J. Cosmet. Sci., 65, 187–195 (May/June 2014)

Stability of urea in solution and pharmaceutical preparations

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Accepted for publication March 10, 2014.

Synopsis

The stability of urea in solution and pharmaceutical preparations was analyzed as a function of temperature (25°–60°C), pH (3.11–9.67), and initial urea concentration (2.5%–20%). This study was undertaken to (i) obtain more extensive, quantitative information relative to the degradation of urea in both aqueous and non-aqueous solutions and in pharmaceutical preparations, and (ii) test the effects of initial urea concentration, pH, buffer, and temperature values on urea degradation. The stability analysis shows that urea is more stable at the pH range of 4–8 and the stability of urea decreases by increase in temperature for all pH values. Within the experimental range of temperature and initial urea concentration values, the lowest urea degradation was found with lactate buffer pH 6.0. The urea decomposition rate in solution and pharmaceutical preparations shows the dependence of the initial urea concentrations. At higher initial urea concentrations, the rate of degradation is a decreasing function with time. This suggests that the reverse reaction is a factor in the degradation of concentrated urea solution. For non-aqueous solvents, isopropanol showed the best effort in retarding the decomposition of urea. Since the losses in urea is directly influenced by its stability at a given temperature and pH, the stability analysis of urea by the proposed model can be used to prevent the loss and optimize the operating condition for urea-containing pharmaceutical preparations.

INTRODUCTION

Being widely used in pharmaceutical and cosmetic products, urea plays a vital role in maintaining the skin's moisture balance and suppleness. Reduced levels of urea, representing 7% of the natural moisturizing factors in the stratum corneum (skin-building layer), lead to a lower water-binding capacity within the skin, which in turn, results in roughness, tightness, flaking, and irritation of the skin. Urea preparations typically range in strength from 3 to 20, in specific preparations up to 40%, and can take many forms, including creams, gels, shampoos, deodorants, foundation, and even toothpaste.

Ever since the decomposition of urea was first presented by Wöhler in 1829 (1), the understanding of its products, by-products, and reaction pathways has been extensively the subject of several studies over the past century (2–11), but little information exists relative to the stability of urea in non-aqueous solutions and pharmaceutical preparations. Urea

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decomposition yields ammonium ions (NH_4^+) and cyanate (CNO^-), further readily undergoing conversion to carbon dioxide (CO_2) and ammonia (NH_3). In aqueous solution, an elimination mechanism for urea decomposition appears to be operative. In contrast, when catalyzed by ureases, urea is generally believed to undergo hydrolysis rather than ammonia elimination (2–6). Although the earlier workers agreed that ammonium cyanate is an intermediate in the decomposition of urea, the numerical magnitudes of the rate constant and the order of reaction reported were quite different. Bull *et al.* (7) reported that urea degradation followed first-order kinetics in both dilute and concentrated solutions as well as Shaw and Bordeaux (8). On the other hand, the hydrolysis of urea was observed by several investigators as a reversible reaction in some specific conditions (9–11).

Many direct approaches for urea determination involving the reactions with urea to form colored products have been described, for instance, the well-known Fearon reaction (12), the reaction with *o*-phathaldehyde, and the reaction with *p*-dimethylaminobenzaldehyde (*p*-DMAB). Because of the use of corrosive reagents or incubation temperature and other disadvantages in diacetylmonoxime and *o*-phathaldehyde assays, in this experiment, the reaction of urea with *p*-DMAB was used. Impressed by Knorst's work (13), we then applied the protocol using *p*-DMAB for the kinetic study of urea degradation both in aqueous solution, non-aqueous, and in pharmaceutical preparations with a broad range of pH and temperature.

MATERIALS AND METHODS

MATERIALS AND REAGENTS

Urea and sulfuric acid were obtained from Merck (Darmstadt, Germany). *p*-DMAB was obtained from Sigma-Aldrich (Steinheim, Germany). Chemicals and solvents were of reagent grade and were used without further purification. Lactate buffers pH 4.5 and 6.0, phosphate buffer pH 6.0, and citrate pH 6.0 were prepared according to European Pharmacopoeia (14). The UV absorption spectra were recorded against a reagent blank using a ThermoScientific (Waltham, MA) Helios Omega spectrophotometer.

DETERMINATION OF UREA DEGRADATION RATE CONSTANTS IN AQUEOUS SOLUTIONS

The various concentrations of urea solutions (2.5%, 5%, and 10% [w/v]) were prepared and subjected to investigate the effect on urea degradation rate constant. 1 *M* NaOH, 1 *M* HCl, and different buffers were used to prepare solutions at different pH intervals between 3.0 and 10.0. At pH values between 3.11 and 4.19, 1 *M* HCl; pH values between 8.40 and 9.67, 1 *M* NaOH; pH values 4.5 and 6.0, lactate buffer; pH values 6.0, phosphate buffer; and for pH values 6.0, citrate buffer solutions were used. The solutions of urea at different pH values were incubated at 25°, 40°, and 60°C, respectively. The residual urea concentration values at a defined pH and temperature values and different time intervals of 3, 7, and 14 days were determined as per the following procedure.

A solution (0.5 ml) containing 4% (w/v) of *p*-DMAB and 4% (v/v) sulfuric acid in 99% ethanol was added to the mixture of 0.05 ml of urea solution and 9.95 ml of water. After

10 min, the absorbance of the solution was measured at 422 nm against a reagent blank. The experiments were performed in triplicate, and mean concentrations were used for determination of rate constants.

DETERMINATION OF UREA DEGRADATION RATE CONSTANTS IN NON-AQUEOUS SOLVENTS

The 2.5% urea solutions with various non-aqueous solvents including propylene glycol, glycerol, ethanol, isopropanol, pentylene glycol, and polyethylene glycol were prepared and incubated at 25° and 40°C. The urea concentration values at defined temperature values and different time intervals of 3, 7, and 14 days were determined as per the following procedure.

A solution (0.5 ml) containing 4% (w/v) of *p*-DMAB and 4% (v/v) sulfuric acid in 99% ethanol was added to the mixture of 0.05 ml of urea solution and 4.95 ml of 99% ethanol. After 10 min, the absorbance of the solution was measured at 422 nm against a reagent blank. The experiments were performed in triplicate, and mean concentrations were used for determination of rate constants.

DETERMINATION OF UREA DEGRADATION RATE CONSTANTS IN PHARMACEUTICAL PREPARATIONS

Pharmaceutical preparations composed of urea at varying concentrations of 2.5%, 5%, 10%, 15%, and 20% (w/w) were prepared using the ingredients shown in Table I. For water phase, ingredients were mixed and heated to 80°C, and then urea was added and

Table I
Ingredients of urea-containing pharmaceutical preparations

	Urea concentration				
Ingredients	2.5%	5%	10%	15%	20%
Water phase					
Glycerol 85%	3.50 g	3.50 g	3.50 g	3.50 g	3.50 g
Magnesiumsulfate-Heptahydrate	0.50 g	0.50 g	0.50 g	0.50 g	0.50 g
Lactic acid solution 9%	1.56 g	1.56 g	1.56 g	1.56 g	1.56 g
Sodium lactate solution 50%	1.72 g	1.72 g	1.72 g	1.72 g	1.72 g
DI-water	29.27 g	28.02 g	25.52 g	23.02 g	20.52 g
Urea	1.25 g	2.50 g	5.00 g	7.50 g	10.00 g
Oil Phase					
Caprylic/capric triglyceride (Myritol 318 PH®)	5.00 g	5.00 g	5.00 g	5.00 g	5.00 g
Decamethylcyclopentasiloxane (Cyclomethicone 5-NF®)	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g
Cetearyl ethylhexanoate/isopropyl myristate (PCL®)	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g
Hexyldecanol/hexyldecyl laurate (Cetiol® PGL)	2.00 g	2.00 g	2.00 g	2.00 g	2.00 g
Polyglyceryl-3 diisostearate (Lameform® TGI)	0.84 g	0.84 g	0.84 g	0.84 g	0.84 g
Polyglyceryl-2 dipolyhydroxystearate (Dehymuls®)	1.66 g	1.66 g	1.66 g	1.66 g	1.66 g
Caprylyl Glycol/ethylhexylglycerin (Sensiva® SC10)	0.20 g	0.20 g	0.20 g	0.20 g	0.20 g

dissolved to get the pH 4.30–4.70. The oil phase was separately heated to 80°C. Water phase was then added to oil phase while stirring. The mixture was homogenized at 65°C for 1 min and was cooled down to 25°C while further stirring. Pharmaceutical preparations composed of urea at varying concentrations of 2.5%, 5%, 10%, 15%, and 20% (w/w) were separated into two groups: pH 4.50 (no further pH adjusting) and pH 6.00 (adjusting with lactate buffer). The urea preparations at different concentrations and pH values were incubated at 25° and 40°C. The residual urea concentration values at a defined pH and temperature values and different time intervals of 3, 7, and 14 days were determined as per the following procedure.

The sample of 1 g urea preparation was diluted to 20 ml with 80% ethanol. The mixture was heated and stirred on a water bath at 70°C for 5 min until dispersed and placed for 30 min at room temperature on a mechanical stirrer. The sample was then centrifuged for 5 min at 8000 rpm at 4°C and filtered through a 0.2 mm filter (Chromafilm® Macherey-Nagel, Düren, Germany). The filtrate (0.05 ml) was diluted with the same solvent to 5 ml. A solution (0.5 ml) containing 4% (w/v) of *p*-DMAB and 4% (v/v) sulfuric acid in 99% ethanol was added to the mixture. After 10 min, the absorbance of the solution was measured at 422 nm against a reagent blank. The experiments were performed in triplicate, and mean concentrations were used for determination of rate constants.

RESULTS AND DISCUSSION

THE KINETICS OF UREA DEGRADATION IN AQUEOUS SOLUTIONS

Urea solutions at different concentrations (2.5%, 5%, and 10% [w/v]), pH (3.11–3.36, 4.08–4.19, 6.43–7.36, 8.40–8.59, 9.40–9.67, 4.5, and 6.0 with lactate buffer, 6.0 with phosphate buffer, and 6.0 with citrate buffer), and temperature values (25°, 40°, and 60°C) were used to determine the effect of concentration, pH, and temperature on the stability of urea.

The linear regression of the decomposition data shows that urea degradation reaction obeys first-order kinetics at all measured pH and temperature values of the experiments. Table II demonstrates the values of the rate constant, k, in h^{-1} calculated from the experimental data of the residual urea concentrations with various initial concentrations of 2.5%, 5%, and 10% as a function of pH and temperature. The values of the urea degradation rate constant are in the same magnitude of those reported by previous investigators (8). Within the experimental range of temperature and initial urea concentration values, the lowest urea degradation was performed at pH 6.43–7.36. There was no significant difference between various buffers, but lactate buffer pH 6.0 showed the minimum degradation rate constant.

Degradation rate constant slightly decreases as the initial urea concentration is increased. Since more ammonium cyanate was produced (in the same time interval) at the higher urea concentration than at the lower ones, this was tentatively attributed to the reverse reaction, thus lowering the urea degradation. The results obtained are in accordance with this argumentation and indicate that the reverse reaction is important when as little as 5% of the urea present initially has decomposed.

The obtained result for the decomposition rate constant at the temperature range of 25°–60°C shows that urea is quite stable in the pH range between 4 and 8 than out of this

Table II

The values of the rate constants, k, in h^{-1} calculated from the experimental data of the residual urea concentrations with various initial concentrations (2.5%, 5%, and 10%) as a function of pH and temperature

	k Value at 25°C			k Value at 40°C			k Value at 60°C		
	2.50%	5%	10%	2.50%	5%	10%	2.50%	5%	10%
3.11–3.36	3.51×10^{-6}	3.34×10^{-6}	3.33×10^{-6}	4.88×10^{-5}	4.84×10^{-5}	4.67×10^{-5}	8.18×10^{-4}	8.17×10^{-4}	8.14×10^{-4}
4.08–4.19	3.09×10^{-6}	3.07×10^{-6}	3.05×10^{-6}	4.34×10^{-5}	4.28×10^{-5}	4.01×10^{-5}	8.06×10^{-4}	7.95×10^{-4}	7.89×10^{-4}
6.43–7.36	3.03×10^{-6}	2.95×10^{-6}	2.90×10^{-6}	4.29×10^{-5}	4.27×10^{-5}	3.98×10^{-5}	7.97×10^{-4}	7.92×10^{-4}	7.81×10^{-4}
8.40–8.59	3.11×10^{-6}	3.10×10^{-6}	3.07×10^{-6}	4.37×10^{-5}	4.28×10^{-5}	4.07×10^{-5}	6.13×10^{-4}	8.01×10^{-4}	7.90×10^{-4}
9.40–9.67	3.92×10^{-6}	3.86×10^{-6}	3.83×10^{-6}	5.27×10^{-5}	5.02×10^{-5}	4.95×10^{-5}	8.20×10^{-4}	8.17×10^{-4}	8.17×10^{-4}
6.00 (lactic buffer)	2.95×10^{-6}	2.79×10^{-6}	2.67×10^{-6}	4.24×10^{-5}	4.08×10^{-5}	3.84×10^{-5}			
6.00 (phosphate buffer)	2.95×10^{-6}	2.83×10^{-6}	2.73×10^{-6}	4.26×10^{-5}	4.14×10^{-5}	3.85×10^{-5}			
6.00 (citric buffer)	2.99×10^{-6}	2.94×10^{-6}	2.82×10^{-6}	4.32×10^{-5}	4.22×10^{-5}	3.88×10^{-5}			
4.50 (lactic buffer)	3.06×10^{-6}	3.07×10^{-6}	2.84×10^{-6}	4.30×10^{-5}	4.27×10^{-5}	3.96×10^{-5}			

range. In acid, a rapid quantitative conversion of the cyanate ion (CNO^-) to ammonium ion (NH_4^+) occurs as shown in equation 1. This reaction is complete at room temperature in sufficiently concentrated acid solution. These findings are in complete agreement with those of Warner (9). The absence of occurrence of reverse reactions in acid media by the rapid hydrolysis of cyanate promotes the increase in urea degradation rate constant in strong acid condition.

$$CNO^- + 2H^+ + 2H_2O \rightarrow NH_4^+ + H_2CO_3 \quad (1)$$

The reversibility is also limited in alkaline solution by the decomposition of cyanate ion in basic environment as depicted in equation 2. This agrees with the increase in urea degradation rate constant in strong basic condition in our experiment.

$$CNO^- + OH^- + 2H_2O \rightarrow NH_4OH + CO_3^{2-} \quad (2)$$

Increase in temperature as well as working out of the range of 4–8 for pH increased the decomposition of urea.

The calculated values of the decomposition free energies as a function of pH and temperature are shown in Table III and can be used for stability analysis of urea. The higher value of the free energy means the more the stability of urea at a given condition (temperature and pH). Therefore, the maximum value of ΔG_i or the maximum stability of urea was obtained at 25°C, the highest initial urea concentration (10%) and pH 6.0 with lactate buffer for the temperature and pH ranges under study.

THE KINETICS OF UREA DEGRADATION IN NON-AQUEOUS SOLVENTS

A variety of non-aqueous solvents used mainly in pharmaceutical preparations (propylene glycol, glycerol, *N*-methylpyrrolidone, dimethylisosorbide, ethanol, isopropanol, pentylene

Table III

The values of the free energies of decomposition, ΔG_i, in kJ/gmol calculated from the experimental data of rate constant with various initial urea concentrations (2.5%, 5%, and 10%) as a function of pH and temperature

	ΔG_i at 25°C (kJ/gmol)			ΔG_i at 40°C (kJ/gmol)			ΔG_i at 60°C (kJ/gmol)		
pH	2.50%	5%	10%	2.50%	5%	10%	2.50%	5%	10%
3.11–3.36	104.16	104.28	104.29	102.67	102.7	102.79	101.59	101.6	101.61
4.08–4.19	104.47	104.49	104.51	102.98	103.02	103.18	101.63	101.67	101.69
6.43–7.36	104.52	104.59	104.63	103.01	103.02	103.2	101.67	101.68	101.72
8.40–8.59	104.46	104.47	104.49	102.96	103.02	103.15	102.39	101.65	101.69
9.40–9.67	103.88	103.92	103.94	102.47	102.6	102.64	101.59	101.6	101.6
6.00 (lactic buffer)	104.59	104.73	104.84	103.04	103.14	103.3			
6.00 (phosphate buffer)	104.59	104.69	104.78	103.03	103.1	103.29			
6.00 (citric buffer)	104.56	104.6	104.7	102.99	103.05	103.27			
4.50 (lactic buffer)	104.5	104.49	104.68	103	103.02	103.22			

glycol, hexylene glycol, and polyethylene glycol) was intended to evaluate the effect comparing to aqueous solution on urea degradation rate constant. However, urea did not dissolve after sonication for 30 min or afterward precipitation in *N*-methylpyrrolidone, dimethylisosorbide, and hexylene glycol. The urea solutions of concentration 2.5% with residual non-aqueous solvents were prepared and incubated at 25° and 40°C.

The linear regression of the decomposition data shows that urea degradation reaction in non-aqueous solvents obeys first-order kinetics at all measured temperature values of the experiments. Table IV demonstrates the values of the rate constant, k, in h^{-1} calculated from the experimental data of the residual urea concentration as a function of solvent and temperature. Both isopropanol and ethanol show lower degradation rate constants (2.70×10^{-6} h^{-1} at 25°C, 3.92×10^{-5} h^{-1} at 40°C; 2.73×10^{-6} h^{-1} at 25°C, 4.02×10^{-5} h^{-1} at 40°C, respectively) than that of water (3.03×10^{-6} h^{-1} at 25°C, 4.29×10^{-5} h^{-1} at 40°C).

The calculated values of the decomposition free energies as a function of temperature are also shown in Table IV and can be used for stability analysis of urea in non-aqueous solvents. The maximum value of ΔG_i or the maximum stability of urea in non-aqueous solvent was performed by isopropanol used as a solvent for the temperature ranges under study.

A work by Alexandrova and Jorgensen (4) also demonstrated that NH_3 elimination assisted by a water molecule was found to have the lowest activation energy, and the preferred reaction route was initiated *via* hydrogen transfer between the two amino groups mediated by one water molecule. The forming zwitterionic intermediate, H_3NCONH, received substantial stabilization *via* extended hydrogen bonding to the solvent, and its subsequent decomposition was found to be rate-determining. A computational study of the solution phase decomposition of urea by Estiu and Merz (3) showed that elimination was favored for the solution phase reaction, which proceeded by H-bond coordination of a water molecule to the amine nitrogen atoms. The coordination of one water molecule greatly facilitates the reaction by allowing it to proceed through a cyclic six-member transition state.

According to both previous studies, we therefore postulate that water molecule acts as a hydrogen shuffle for the first step of the elimination reaction. In the elimination step, the hydrogen atom can easily undergo nucleophilic attack by nitrogen atom of one NH_2 group followed by concerted H-transfer. Alcohols with various dipole moments in O–H

Table IV
The values of rate constants, k, in h^{-1} and the free energies of decomposition, ΔG_i, in kJ/gmol calculated from the experimental data of rate constant as a function of temperature

	25°C		40°C	
Non-aqueous solvents	k Value	ΔG_i	k Value	ΔG_i
Isopropanol	2.70×10^{-6}	104.81	3.92×10^{-5}	103.24
Ethanol	2.73×10^{-6}	104.78	4.02×10^{-5}	103.18
Water	3.03×10^{-6}	104.52	4.29×10^{-5}	103.01
Pentylene glycol	5.00×10^{-6}	103.28	6.85×10^{-5}	101.79
Propylene glycol	5.07×10^{-6}	103.25	6.85×10^{-5}	101.79
Glycerol	5.43×10^{-6}	103.08	6.93×10^{-5}	101.76
Polyethylene glycol	5.72×10^{-6}	102.95	6.97×10^{-5}	101.75

bond can act in the same way to water molecule as hydrogen shuffle. This can imply that increase in dipole moment in O–H bonds of different solvents increased the electron attracting ability of oxygen atom, as a result, facilitating the elimination step and thus the urea degradation. The effect of isopropanol in retarding the decomposition of urea in solution is explained by our hypothesis described above, with which it is in perfect agreement with the lowest dipole moment.

THE KINETICS OF UREA DEGRADATION IN PHARMACEUTICAL PREPARATIONS

According to the optimum result in retarding urea decomposition in aqueous solution, preparations adjusting with lactate buffer pH 6.0 were subjected to study in this experiment. Pharmaceutical preparations composed of urea at varying concentrations of 2.5%, 5%, 10%, 15%, and 20% (w/w) with pH 4.50 (no further pH adjusting) and pH 6.00 (adjusting with lactate buffer) were examined. Table V demonstrates the values of the rate constant, k, in h^{-1} calculated from the experimental data of the residual urea concentration as a function of temperature. Within the experimental range of temperature and initial urea concentration values, the lowest urea degradation was found with lactate buffer pH 6.0. Degradation rate constant slightly decreases as the initial urea concentration is increased. Since more ammonium cyanate was produced (in the same time interval) at the higher urea concentration than at the lower ones, this was tentatively attributed to the reverse reaction as also observed in solution, thus lowering the urea degradation.

CONCLUSION

The proposed model for prediction of the combined effect of pH and temperature on decomposition of urea was used to investigate the decomposition rate constants and the stability of urea for pH values between 3.11 and 9.67 and a temperature range of 25°–60°C. The rate constant values obtained from the experiment are in good agreement with those of the literature values. The urea decomposition rate in aqueous solution, represented by the first-order reaction kinetics, shows the dependence of the initial urea concentrations. At higher initial urea concentrations, the rate of degradation is a decreasing function with time. This suggests that the reverse reaction is a factor in the degradation of concentrated urea solution. The obtained results also show that urea is more unstable

Table V
The values of the rate constants, k, in h^{-1} calculated from the experimental data of the residual urea concentrations with various initial concentrations as a function of temperature

	k Value at 25°C		k Value at 40°C	
Concentration (%)	Normal cream	Cream pH 6 (lactate)	Normal cream	Cream pH 6 (lactate)
2.50	8.59×10^{-7}	8.37×10^{-7}	7.13×10^{-6}	6.86×10^{-6}
5	8.44×10^{-7}	8.05×10^{-7}	7.05×10^{-6}	6.81×10^{-6}
10	8.39×10^{-7}	7.96×10^{-7}	7.03×10^{-6}	6.80×10^{-6}
15	8.38×10^{-7}	7.93×10^{-7}	7.01×10^{-6}	6.70×10^{-6}
20	8.23×10^{-7}	7.08×10^{-7}	6.87×10^{-6}	6.58×10^{-6}

at higher temperature and at pH values more than 8.0 and less than 4.0. Therefore, urea recovers in the range of pH 5.0–8.0, and the minimum possible temperature reduces the losses of product. In non-aqueous solvents, isopropanol showed the best effect in retarding the decomposition of urea. Within the experimental range of temperature and initial urea concentration values, the lowest urea degradation was performed with lactate buffer pH 6.0.

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