTANTS OF CYANIC ACID AND CYANATE ACCORDING TO THE REACTIONS (2a), (2b) AND (2c) AT AN IONIC STRENGTH  $I = 0.25$ 

Temp.	$k_{2a} (M^{-1} \cdot \text{min}^{-1})$	$k_{2b} (\text{min}^{-1})$	$k_{2c} (\text{min}^{-1})$
0°	0.69	0.011	$2.74 \cdot 10^{-8}$
18°	3.60	0.066	$4.06 \cdot 10^{-7}$
25°	6.70	0.112	$10.7 \cdot 10^{-7}$

TABLE V

DISSOCIATION CONSTANTS OF CYANIC ACID, AMMONIA AND WATER AT AN IONIC STRENGTH  $I = 0.25$ 

Temp.	$\text{HNCO} (pK_a)$	$\text{NH}_3 (pK_b)$	$\text{H}_2\text{O} (pK_w)$
20°	3.43	10.08	14.81
25°	3.42	9.24	13.87

*Reaction (2)*

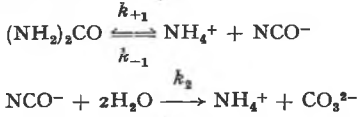
The kinetics of the decomposition of cyanic acid and cyanate into ammonia and  $\text{CO}_2$  has been studied under several conditions<sup>14,19,27</sup>. In our calculations we made use of the experimental results of LISTER<sup>14</sup> and JENSEN<sup>27</sup>. Corrections for the activity coefficients were made in order to obtain the values at an ionic strength  $I = 0.25$ . The resulting rate constants are summarized in Table IV.

At a fixed pH the effective rate of decomposition of cyanic acid (*i.e.*  $k_2 = k_{2a} + k_{2b} + k_{2c}$ ) may be calculated from the results of Table IV and with the aid of the ionization constants of cyanic acid<sup>14,27,28</sup>, ammonia<sup>29</sup> and water. The values of these constants at ionic strength  $I = 0.25$  are given in Table V.

From the data collected in Tables IV and V we calculated the value of  $k_2$  at different hydrogen ion concentrations and at two different temperatures (Table VI).

TABLE VI

CALCULATED RATE CONSTANTS OF THE REACTION SCHEME:



Temp. (°)	pH	$k_{+1}$ (min <sup>-1</sup> )	$k_{-1}$ (M <sup>-1</sup> ·min <sup>-1</sup> )	$k_2$ (min <sup>-1</sup> )
25°	10.0	$5.04 \cdot 10^{-8}$	$2.04 \cdot 10^{-4}$	$1.10 \cdot 10^{-6}$
	9.0	$5.04 \cdot 10^{-8}$	$8.15 \cdot 10^{-4}$	$1.31 \cdot 10^{-6}$
	8.0	$5.04 \cdot 10^{-8}$	$1.31 \cdot 10^{-3}$	$4.02 \cdot 10^{-6}$
	7.0	$5.04 \cdot 10^{-8}$	$1.37 \cdot 10^{-3}$	$3.06 \cdot 10^{-5}$
	6.0	$5.04 \cdot 10^{-8}$	$1.38 \cdot 10^{-3}$	$2.96 \cdot 10^{-4}$
	5.0	$5.04 \cdot 10^{-8}$	$1.35 \cdot 10^{-3}$	$2.95 \cdot 10^{-3}$
	4.0	$5.04 \cdot 10^{-8}$	$1.01 \cdot 10^{-3}$	$2.96 \cdot 10^{-3}$
0°	10.0	$3.54 \cdot 10^{-10}$	$2.52 \cdot 10^{-6}$	$3.0 \cdot 10^{-8}$
	9.0	$3.54 \cdot 10^{-10}$	$4.27 \cdot 10^{-6}$	$5.7 \cdot 10^{-8}$
	8.0	$3.54 \cdot 10^{-10}$	$4.58 \cdot 10^{-5}$	$3.23 \cdot 10^{-7}$
	7.0	$3.54 \cdot 10^{-10}$	$4.61 \cdot 10^{-5}$	$3.00 \cdot 10^{-6}$
	6.0	$3.54 \cdot 10^{-10}$	$4.6 \cdot 10^{-5}$	$2.96 \cdot 10^{-5}$
	5.0	$3.54 \cdot 10^{-10}$	$4.5 \cdot 10^{-5}$	$2.96 \cdot 10^{-4}$
	4.0	$3.54 \cdot 10^{-10}$	$3.6 \cdot 10^{-5}$	$2.96 \cdot 10^{-3}$
3.0	$3.54 \cdot 10^{-10}$	$1.25 \cdot 10^{-5}$	$3.16 \cdot 10^{-2}$	

*Calculation of the cyanate concentration*

From the reactions (1) and (2) it can be derived that the differential equation of the formation of ammonium ions and cyanate ions in an aqueous solution of pure urea at a given pH, ionic strength and temperature becomes:

$$\frac{d[\text{NCO}^-]}{dt} = k_{+1}[(\text{NH}_2)_2\text{CO}] - k_{-1}[\text{NH}_4^+][\text{NCO}^-] - k_2[\text{NCO}^-]$$

$$\frac{d[\text{NH}_4^+]}{dt} = k_{+1}[(\text{NH}_2)_2\text{CO}] - k_{-1}[\text{NH}_4^+][\text{NCO}^-] + k_2[\text{NCO}^-]$$

At zero time:  $[\text{NCO}^-] = 0$  and  $[\text{NH}_4^+] = 0$ .

These differential equations cannot easily be resolved. For that reason we developed a computer program (Algol 60) which enabled us to obtain a numerical solution of these equations. This solution gives the concentration of cyanate as a function of temperature and time.

## RESULTS

Tables I–VI and Figs. 1–4 summarize our results. The results represented in Tables I–VI have been discussed above. Figs. 1 and 2 give graphic representations of the accumulation of cyanate in 8 M urea at a temperature of 0 and 25° after prolonged standing. Figs. 3 and 4 show the accumulation of cyanate in 8 M urea after 3 days. The calculations have been carried out for 8 M urea. In the range from 1–9 M urea (the most important in protein chemistry) there exists a linear relationship between cyanate and urea concentration. Hence the cyanate concentration in aqueous solutions of 1–9 M urea can be easily derived from Figs. 1–4.

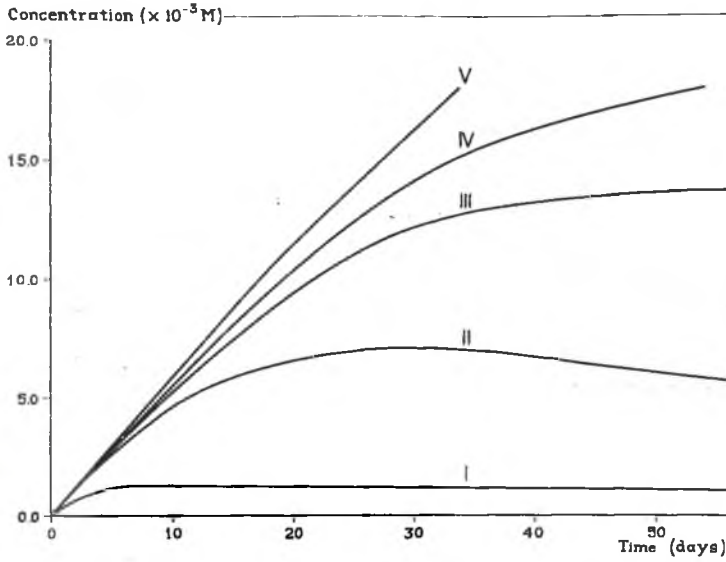


Fig. 1. The accumulation of cyanate in an aqueous solution of 8 M urea at 25° and  $I = 0.25$  over a period of two months. I, pH 4; II, pH 5; III, pH 6; IV, pH 7; V, pH  $\geq 8$ .

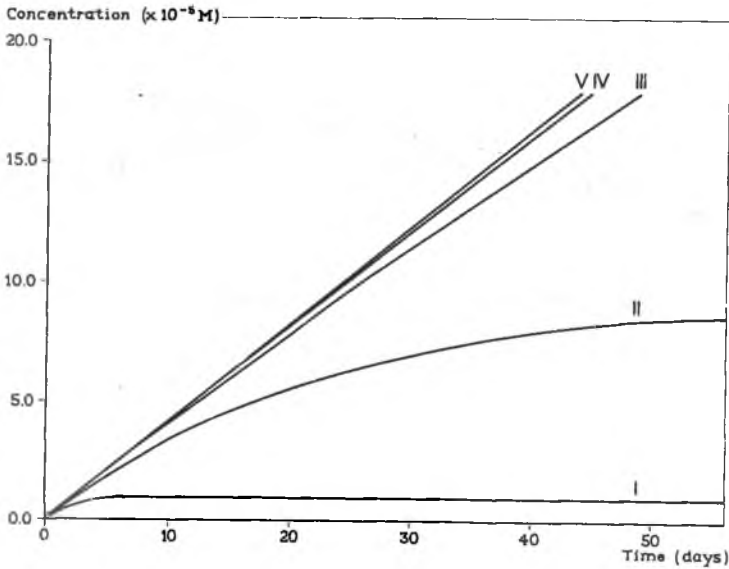


Fig. 2. The accumulation of cyanate in an aqueous solution of 8 M urea at 0° and  $I = 0.25$  over a period of two months. I, pH 3; II, pH 4; III, pH 5; IV, pH 6; V, pH  $\geq 7$ .

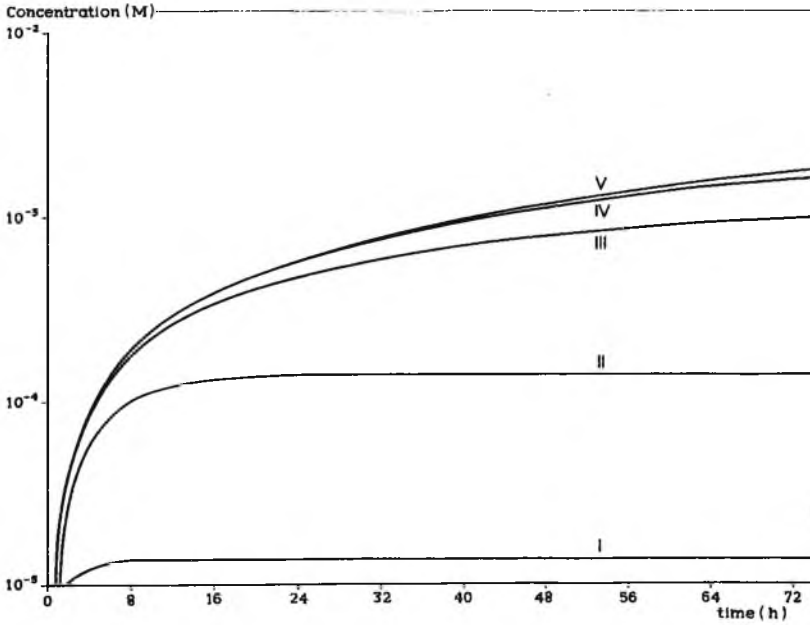


Fig. 3. The accumulation of cyanate in an aqueous solution of 8 M urea during the first three days at 25°. I, pH 4; II, pH 5; III, pH 6; IV, pH 7; V, pH  $\geq 8$ .

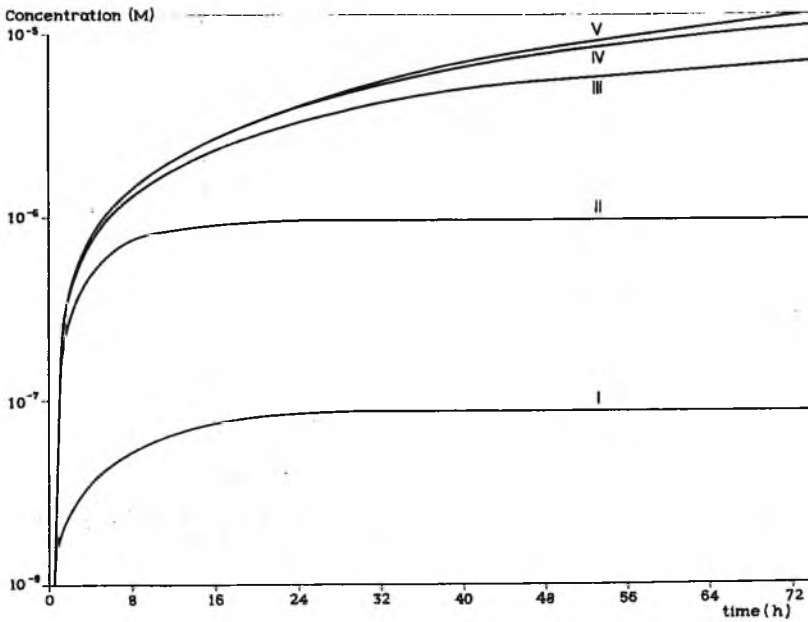


Fig. 4. The accumulation of cyanate in an aqueous solution of 8 M urea during the first three days at 0°. I, pH 3; II, pH 4; III, pH 5; IV, pH 6; V, pH  $\geq 7$ .

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