Effect of Buffer Solution and Temperature on the Stability of Penicillin G

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The effect of medium, molar ratio of buffer to penicillin G sodium, pH, and temperature on the stability of penicillin G sodium was investigated. The stability of penicillin G sodium in various media is in the order of: citrate buffer > acetate buffer > phosphate buffer > sodium bicarbonate > 0.9 % NaCl and 5 % glucose. In the case of the citrate buffer, to maintain stability for a long time, the molar ratio should be higher than 0.75. The degradation kinetics of penicillin in citrate buffer was determined over a pH range of 4.0 to 10.0 and temperatures from (5 to 50) °C. By plotting the data of log k versus pH at constant temperature, V-shaped curves were obtained with a minimum around pH 7.0. The plots of ln k versus $1/T$ at constant pH are linear, and the lines at all pH values are almost parallel to each other. By fitting to the Arrhenius equation, the activated energy obtained is 83.5 kJ·mol^{-1}·K^{-1}. The best condition to maintain reasonable stability of penicillin G sodium is using the citrate buffer solution with a molar ratio of buffer to penicillin ≥ 0.75, pH ≈ 7.0, temperature ≤ 25 °C.

1. Introduction

β-Lactam antibiotics are among the most widely used antibacterial agents on account of their bactericidal activity, broad spectrum, low toxicity, and excellent distribution throughout the body.[1] The earliest generation of these compounds was composed of penicillins which have wide usage for their antimicrobial activity against both gram-positive and gram-negative organisms. The basic structure of penicillin consists of a thiazolidine ring, an attached β-lactam ring, and a side chain. The properties of penicillin, such as the antibacterial spectrum, β-lactamase susceptibility, and pharmacokinetics could be altered through manipulation of the side chain. Throughout the years, penicillin has remained a useful antibiotic against many bacteria for which it was initially introduced.

The stability of penicillin has to be taken into account during its production, storage, and use. The problem of penicillin stability in aqueous solution is still a topical issue. Dominating reactions of penicillin are at the highly labile β-lactam ring carbonyl at the 7-position, usually by nuclear attack. The penicillins are not stable in the presence of acid, alkaline, or penicillinases.[2–5] Just as the route by which penicillins exert their antibacterial activity, opening of the β-lactam ring has been shown to be the primary mode of degradation of penicillins in acid media. Penicillin G is thought to be degraded to penicillenic acid initially, which is a short-lived intermediate, and then further to a range of products. The degradation rate of penicillin salts is also dependent upon its purity grade.[6] Once acid hydrolysis begins, it is autocatalytic, owing to formation of acid degradation products. Penicillin G is susceptible to polymer formation by cleavage of the β-lactam ring, and this is one of the causes of their acquisition of antigenicity and allergenicity.[7]

The stability of penicillin G in aqueous solutions has been investigated in a few studies.[8–10] Lindsay and Tsuji have studied the effect of surfactants and ionic strength on the degradation of penicillins.[11,12] Ong found that hydroxypropyl β-cyclodextrin can enhance the stability of penicillin G in an acidic environment.[13] Zhao investigated the effects of temperature and moisture on the stability of penicillin.[14] Das Gupta and Michnik have studied the effect of magnesium supplements, which are sometimes coadministered with antibiotics, on the stability of penicillin G.[15,16] The deterioration of penicillin was particularly rapid when it was stored over a prolonged period in warm and moist rooms.[17] In fact, when the penicillin salts were stored in a sealed ampule in which the moisture and heavy ions like Zn^{2+} or Cu^{2+} were totally excluded, degradation of penicillin was negligible even after very prolonged storage at room or slightly higher temperature.

By the conventional manufacture process, penicillin is extracted from the metabolism solution by an organic solvent at low pH values and re-extracted by alkali solution. Further purification is carried out by gel filtration using buffer solutions as the mobile phase. During injection, 0.9 % sodium chloride and 5 % glucose aqueous solution are usually used as infusion fluids. Therefore, it is of significance to study the effect of various kinds of buffer solution and aqueous solutions of sodium chloride solution and glucose on the stability of penicillin. Furthermore, during the processing of penicillin, the pH value of the buffer solution and temperature should be considered as well. The present work intended to study systematically the effect of all these factors on the stability of penicillin. The methodology was to determine the kinetics of the degradation reaction of penicillin under various combinations of the factors

Table 1. Percent of Mobile Phase A and B at Time t for Determination of PGS

t/min	mobile phase A[a]	mobile phase B[b]	notes
0 to 20	100 → 0	0 → 100	linear gradient
20 to 45	0	100	Isocratic
45 to 58	100	0	displacement

[a] Mobile phase A: Mix 10 volumes of a 68 g·L^{-1} solution of potassium dihydrogen phosphate adjusted to pH 3.5 with a 500 g·L^{-1} solution of dilute phosphoric acid, 30 volumes of methanol, and 60 volumes of water. [b] Mobile phase B: Mix 10 volumes of a 68 g·L^{-1} solution of potassium dihydrogen phosphate adjusted to pH 3.5 with a 500 g·L^{-1} solution of dilute phosphoric acid, 50 volumes of methanol, and 40 volumes of water.

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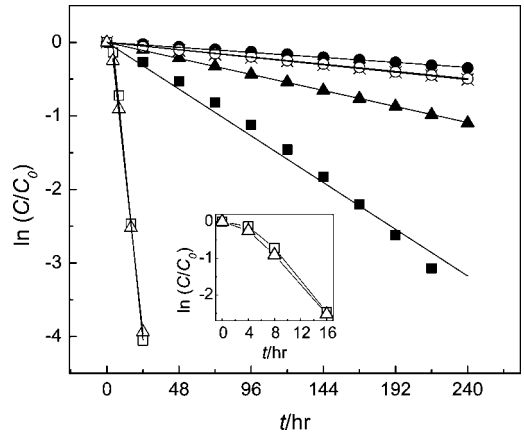

Figure 1. Plots of $\ln(C/C_0)$ of PGS vs time t in various media at 37 °C. •, sodium citrate buffer (pH = 7.0); ○, sodium acetate buffer (pH = 7.0); ×, ammonium acetate buffer (pH = 7.0); ▲, sodium phosphate buffer (pH = 7.0); ■, sodium bicarbonate (pH = 8.44); □, 0.9 % sodium chloride (pH = 5.72); Δ, 5 % glucose (pH = 4.76).

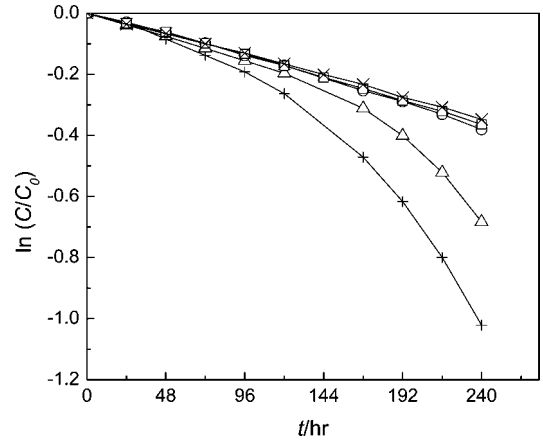

Figure 2. Plots of $\ln(C/C_0)$ of PGS samples versus time t in citrate buffer with various MR at 37 °C. Concentration of PGS: 3.37 mM. MR: ×, 10; □, 1; ○, 0.75; Δ, 0.5; +, 0.25.

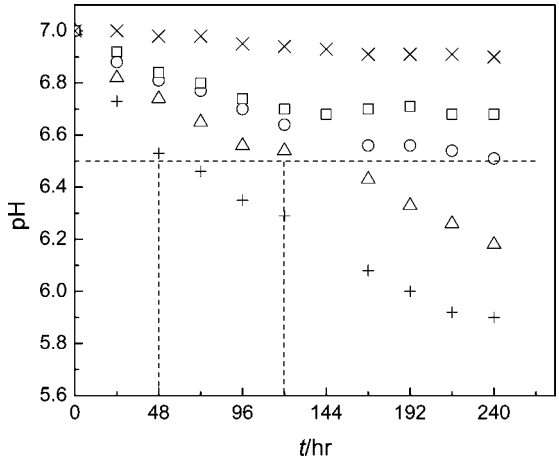

Figure 3. Plots of the pH values of PGS samples versus time t in citrate buffer with various MR at 37 °C. Concentration of PGS: 3.37 mM. Molar ratio: ×, 10; □, 1; ○, 0.75; Δ, 0.5; +, 0.25.

mentioned above. On the basis of the results, correlations between the stability of penicillin and the factors could be quantitatively obtained.

2. Materials and Methods

2.1. Chemicals and Reagents. Penicillin G sodium (PGS) was purchased from the North China Pharmaceutical Group Co., Shijiazhuang, China. Methanol was HPLC grade (Tedia Co., Inc., USA). Ultrapure water was freshly prepared by a Nanopure Barnstead deionization system (Barnstead/ Thermodyne. Co., Dubuque, USA) and was boiled before use. All other reagents were of AR grade.

2.2. Experiments of Degradation Kinetics. The experimental procedures of the degradation kinetics of penicillin were as follows.

Effect of Media. The media studied were 0.9 % sodium chloride and 5 % glucose aqueous solutions and 0.06 M buffer solutions of sodium citrate, sodium acetate, ammonium acetate, sodium phosphate, and sodium bicarbonate. PGS (300 mg) was weighed into a 250 mL volumetric flask, and one of the prepared media was added into the volumetric flask to graduation. The flask was shaken to dissolve the PGS. The concentration of PGS in the obtained solution was 1.2 $mg \cdot mL^{-1}$ (3.37 $mmol \cdot L^{-1}$) for all the media. The solution was transferred into a 250 mL Erlenmeyer flask. The flasks with the PGS solutions were set in an air thermostat with a rocking mechanism of 100 rpm, and the temperature was maintained at 37 °C. Every 24 h, the flask was moved into a water bath at 37 °C; about 2 mL of sample was taken by a syringe; and then the concentration of PGS was determined by HPLC immediately.

Effect of Molar Ratio of Citrate Buffer to Penicillin. The procedure was the same as mentioned above, but the media were citrate buffers of a series of concentration of (0.84, 1.68, 2.52, 3.37, and 33.7) $mmol \cdot L^{-1}$. The corresponding molar ratios of the buffer to penicillin were 0.25, 0.5, 0.75, 1, and 10 respectively. The pH of the solution was determined by a pH meter before taking the sample.

Effect of pH and Temperature. Trisodium citrate solution (0.06 M) was prepared in a beaker. The beaker was kept in a thermostat at a definite temperature of (5, 15, 25, or 37) °C. Buffer solutions with different pH values were prepared by adding citric acid while monitoring with a pH meter. The prepared buffers were used as the media, and the experiments were run the same way as above while the temperature of the thermostat was kept at the temperature desired. The repeatability of the method was examined in the pH range of 4.0 to 10.0 at 37 °C. The deviations of rate constants k were 4.4 %, 3.6 %, 5.2 %, 6.8 %, 4.3 %, 9.1 %, and 8.6 % for pH 4.0, 5.0, 6.0, 7.0, 7.5, 9.0, and 10.0, respectively. Data obtained by this method are repeatable and credible.

2.3. Determination of PGS Concentration by HPLC. The HPLC system consisted of a Waters (Milford, MA, USA) 1525 binary pump, a 717 plus autosampler, a 2487 dual λ absorbance detector, and a thermostat of the column. The chromatographic column was a Waters Sunfire C_{18} column (5 μm, 250 × 4.6 mm I.D.) equipped with a C_{18} guard column. The gradient elution procedure was similar to the method recorded by European Pharmacopoeia[18] as shown in Table 1 (1.0 $mL \cdot min^{-1}$, 254 nm, 30 °C). The injection volume of all the samples was 10 μL. The peak area on the chromatogram of the sample of PGS in the initial solution was determined right after it was prepared. The ratio of the concentration of samples at time t to the initial solution (C/C_0) was taken as the ratio of the peak area of the sample at time t to that of the initial solution.

3. Result and discussion

3.1. Effect of the Medium. As the experiment data show in Figure 1, straight lines of $\ln(C/C_0)$ versus t are obtained. Thus, the PGS concentration decays exponentially, typical of a first-

Table 2. Degradation Rate k of PGS in Temperature T Range of (278.15 to 323.15) K and pH Range of 4.0 to 10.0

	$k \cdot 10^{-4}/h^{-1}$				
pH	T/K = 278.15	T/K = 288.15	T/K = 298.15	T/K = 310.15	T/K = 323.15
4.0	54.0 ± 0.90	211 ± 0.51	822 ± 11	2850 ± 56	7900 ± 110
5.0	7.35 ± 0.094	25.3 ± 0.051	94.0 ± 0.060	331 ± 0.98	935 ± 0.25
6.0	0.891 ± 0.012	3.63 ± 0.014	12.9 ± 0.013	55.0 ± 0.014	141 ± 0.018
6.5	0.521 ± 0.012	1.64 ± 0.060	6.06 ± 0.086	21.5 ± 0.060	67.0 ± 0.065
7.0	0.297 ± 0.057	1.10 ± 0.019	3.93 ± 0.063	14.1 ± 0.019	47.5 ± 0.097
7.5	0.339 ± 0.048	1.29 ± 0.088	4.16 ± 0.058	15.8 ± 0.088	52.6 ± 0.011
9.0	5.85 ± 0.48	30.0 ± 0.90	86.0 ± 0.40	304 ± 0.64	1220 ± 12
10.0	63.5 ± 0.84	215 ± 0.86	754 ± 0.26	2250 ± 22	8620 ± 71

order reaction. The experimental data were fitted to the first order kinetic equation

$$\ln(C/C_0) = -kt \quad (1)$$

where C_0 is the initial concentration of PGS and C is the concentration at time t. As shown in Figure 1, the experimental data fit eq 1 quite well, with all correlation coefficients greater than 0.986.

By comparing the lines in Figure 1, the media can be divided into three groups according to the effect on the degradation rate of PGS.

Aqueous Solution without Buffer Agent, pH < 7 (Sodium Chloride and Glucose Solution). The degradation rate in these media is much greater than in all other buffer solutions, as PGS is almost completely exhausted within 24 h. Furthermore, the kinetic curves show that the difference between the degradation rates of these two media is negligible. It can be seen from the kinetic lines that in the period of the first 4 h the incubation is like an induction for hydrolysis with a small degradation rate of PGS, after the induction the PGS degrades rapidly and $\ln(C/C_0)$ varies linearly with time. The rate constants k are (0.200 ± 0.0058 and 0.187 ± 0.0058) h^{-1} for sodium chloride and glucose, respectively. The pH values of the PGS solution changed significantly during the course of the degradation especially in the first 4 h. This could be attributed to the acid products formed by degradation.

Aqueous Solution without Buffer Agent, pH > 7 (Sodium Bicarbonate Solution). The degradation rate in this medium is rather significant but is much less than in the former media. The rate constant k is $(1.32 \pm 0.013) \cdot 10^{-2}$ h^{-1}. In this alkaline medium, the direction of the change of pH is opposite to that in the acidic medium, as it increases from an initial pH = 8.4 to 8.71 after 10 days at 37 °C. So far this phenomenon cannot be explained.

Aqueous Buffer Solutions, pH = 7 (Phosphate, Acetate, and Citrate). The degradation rates in these media are the least. With regard to the difference between various kinds of buffer solution, the order of the degradation rate is: sodium phosphate > ammonium acetate > sodium acetate > sodium citrate, with rate constants k of [(4.54 ± 0.11), (2.11 ± 0.11), (2.10 ± 0.11), and (1.41 ± 0.11)]$\cdot 10^{-3}$ h^{-1}, respectively. Therefore, citrate is the best buffer to be used during the processing of PGS. In addition, it can be seen that there is no significant difference between the degradation rates in sodium acetate and ammonium acetate. This indicates that the cations, Na^+ and NH_4^+, have no catalytic effect on the degradation process. Since the cation of all the buffers other than ammonium acetate is Na^+, the difference of the effect between the media can be attributed mainly to the anion. The change of pH value is much smaller in buffer media of this group, at 37 °C, and the final pH in the four buffers is in the range of 6.43 to 6.95 after 10 days.

3.2. Effect of the Molar Ratio of Citrate Buffer to Penicillin. Since stability in the citrate buffer solution is the highest among all the media studied, a further study was carried out concerning the effect of molar ratio (MR) of citrate buffer to PGS on the stability of PGS. The results in Figure 2 show that the stability of PGS in general increases with MR. In addition, three kinetic curves with MR ≥ 0.75 are quite close to each other up to 10 days. With regard to the other two cases with MR ≤ 0.5, $\ln(C/C_0)$ decreases linearly with t at a definite time, then the degradation of PGS does not obey the first-order rate anymore. This means that the rate of degradation is accelerated, and such phenomena can be attributed to the decrease of pH values in PGS solutions as shown in Figure 3. The lower the pH values of the solutions are, the faster the PGS degrades. The buffer capacity of the solutions is weak with low MR, so that during the decomposition of PGS the pH values of the solutions decrease greatly with an increasing amount of acid degradation products. The rate of degradation will continue to accelerate until all the PGS has been exhausted.

3.3. Effect of Temperature and pH of the Buffer Solution. The effect of temperature and pH of the buffer solution on the stability of PGS was investigated at (5 to 50) °C. In the pH range of 4.0 to 7.5, citrate was used as the buffer solution, while at pH = 9.0 and 10.0, the buffer was glycine−NaCl−NaOH.

The degradation kinetics data were plotted as $\ln(C/C_0)$ versus time t and fitted to eq 1, and all the correlation coefficients were greater than 0.991. The obtained rate constants are listed in Table 2. It can be seen, for instance, at 5 °C that the degradation rate constant k is $(54.0 \pm 0.90) \cdot 10^{-4}$ h^{-1} at pH = 4.0, then decreases drastically to $(7.35 \pm 0.094) \cdot 10^{-4}$ h^{-1} at pH = 5.0, and further to $(0.891 \pm 0.012) \cdot 10^{-4}$ h^{-1} at pH = 6.0. On the other hand, k decreases drastically from [$(63.5 \pm 0.84) \cdot 10^{-4}$ to $(0.339 \pm 0.048) \cdot 10^{-4}$] h^{-1} as pH decreases from 10.0 to 7.5. Conversely k does not change significantly in the range of pH 6.5 to 7.5. These same phenomena are observed at all other temperatures.

The profile of k versus pH was obtained by plotting log k against pH in the temperature range of (5 to 50) °C (Figure 4). The V-shape of the curves indicates that the degradation of PGS is catalyzed by both a hydrogen ion and a hydroxide ion. The curves are almost symmetrical to the axis of pH 7.0, which implies that the catalysis ability of the hydrogen ion and the hydroxide ion is nearly the same. PGS is most stable in the plateau region of the curves corresponding to the pH range of 6.5 to 7.5. The smallest value of log k at pH 7.0 suggests the greatest stability of PGS in the neutral condition.

Kheirolomoom has studied the combined effects of pH and temperature on penicillin G decomposition,[19] and the degradation rate constants k obtained by Kheirolomoom at pH 7.0 are ten times larger compared with data listed in Table 2. This may be due to the different buffer and different analysis method which were used in the stability studies. Phosphate buffer was

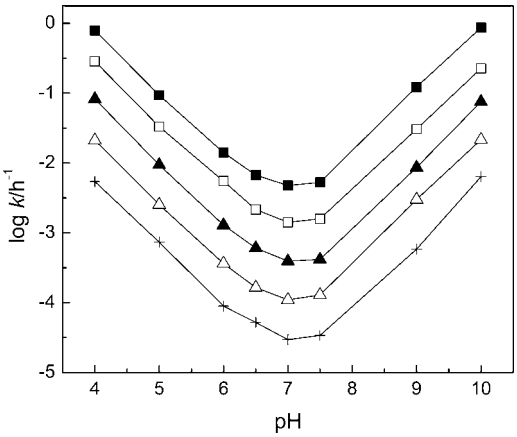

Figure 4. Plots of log k versus the pH at different temperatures for PGS. ■, 50 °C; □, 37 °C; ▲, 25 °C; □, 15 °C; +, 5 °C.

used at the pH range of 6.0 to 8.0 by Kheirolomoom in the research of PGS stability. However, the stability of PGS is lower in phosphate than in citrate which has been shown above. This is the major factor that causes the difference in the degradation rate constants k. In the phosphate buffer solution at 37 °C and pH 7.0, the k obtained by us is $4.54 \cdot 10^{-3}$ h^{-1}, compared with the result of about $1 \cdot 10^{-2}$ h^{-1} obtained by Kheirolomoom. This difference may be attributed to the different analysis method used for determining the concentration of PGS. The hydroxylamine method was used by Kheirolomoom, and the results may be interfered with by the degradation product or phosphate buffer.

Because of the labile β-lactam ring at the 7-position, PGS has limited stability. It degrades through different routes under different pH conditions. This can be proved by the chromatograms of acidic, neutral, and basic solutions as shown in Figure 5. PGS is eluted at 23 min. In acidic solution, PGS initially degrades to benzylpenicillenic acid which is a metastable intermediate tautomeric with penicillin G, and then benzylpenicillenic acid degrades to a series of products,[2,3] like benzylpenillic acid, benzylpenamaldic acid, benzylpenicilloic acid, and benzylpenilloaldehyde. In basic solution, PGS mainly degrades to benzylpenicilloic sodium. In neutral solution, PGS has the maximal stability. Mitsumori[20] studied the degradation of penicillin G in phosphate buffer at pH 7.0 and reached the conclusion that penicillin G degrades to benzylpenicilloic acid, then to secondary products. Jemal[21] found penicillamine as a degradation product at neutral pH and proposed that it originated from benzylpenicillenic acid.

At a definite pH value, the relationship between k and temperature can be expressed with the Arrhenius equation

$$\ln\ k = \ln\ A - \frac{E_a}{R} \cdot \frac{1}{T} \qquad (2)$$

where A is the pre-exponential factor; E_a is the activation energy; R is the universal gas constant; and T is the absolute temperature. The $\ln k \sim 1/T$ plots are shown in Figure 6. The correlation coefficients of the linear regressions are all greater than 0.997. As can be seen from Figure 6, all the lines are almost parallel to each other, indicating that the activation energy is almost independent of pH. The obtained average activation energy is 83.5 $kJ \cdot mol^{-1} \cdot K^{-1}$, with an RSD of 1.97 %.

4. Conclusions

Penicillin G sodium degrades most rapidly in aqueous solution without buffer agent. Penicillin G sodium is much more stable

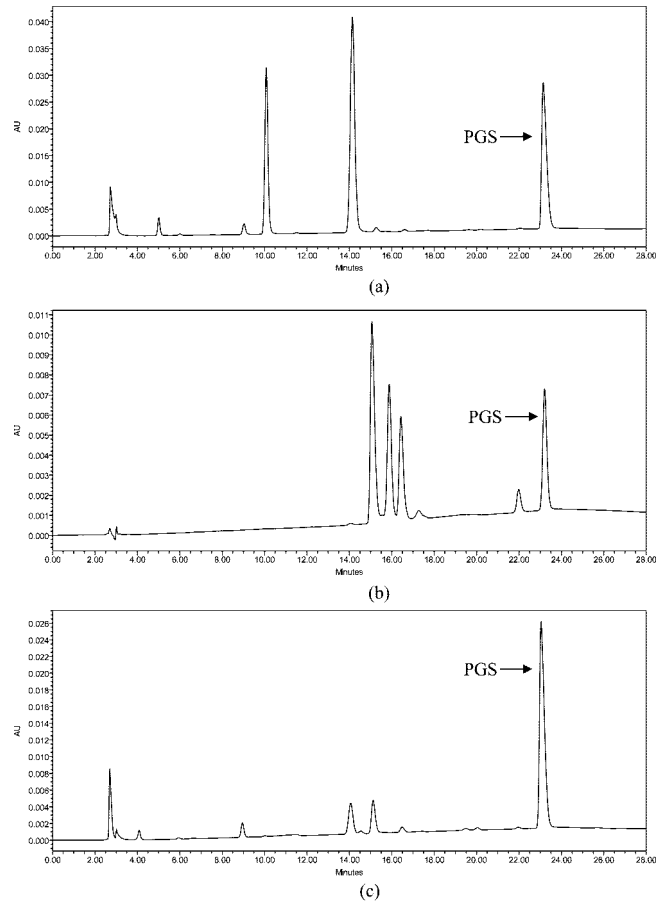

Figure 5. Chromatogram of degraded PGS solutions incubated at 37 °C. Concentration of citrate buffer: 0.06 M. (a) In citrate buffer with pH 5.0 at 8 h. (b) In glycine−NaCl−NaOH buffer with pH 10.0 at 8 h. (c) In citrate buffer with pH 7.0 at 10 days.

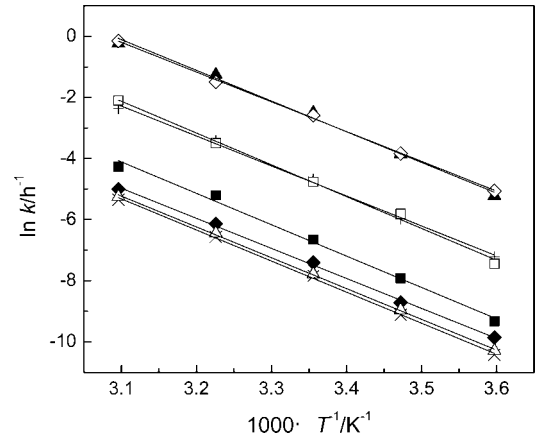

Figure 6. Plots of ln k versus $1000 \cdot T^{-1}$ for PGS solutions at different pH. ▲, pH 4.0; +, pH 5.0; ■, pH 6.0; ♦, pH 6.5; ×, pH 7.0; Δ, pH 7.5; □, pH 9.0; ◇, pH 10.0.

in buffer solution than without buffer agent, and among the buffers examined in this work citrate is the most suitable medium. In citrate buffer, the appropriate pH range of the buffer is 6.5 to 7.5. A higher molar ratio of citrate buffer to penicillin (MR) is better, and a MR value greater than 0.75 is recommended.

Supporting Information Available:

Plots of $\ln(C/C_0)$ of PGS versus time t. ×, 5 °C; □, 15 °C; Δ, 25 °C; ▲, 37 °C; •, 50 °C. This material is available free of charge via the Internet at http://pubs.acs.org.

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