Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 273 (2004) 149–158
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Influence of magnesium glutamate on stability of penicillin G aqueous solution

A. Michnik [a,*], K. Michalik [a], W. Marcoin [b]

[a] *Department of Medical Physics, Institute of Physics, University of Silesia, ul. Uniwersytecka 4, 40-007 Katowice, Poland*
[b] *Chair and Department of Pharmaceutical Technology, Medical Academy of Silesia, 41-200 Sosnowiec, Poland*

Received 27 June 2003; received in revised form 5 January 2004; accepted 6 January 2004

Abstract

Differential scanning microcalorimetry (DSC) has been used to determine the influence of magnesium glutamate on the stability of penicillin G in aqueous solution. The degradation of penicillin is accompanied by an evolution of heat and has been observed as an irreversible, scan rate dependent, broad exothermic transition. The increase of the transition temperature T_m and enthalpy change ΔH with increasing magnesium glutamate concentration indicates the increase of penicillin G stability. The kinetic parameters describing the penicillin decomposition process, obtained for a reaction following a first-order course, suggest maximum penicillin G stability if about two molecules of salt per one penicillin molecule are present in solution.

Keywords: DSC; Penicillin aqueous solution; Stability; Thermal decomposition

1. Introduction

Penicillins are a class of β-lactam antibiotics, a group of antibacterial compounds inhibiting bacterial cell wall synthesis. They are highly sensitive to heat, acids and penicillinases. The degradation of penicillins is influenced by different factors like temperature, pH, ionic strength, metal ions, degree of crystallinity, solvent composition (Arnott and Weatherley, 1995; Degelaen et al., 1979; Gensmantel et al., 1978, 1980; Kheirolomoom et al., 1999; Pikal et al., 1978; Sher et al., 1997). Numerous reports show that the degradation of penicillin in solution can be retarded in the presence of certain chemical additives (Hou and Poole, 1971). The nature of the degradation products is modified by the pH of the solution (Bird et al., 1986; Blaha et al., 1976; Gensmantel et al., 1978). Between pH 4 and 8 the initial product, penicillenic acid, undergoes a rapid base-catalysed hydrolysis to penicilloic acid which is formed directly above pH 8. The degradation of benzylpenicillin in unbuffered aqueous solution produces *N*-formylpenicillamine (a maximum rate and extent of conversion occurs at pH 5). Very little penicillamine is formed under these conditions (Bird et al., 1986). Penicillin G is more unstable at higher temperatures and at pH values more than 8.0 and less than 4.0 (Kheirolomoom et al., 1999). Its decomposition reaction in aqueous solution obeys first-order irreversible kinetics at broad pH and temperature ranges (Kheirolomoom et al., 1999).

Problem of penicillin stability in aqueous solution is still topical issue. Chemical substances which could improve this stability are still searching for. The thing is that such substances should not be harmful

* Corresponding author. Tel.: +48-32-2588211; fax: +48-32-2588431.
E-mail address: michnik@us.edu.pl (A. Michnik).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.01.002

but on the contrary, they should be working on the human body adventage. Application of antibiotic therapy causes weakening of immunological system, accompanied by magnesium deficiency. In such situation antibiotics are sometimes coadministered with magnesium supplements. Magnesium may impair the properties of several drugs (Durlach, 1993). Inorganic magnesium ions reduce the bioavailability of antibiotics (Satterwhite et al., 1992). The most widely used magnesium salt in parenteral magnesium therapy is magnesium sulphate. This salt has, however, among the soluble salts of magnesium the least advantageous pharmacological properties (Durlach, 1993). Magnesium sulphate belongs to the group of osmotic (Laxantia) and in digestive tract it absorbs in minimal degree. Administered parenterally $MgSO_4$ causes narcotic effect by inhibiting activities of centrifugal and circumferential nervous system. A significant association between high-dose tocolytic magnesium sulphate and perinatal mortality was found (Scudiero et al., 2000). At the pharmacological level it appears more important to determine which are the most active and useful magnesium salts. The organic salts seem to be promising, though it was reported that inorganic magnesium salts would have bioavailability equivalent to organic magnesium salts (Firoz and Graber, 2001). The magnesium ions better penetrate through biological membranes in form of chelates. For magnesium supplementation Mg chelate with aminoacids are the best to ensure the highest percentage of Mg absorption. The specific properties of anions of organic salts, i.e. acetate, citrate, methionate, aspartate, lactate, glutamate may have their own importance (Marcoin and Szulc, 2002). Taking into consideration foregoing information one could ask a question about influence of glutMg on penicillin.

The calorimetric approach would be useful in kinetic studies on decomposition processes of drugs and substances of pharmaceutical interest. Thermal stability of antibiotics solutions can be investigated by differential scanning microcalorimetry (DSC) both in conventional mode—at controlled increase of temperature in desirable range, and with use of the option of isothermal measurement (Beezer et al., 1999; Rodante et al., 2002; Selzer et al., 1998).

The aim of the present study was to examine the influence of magnesium glutamate (glutMg) on the stability of penicillin G aqueous solution. For better understanding of the nature of penicillin stability modification by glutMg and the role of magnesium ion and glutamate part in this process, the effect of two other magnesium salts: chloride and sulphate was investigated. DSC and UV-Vis spectrophotometry were used as the main and assistant measurement methods, respectively.

2. Materials and methods

2.1. Materials

Penicillin G Benzatine Salt was purchased from Sigma Chemical Co. (Lot 50H0450). Magnesium glutamate (glutMg) was produced by Marcoin et al. as described earlier (Marcoin and Ryszka, 1991). Magnesium chloride and magnesium sulphate anhydrous pure were purchased from POCH, Gliwice (Poland).

Aqua pro injection was used as solvent in all experiments.

2.2. Preparation of samples

An accurately weighed amount of penicillin was dissolved in distilled water or in appropriate magnesium salt solution to produce a final concentration of $0.33\,mM\,l^{-1}$. Magnesium salt solutions were prepared with concentrations in the range from 0.1 to $0.5\,mg\,ml^{-1}$. pH of solutions was 5.5 ± 0.3.

2.3. Measurements

DSC scans were performed using the VP DSC ultrasensitive microcalorimeter (MicroCal Inc., Northampton, MA) with cell volumes at 0.5 ml. Heat capacity versus temperature profiles were obtained for scanning rates of 0.67, 1 and $1.5\,^{\circ}C\,min^{-1}$ in the temperature range 20–130 °C. Additional constant pressure of about 1.8 atm over the liquids in the cells was applied. Samples were degassed immediately prior to loading the cells. An aliquot of the solution was loaded into the calorimeter 40 min after the dissolution of the penicillin. The sample was initially heated from 20 to 30 °C. Next, after rapid cooling, the sample was scanned three times in the whole temperature range. The pre-heating DSC curve overlapped with the adequate initial fragment of DSC curve obtained in the

first full range scan. It ascertained us that all penicillin got dissolved in 20 °C.

UV-Vis spectra of fresh and aged penicillin solutions were recorded in the wavelength range of 190–500 nm on JASCO V-530 spectrophotometer with 2 nm band-width.

2.4. Analysis

The calorimetric data were corrected for the instrumental baseline and for the difference in heat capacity between the initial and the final state by using a linear baseline. DSC curves were analysed with MicroCal Origin software.

Statistical analysis of the results was done with Statistica 5.1 using ANOVA. Schapiro–Wilk and Levene tests were performed to check the normality of the distributions and homogeneity of variance, respectively.

3. Results

The representative original DSC recordings of the two subsequent penicillin scans and water–water scan are shown in Fig. 1. It should be noted that the observed thermal transitions are not reproducible on reheating a sample. Below 110 °C the shape of the repeat scan is almost identical to the baseline. It is noteworthy that the thermal profile of a third scan (not shown) of the same sample is essentially identical to the profiles of the second scan.

The DSC curves in Fig. 2 were obtained by subtracting the profile of the second penicillin scans from the profile for the first scans. The DSC curves for all penicillin G solutions show relatively small and broad endothermic transitions between 35 and 65 °C followed by a marked exothermic peak. It seems most likely that the observed endothermic transition corresponds to the polymerisation reaction. It was reported that in an aqueous solution penicillin G polymers were formed (Ueno et al., 1984). Taboada et al. (2002) showed that the process of aggregation formation of penicillin V in aqueous solutions was endothermic with a positive value (about 0.8 kJ mol^{-1}) for the enthalpy change at 25 °C. In this work the endothermic peak will not be analysed in detail.

The exothermic peak can be attributed to decomposition of drug. The transition temperature of the exothermic process increases with the rise of the scan rate. This scan-rate dependence and the fact that calorimetric traces were found to be irreversible indicate that process is kinetically controlled.

Fig. 3 illustrates the effect of glutMg concentration on DSC curves of penicillin G. All curves were obtained at constant scan rate 1 °C min^{-1}. As seen in Fig. 3a, the transition temperature T_m of the

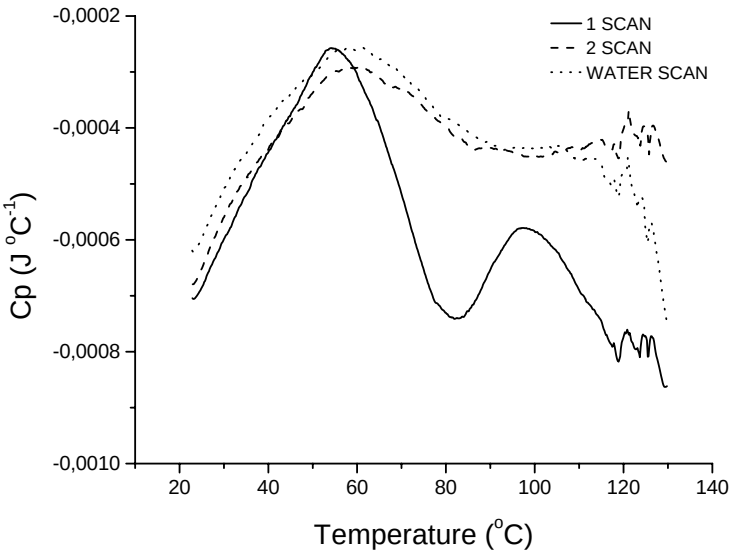

Fig. 1. The raw heat capacity data for penicillin G aqueous solution (concentration 0.33 mM l^{-1}, scan rate 1 °C min^{-1}).

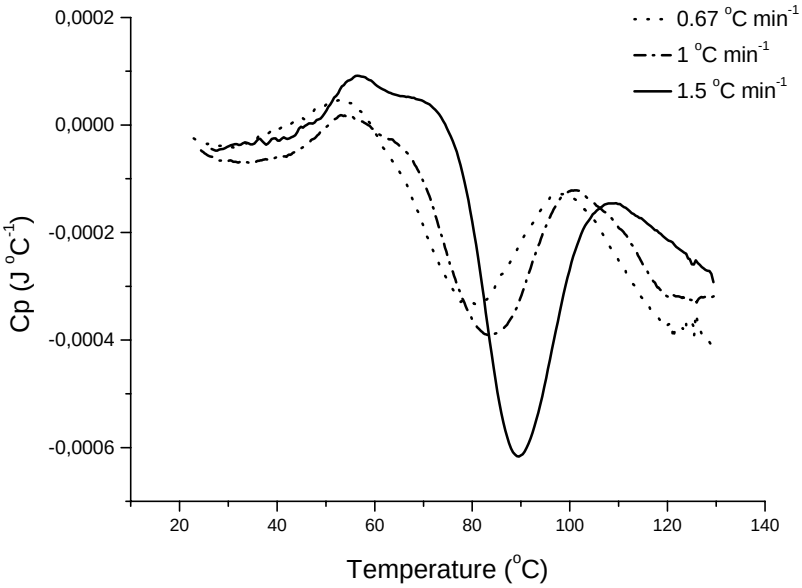

Fig. 2. DSC curves of the relative heat capacity vs. temperature for penicillin G aqueous solution (0.33 mM l^{-1}) at different scan rates.

exothermic process increases with the rise of the glutMg concentration. At about 0.2 mg ml^{-1} a saturation phenomenon is observed. The minima in DSC profiles for glutMg concentrations between 0.2 and 0.5 mg ml^{-1} are very similar (Fig. 3b). The average values of changes of T_m and ΔH (representing the total heat of reaction) with glutMg concentration are shown in Table 1. The differences for T_m are statistically essential ($P \ll 0.05$) between all concentration groups with glutMg concentration $\leq$0.2 mg ml^{-1}. The differences are not essential between groups with glutMg concentration above 0.2 mg ml^{-1}. Taking into account ΔH, statistical analysis shows essential differences only between "0" (penicillin aqueous solution) and concentration groups with glutMg $\geq$0.3 mg ml^{-1}.

Table 1
The transition parameters (T_m and $\Delta H \pm$ S.E.M.[a]) for penicillin G at different glutMg concentrations

glutMg concentration (mg ml^{-1})	T_m (°C)	ΔH (kJ mol^{-1})
0	86 ± 1	45 ± 4
0.1	92 ± 1	45 ± 5
0.15	101 ± 3	49 ± 5
0.2	107 ± 1	48 ± 6
0.3	107 ± 1	56 ± 5
0.5	110 ± 2	57 ± 5

[a] S.E.M.: standard error of the mean.

DSC curves presented in Fig. 4 show that in the case of magnesium chloride and magnesium sulphate, the minimum of the penicillin exothermic peak does not shift to higher temperature in comparison with water solution.

It was assumed that the observed irreversible exothermic process can be represented as:

$$A \xrightarrow{k} B$$

where A is the initial state, B is the final state and k is a first-order kinetic constant, which changes with temperature according to the Arrhenius equation. From the kinetic equation one derives (Sanchez-Ruiz et al., 1988):

$$k(T) = \frac{\nu c_p(T)}{[Q_t - Q(T)]}$$

where ν (K min^{-1}) stands for the scan rate, c_p for the excess heat capacity, Q_t for the total heat of the process, and Q for the heat evolved at a given temperature, T.

The energy of activation can be calculated from the slope of the Arrhenius plot, $\ln k$ versus $1/T$—we name this method "A". In method "B" we calculated the activation energy from the heat capacity at the

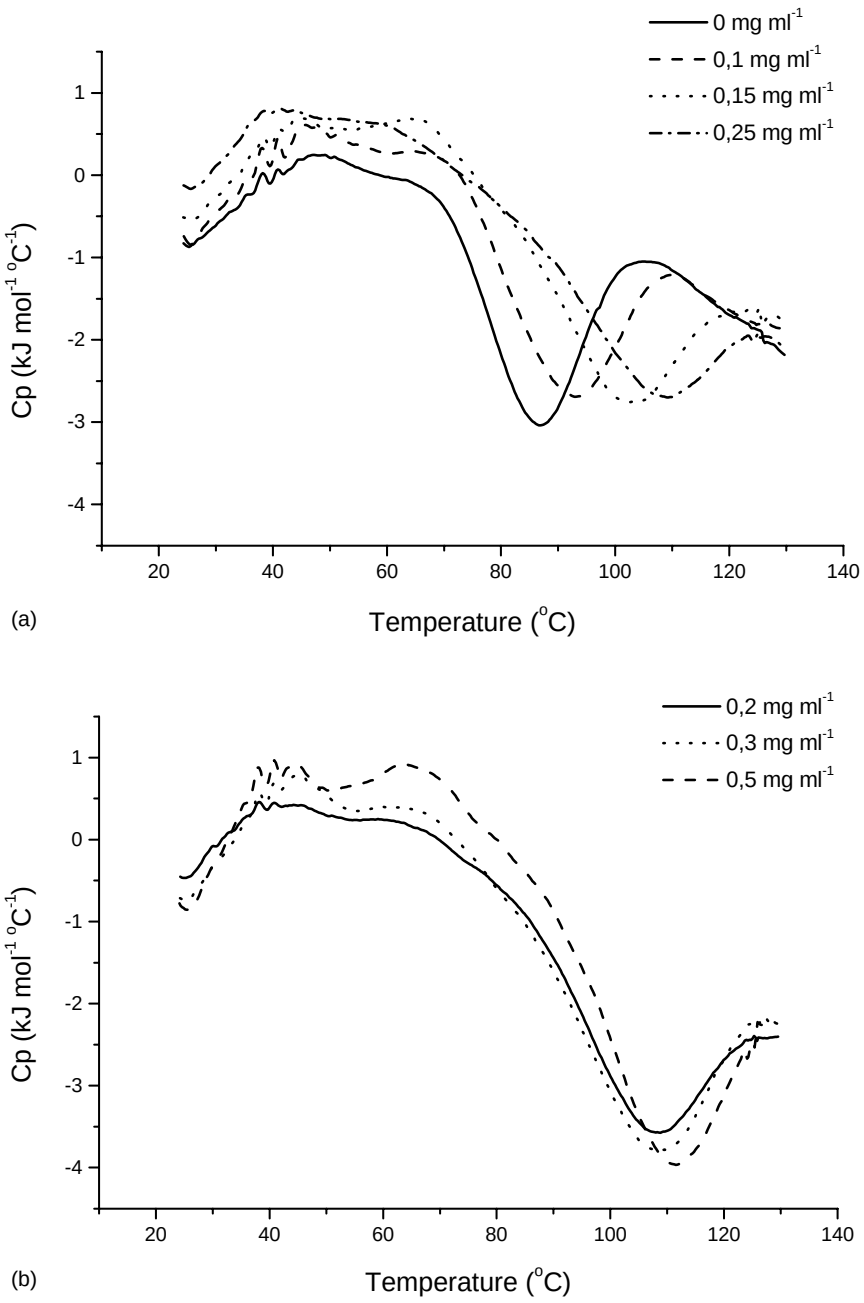

Fig. 3. Effect of glutMg concentration on DSC curves of penicillin G (penicillin concentration 0.33 mM l^{-1}, scan rate 1 °C min^{-1}).

minimum of the trace, c_p^m, according to

$$E = \frac{eRc_p^m T_m^2}{Q_t}$$

where T_m is the temperature value corresponding to the minimum of the heat capacity peak, e is the base of natural logarithm, R is the gas constant (see Appendix in Sanchez-Ruiz et al., 1988).

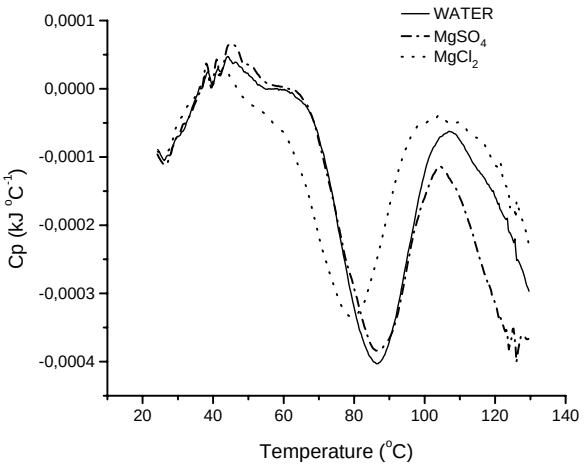

Fig. 4. DSC curves of penicillin G in aqueous (—) and 0.1 mg ml^{-1} salt solutions: magnesium sulphate (–·–·–), magnesium chloride (· · ·); scan rate 1 °C min^{-1}.

Fig. 5 shows the mean activation energies obtained by methods A and B. The errors (standard deviations and standard errors of the mean) associated with the calculated values are also presented. The tendency of the activation energy to decrease with increasing glutMg can be seen. The differences are statistically essential between "0" and "0.2–0.3" mg ml^{-1} (for method "A" $P = 0.01$, 0.04, 0.03 in NIR, Scheffe and RIR Tukey tests, respectively, for method "B" $P = 0.02$, 0.05, 0.04 in NIR, Scheffe and RIR Tukey tests, respectively). The differences between methods "A" and "B" are not statistically essential except that for "0.1–0.15" glutMg concentration range ($P = 0.0498 < 0.05$).

The calculations of the activation energy were also done for averaged scans (MicroCal Origin gives such possibility) obtained at each particular glutMg concentration. The results of methods "A" and "B" are listed in Table 2. We did not show the errors in this case because we were not able to determine the error associated with averaging of scans. The natural logarithm of k versus the reciprocal of the absolute temperature T^{-1} (K^{-1}) plotted for different glutMg concentrations are shown in Fig. 6. These plots were extrapolated to lower temperatures in order to predict the room temperature stability of penicillin solutions. The values of the rate constants k and half-lives at 20, 25 and 37 °C are presented in Table 3. The lowest rate constant was found for 0.2 mg ml^{-1} glutMg. At this salt concentration the estimated half-life time of penicillin solution

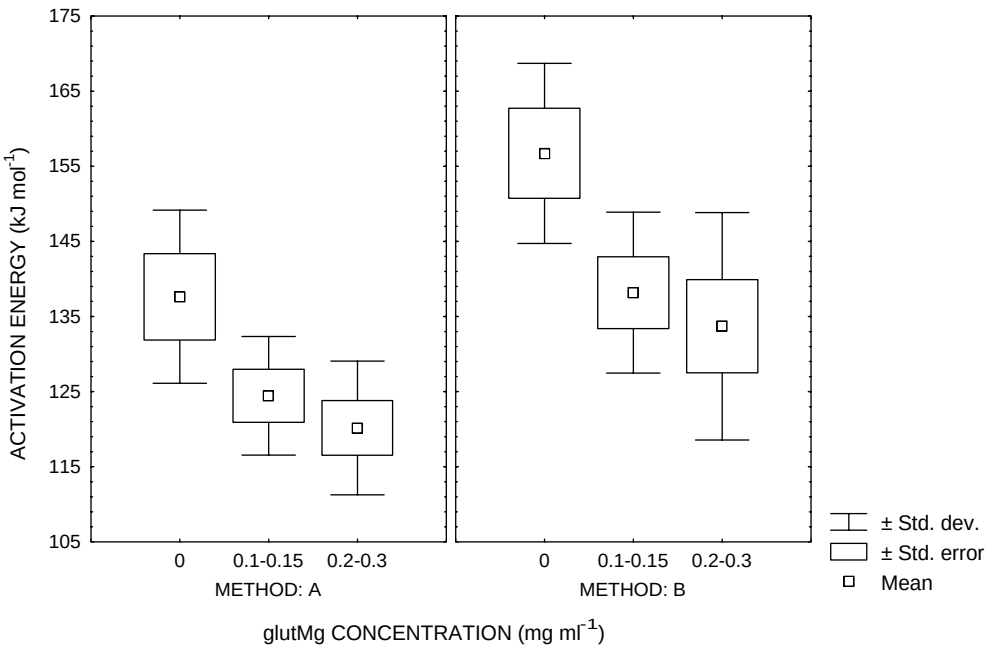

Fig. 5. Activation energies calculated by methods A and B.

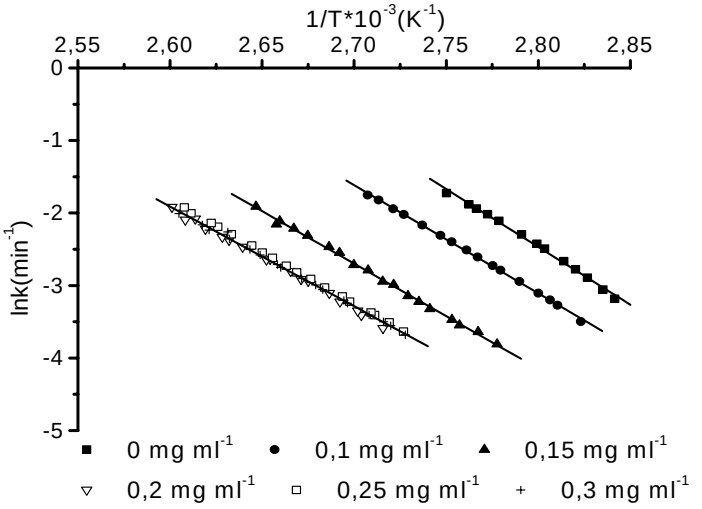

Fig. 6. The Arrhenius plots for penicillin G solutions at different glutMg concentrations.

at temperature 25 °C is about 170 days, i.e. 4.5 times longer than in pure water.

In Fig. 7 the UV-Vis spectrum of aged in room temperature penicillin aqueous solution is compared with the spectra of the samples: heated to 80 °C and to 130 °C during DSC experiment. A maximum at about 320 nm, characteristic for penicillenic acid (Gensmantel et al., 1978) can be seen in the first two spectra. The third spectrum indicates the formation of other penicillin degradation products during DSC runs.

Table 2
The activation energies obtained by methods A and B for averaged at given glutMg concentration penicillin G scans performed at scan rate 1 °C min^{-1}

glutMg concentration (mg ml^{-1})	Method	Activation energy (kJ mol^{-1})
0	A	132
	B	161
0.1	A	124
	B	147
0.15	A	118
	B	127
0.2	A	120
	B	135
0.25	A	116
	B	126
0.3	A	113
	B	122

Table 3
glutMg concentration dependence of the rate constant (k) and half-life ($t_{1/2}$) obtained at 20, 25 and 37 °C

glutMg concentration (mg ml^{-1})	T (°C)	k (min^{-1})	$t_{1/2}$ (days)
0	20	5.1×10^{-6}	96
	25	1.2×10^{-5}	39
	37	9.7×10^{-5}	5
0.1	20	4.7×10^{-6}	101
	25	1.1×10^{-5}	43
	37	7.7×10^{-5}	6
0.15	20	3.2×10^{-6}	149
	25	7.1×10^{-6}	67
	37	4.3×10^{-5}	11
0.2	20	1.2×10^{-6}	392
	25	2.8×10^{-6}	171
	37	1.8×10^{-5}	26
0.25	20	1.7×10^{-6}	276
	25	3.8×10^{-6}	123
	37	2.4×10^{-5}	20
0.3	20	2.3×10^{-6}	204
	25	5.1×10^{-6}	94
	37	2.9×10^{-5}	16

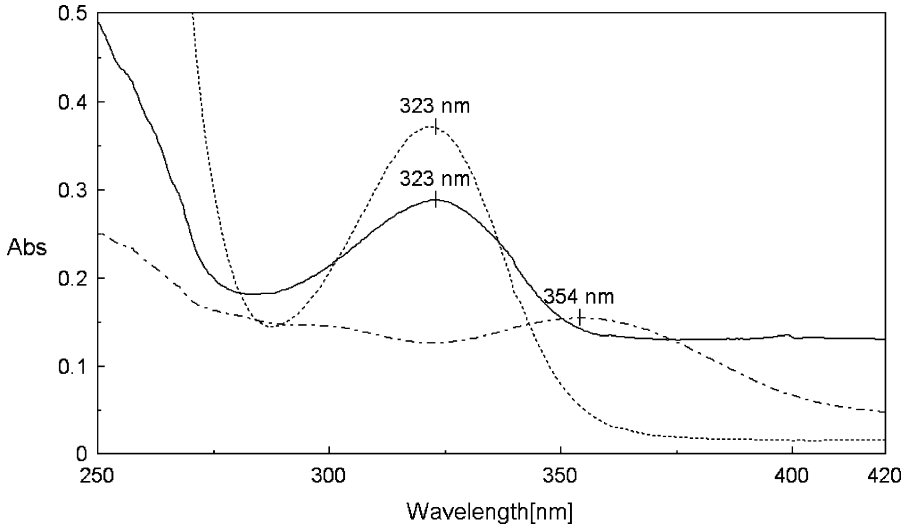

Fig. 7. UV-Vis spectra of penicillin G aqueous solutions: (—) stored 1 week in 20 °C, (···) heated to 80 °C, (–·–·–) after DSC experiment.

4. Discussion

The decomposition of drug is usually accompanied by an evolution of heat (Pikal and Dellerman, 1989; Selzer et al., 1998). An effective exothermic enthalpy of reaction is the sum of the endothermic bond breaking and the exothermic bond forming reaction. The rupture of the β-lactam ring is probably exothermic (Pikal and Dellerman, 1989) because of the large amount of strain energy of the four membered ring which is released upon carbon–nitrogen bond fission. Penicillins have been subjected to facile cleavage of their β-lactam bonds in aqueous solution (Hou and Poole, 1971).

The heats of reaction for the dry solids are roughly a factor of 5 higher than the values for the corresponding solutions. Heats of reaction on the order of several hundred kJ mol^{-1} were observed for decomposition of cephalosporins in solid states (Pikal and Dellerman, 1989).

The results of our experiment show that decomposition of penicillin G in aqueous solutions is exothermic with enthalpy change about 45 kJ mol^{-1} (Table 1).

Gensmantel et al. (1978) have studied the kinetic of the decomposition of benzylpenicillin in water at various pH at 30 °C. At pH 5.4, the rate constant $0.079 \times 10^{-5}\ s^{-1}$ ($4.7 \times 10^{-5}\ min^{-1}$) was obtained for reaction following a first-order course. The calculated by us at 30 °C mean value of k is $4.9 \times 10^{-5}\ min^{-1}$ with 95% confidence interval (1.6×10^{-5} and $8.3 \times 10^{-5}\ min^{-1}$). In the same conditions we obtained the value $3 \times 10^{-5}\ min^{-1}$ for averaged scan of penicillin in water.

In weak acid or in neutral solution, penicillins inevitably undergo transformation to penicillenic acid, which possesses a characteristic UV band near 320 nm, regardless of the type of penicillin. The first-order rate constant k for penicillenic acid formation (at 30 °C, pH 5.4) were calculated from the initial increase in absorbance at 318 nm as $0.118 \times 10^{-5}\ s^{-1}$ (Gensmantel et al., 1978).

Penicillenic acid is very unstable; it quickly isomerises to either penicilloic or penillic acid, depending on the pH of the solution (Hou and Poole, 1971). In our experiment we observed for aged (in room temperature, pH ≈ 5.5) penicillin solutions the increase in absorbance at about 320 nm thus the penicillenic acid formation. However in UV-Vis spectra of penicillin solutions made after DSC measurements this band was shifted to longer wavelengths with maximum at about 350 nm (Fig. 7).

Between pH 4 and 8 the penicillenic acid undergoes hydrolysis to penicilloic acid. At pH about 5 *N*-formylpenicillamine has been reported as a product

of degradation of benzylpenicillin in aqueous solution (Bird et al., 1986; Ueno et al., 1984). The parallel reaction pathways leading to the different potential products of the penicillin degradation are possible (Arnott and Weatherley, 1995; Blaha et al., 1976; Gensmantel et al., 1978; Degelaen et al., 1979). Additionally the formation of the polymers or the aggregates should be taken into account (Taboada et al., 2002; Ueno et al., 1984). This makes for a difficult description of the complex process of penicillin decomposition.

Our experimental results show the increase of T_m and ΔH for the observed exothermic transition (connected with penicillin solution decomposition) with increasing glutMg concentration. It indicates the increase of penicillin G stability in the presence of glutMg. The two other magnesium salts (magnesium chloride and magnesium sulphate) have no similar stabilising effect on penicillin G aqueous solutions, suggesting that the magnesium ion is not the deciding factor.

The glutMg probably makes carbon-β-lactam nitrogen bond fission more difficult. It is possible that penicillin–glutamate complexes are formed. This hypothesis is supported by the fact that observed effect shows a saturation phenomenon with varying glutMg concentration. Taking into account that for $0.33\,mM\,l^{-1}$ penicillin concentration saturation occurs at $0.2\,mg\,ml^{-1}$ glutMg and taking 318.5 as the molecular weight of glutMg, one can conclude that the saturation occurs at glutMg/penicillin molar ratio ∼2. If more than two glutMg molecules per one penicillin molecule occur, the stability of penicillin G solution does not increase further. The calculated rate constants and half-lives (Table 3) indicate that above this molar ratio value (which corresponds to $0.2\,mg\,ml^{-1}$ glutMg), the stability even fell with increasing glutMg concentration. Thus, penicillin G is most stable in solution when it is protected by two salt molecules.

In light of this interpretation a surprise is the obtained glutMg concentration dependence of the activation energy. It is not clear why the activation energy of the penicillin decomposition process shows the tendency to decrease with glutMg concentration increasing. The single-step kinetic approach may be the reason of discrepancy, because several steps with different activation energies may occur in decomposition process of penicillin.

5. Conclusion

The magnesium glutamate hinders the destruction of penicillin G in aqueous solution.

Acknowledgements

We would like to thank Professor Zofia Drzazga for helpful discussion.

References

Arnott, A., Weatherley, L.R., 1995. The stability of penicillin G during recovery by electrically enhanced extraction. Process Biochem. 30, 447–455.

Beezer, A.E., Gaisford, S., Hills, A.K., Willson, R.J., Mitchell, J.C., 1999. Pharmaceutical microcalorimetry: applications to long term stability studies. Int. J. Pharm. 179, 159–165.

Bird, A.E., Jennings, K.R., Marshall, A.C., 1986. *N*-Formylpenicillamine and penicillamine as degradation products of penicillins in solution. J. Pharm. Pharmacol. 38, 913–917.

Blaha, J.M., Knevel, A.M., Kessler, D.P., Mincy, J.W., Hem, S.L., 1976. Kinetic analysis of penicillin degradation in acidic media. J. Pharm. Sci. 65, 1165–1170.

Degelaen, J.P., Loukas, S.L., Feeney, J., Roberts, G.C.K., Burgen, A.S.V., 1979. A nuclear magnetic resonance study of degradation of penicyllin G in acidic solution. J.C.S. Perkin II, 86–90.

Durlach, J., 1993. Present and future of magnesium research. J. Jpn. Soc. Mag. Res. 12, 113–135.

Firoz, M., Graber, M., 2001. Bioavailability of US commercial magnesium preparations. Mag. Res. 14, 257–262.

Gensmantel, N.P., Gowling, E.W., Page, M.I., 1978. Metal ion catalysis in aminolysis of penicyllin. J.C.S. Perkin II, 335–342.

Gensmantel, N.P., Proctor, P., Page, M.I., 1980. Metal-ion catalysed hydrolysis of some β-lactam antibiotics. J.C.S. Perkin II, 1725–1732.

Hou, J.P., Poole, J.W., 1971. β-Lactam antibiotics: their physicochemical properties and biological activities in relation to structure. J. Pharm. Sci. 60, 503–532.

Kheirolomoom, A., Kazemi-Vaysari, A., Ardjmand, M., Baradar-Khoshfetrat, A., 1999. The combined effect of pH and temperature on penicillin G decomposition and its stability modelling. Process Biochem. 35, 205–211.

Marcoin, W., Ryszka, F., 1991. Selected magnesium compounds of expected pharmacological activity. Ann. Acad. Med. Siles. 23, 45–53.

Marcoin, W., Szulc, B., 2002. Influence of anions aminoacids on the absorption process of Mg^{2+} ions in vitro. Sci. Pharm. 70, 29–37.

Pikal, M.J., Dellerman, K.M., 1989. Stability testing of pharmaceuticals by high–sensitivity isothermal calorimetry at 25 °C: cephalosporins in the solid and aqueous solution states. Int. J. Pharm. 50, 233–252.

Pikal, M.J., Lukes, A.L., Lang, J.E., Gaines, K., 1978. Quantitative crystallinity determinations for β-lactam antibiotics by solution calorimetry: correlations with stability. J. Pharm. Sci. 67, 767–772.

Rodante, F., Catalani, G., Vecchio, S., 2002. Kinetic analysis of single or multi-step decomposition processes. Limits introduced by statistical analysis. J. Therm. Anal. Cal. 68, 689–713.

Sanchez-Ruiz, M.J., Lõpez-Lacomba, J.L., Cortijo, M., Mateo, P.L., 1988. Differential scanning calorimetry of the irreversible thermal denaturation of thermolysin. Biochemistry 27, 1648–1652.

Satterwhite, J.H., Cerimele, B.J., Coleman, D.L., Hatcher, B.L., Kisicki, J., DeSante, K.A., 1992. Pharmacokinetics of cefaclor AF: effect of age, antacids and H2-receptor antagonists. Postgrad. Med. J. 68, 3–9.

Scudiero, R., Khoshnood, B., Pryde, P.G., Lee, K.S., Wall, S., Mittendorf, R., 2000. Perinatal death and tocolytic magnesium sulfate. Obstet. Gynecol. 96, 178–182.

Selzer, T., Radau, M., Kreuter, J., 1998. Use of isothermal heat conduction microcalorimetry to evaluate stability and excipient compatibility of a solid drug. Int. J. Pharm. 171, 227–241.

Sher, A., Veber, M., Marolt-Gomišćek, M., 1997. Spectroscopic and polarographic investigations: copper (II)-penicillin derivatives. Int. J. Pharm. 148, 191–199.

Taboada, P., Perez-Rodriguez, M., Varela, L.M., Barbosa, S., Garcia, M., Mosquera, V., 2002. Permitivity of penicillin V: a thermodynamic study. Thermochim. Acta 394, 39–43.

Ueno, H., Nishikawa, M., Suzuki, S., Muranake, M., 1984. Chromatographic separation and chemical analysis of polymers formed by penicillin G. J. Chromatogr. 288, 117–126.