Possibilities to Improve Benzylpenicillin and Phenoxymethylpenicillin Stability in Acidic Environment

Rad I[1], Croitoru MD[2], Gyéresi Á[3]

[1] Dimitrie Cantemir College, Tîrgu Mureş, Romania
[2] Department of Toxicology-Biopharmacy, Faculty of Pharmacy, University of Medicine and Pharmacy, Tîrgu Mureş, Romania
[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy, Tîrgu Mureş, Romania

Background: Benzylpenicillin is water soluble natural penicillin used only by parenteral administration. Fast decomposition in acidic environment is the reason why benzylpenicillin can not be orally used. Phenoxymethylpenicillin is natural penicillin used in therapy by oral administration (filmed tablets, tablets, syrup, and suspension). It is relatively stable in acidic environment and has a bioavailability of about 50%.
Objective: The purpose of this study was to increase the stability of these penicillins in acidic environment (pH = 1.2) by using auxiliary substances: β-cyclodextrin, 2-hydroxypropil-β-cyclodextrin, magnesium glutamate and magnesium aspartate.
Methods: Improvements of stability were measured by high performance liquid chromatography in the absence and presence of the mentioned auxiliary substances.
Results: Cyclodextrins significantly improved the stability of the studied penicillins in acidic environment. Glutamate and aspartate showed no effect on this regard.
Conclusions: Significant improvement of benzylpenicillin and phenoxymethylpenicillin stability and possible pharmacokinetics can be achieved by using cyclodextrins.

Keywords: benzylpenicillin, phenoxymethylpenicillin, stability, decomposition kinetics, cyclodextrins

Introduction

Sodium benzylpenicillin is water soluble natural penicillin conditioned as flasks with powder for injectable solution. Two concentrations are available: 400,000 and 1,000,000 IU [1]. Because of the poor stability in the acidic environment of the stomach this penicillin is used as parenteral administration only [1].

Potassium phenoxymethylpenicillin is water soluble natural penicillin used in therapy as oral administration only as filmed tablets, tablets, syrup [2], and suspension. It is relatively stable to the acidic environment of the stomach. Its bioavailability is about 50%, but coadministered with food leads to a decreased absorption so it should be taken before meal [3]. Pharmacokinetics modification by food intake can lead to poor correlations between dose and therapeutic response and also to increased interindividual variations.

There are studies in the scientific literature that show that there are possibilities to increase the stability of penicillins in the acidic environment. Ampicillin [4–6], amoxicillin [6–8] and benzylpenicillin [6] were stabilized in an acidic environment with a pH of 1.2 by using cyclodextrins [4–8] or magnesium salts of aspartic acid and glutamic acid in different mole ratios [9].

The purpose of this study was to check if benzylpenicillin and phenoxymethylpenicillin can be stabilized in acidic environment. If a significant stability increase occurs benzylpenicillin could be proposed for oral administration and pharmacokinetics of phenoxymethylpenicillin will be greatly improved.

A high pressure liquid chromatography technique (HPLC) was used for concentration measurements, during the decomposition process, in the presence and absence of auxiliary substances [10,11]. Obtained results were used to assess if auxiliary substances are able to change the parameters of the curves that describe the decomposition process.

Materials and methods

Reagents

- Benzylpenicillin sodium (BP), pharmaceutical quality, Antibiotice Romania;
- Phenoxymethylpenicillin potassium (PMP) analytical purity, Sigma Aldrich;
- β-Cyclodextrin (β-CD) and 2-Hydroxypropyl-β-Cyclodextrin (2-HP-β-CD), analytical purity, from Molekula England;
- Clark and Lubs buffer pH 1.2 (HCl – 1/5 M, KCl – 1/5 M, water) [5];
- Phosphoric acid, analytical purity, Merck KgaA;
- Dihydrogen phosphate 0.2 M, analytical purity, from Merck KgaA;
- Methanol, HPLC gradient grade, from Merck KgaA;
- L-Glutamic acid hemimagnesium salt tetrahydrate (Mg-Glu), analytical purity, from Sigma Aldrich;
- DL-aspartic acid hemimagnesium salt tetrahydrate (Mg-Asp), pharmaceutical purity, from chemBlink;
- solutions for calibration curve were prepared by dissolving BP or PMP in HPLC quality water (concentrations ranged between 1 and 125 μg/ml).

Sample preparation

The stability of BP and PMP was studied in a pH = 1.2 acidic environment. This was done by obtaining a BP or

Table I. thalfs calculated for BP in the presence and absence of cyclodextrins

	BP	BP:β-CD mole ratio					BP:2-HP-β-CD mole ratio			
		1:1	1:2	1:3	1:5	1:7	1:1	1:2	1:3	1:5
thalf (min)	5.46	8.55	11.03	13.28	18.93	23.76	7.2	8.52	10.17	13.4
kd (min^{-1})	0.127	0.081	0.063	0.052	0.037	0.029	0.096	0.081	0.068	0.052

Table II. thalfs calculated for PMP in the presence and absence of cyclodextrins

	PMP	PMP:β-CD mole ratio					PMP:2-HP-β-CD mole ratio				
		1:1	1:2	1:3	1:5	1:7	1:1	1:2	1:3	1:5	1:7
thalf (min)	2.83	4.01	6.49	8.02	11.21	13.92	3.89	5.25	6.38	8.56	9.87
kd (min^{-1})	0.245	0.173	0.107	0.086	0.062	0.050	0.178	0.132	0.109	0.081	0.070

PMP solution in Clark-Labs buffer [6], adding an auxiliary substance and measuring at different time intervals the concentration of the non-decomposed active substance by an HPLC technique.

The auxiliary substance was weighted in 5 ml polypropylene test tubes and dissolved in 4 ml Clark-Lubs buffer, then 40 µl of a concentrated solution of BP (50 mg/ml of sodium benzylpenicillin) or PMP (50 mg/ml potassium phenoxymethylpenicillin) was added, followed by mixing and immediately sampling the solution in order to obtain the time zero concentration.

The following excipient quantities and mole ratios were used: no excipient (1); β-cyclodextrin mole ratio 1/1 (2); β-cyclodextrin mole ratio 1/2 (3); β-cyclodextrin mole ratio 1/3 (4); β-cyclodextrin mole ratio 1/5 (5); β-cyclodextrin mole ratio 1/7 (6); magnesium glutamate mole ratio 1/2 (7); magnesium aspartate mole ratio 1/2 (8); 2-HP-β-cyclodextrin mole ratio 1/1 (9); 2-HP-β-cyclodextrin mole ratio 1/2 (10); 2-HP-β-cyclodextrin mole ratio 1/3 (11); 2-HP-β-cyclodextrin mole ratio 1/5 (12); 2-HP-β-cyclodextrin mole ratio 1/7 (13).

At every sampling time 100 µl sample was mixed with 900 ml of a pH = 9 phosphate buffer, followed by injection of 30 µl mixture into the HPLC system.

Sampling times were 0, 10, 25, 45, 55, and 70 minutes for BP, and 0, 30, 65, 90, 120, 240, and 480 minutes for PMP.

HPLC method

BP: for measuring the concentrations of benzylpenicillin in the samples a European Pharmacopoeia method was used [10].

- Merck D7000 HPLC with DAD detector;
- LiChroCART 250-4 LiChrospher 100 RP-18 (5 µm) Merck column;
- Mobile phase A. Mix 10 volumes of a 68 g/l solution of potassium dihydrogen phosphate R adjusted to pH 3.5 with a 500 g/l solution of dilute phosphoric acid R, 30 volumes of methanol R and 60 volumes of water R;
- Mobile phase B. Mix 10 volumes of a 68 g/l solution of potassium dihydrogen phosphate R adjusted to pH 3.5 with a 500 g/l solution of dilute phosphoric acid R, 40 volumes of water R and 50 volumes of methanol R;

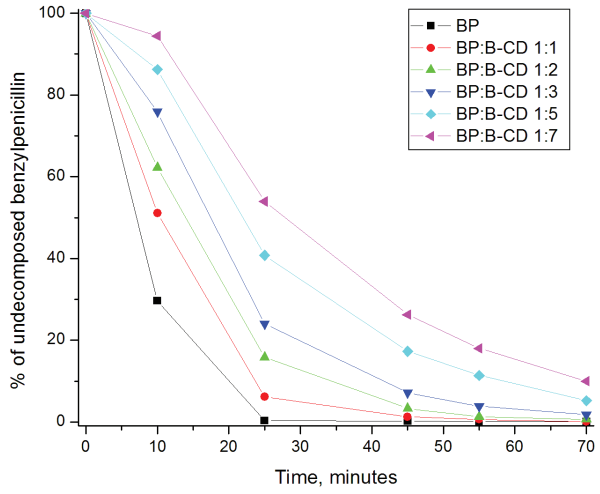

Fig. 1. Changes in BP stability in acidic environment by β-cyclodextrin addition

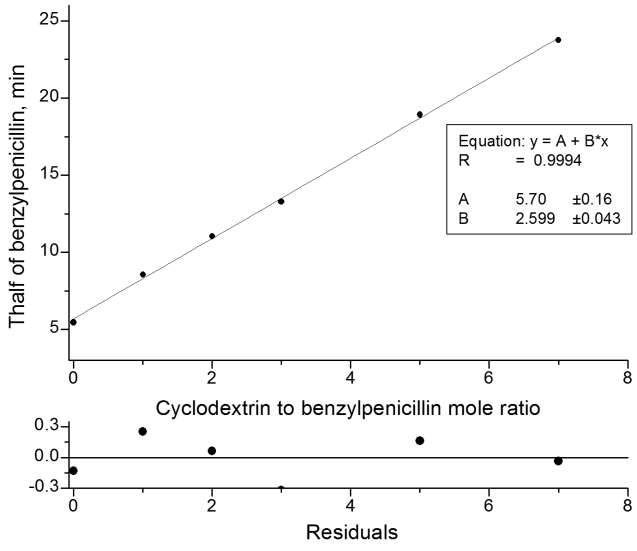

Fig. 2. Linear response between thalf of BP and BP:β-cyclodextrin mole ratio; residuals plotted

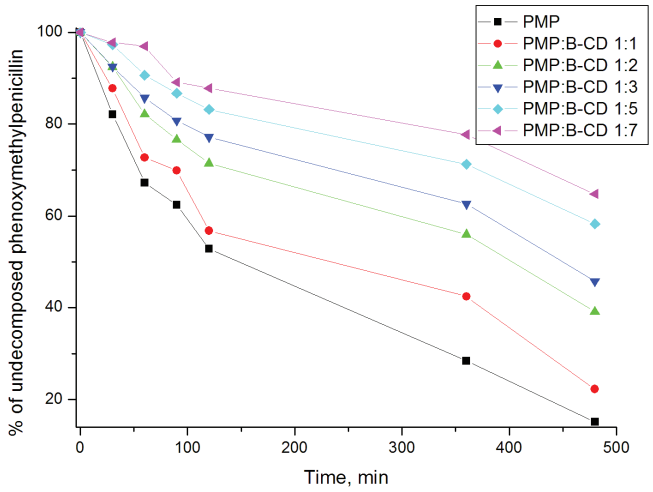

Fig. 3. Changes in PMP stability in acidic environment by β-cyclodextrin addition

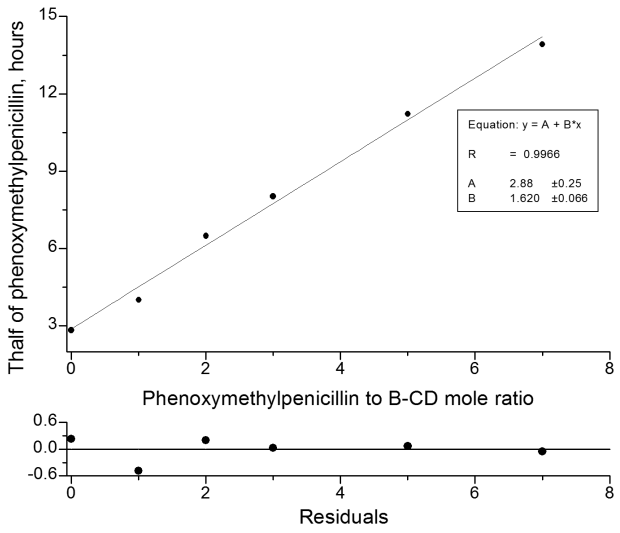

Fig. 4. Linear response between thalf of PMP and PMP:β-cyclodextrin mole ratio; residuals plotted against PMP:β-cyclodextrin mole ratio

- Mobile phase ratio A:B of 70:30;
- flow: 1 ml/min;
- injected volume 20 µl;
- detection wavelength: 225 nm.

PMP: for measuring the concentrations of phenoxymethylpenicillin in the samples a European Pharmacopoeia method was used [10].

- LiChroCART 250-4 LiChrospher 100 RP-18 (5 µm) Merck column;
- Mobile phase A. Mix 10 volumes of a 68 g/l solution of potassium dihydrogen phosphate R adjusted to pH 3.5 with a 500 g/l solution of dilute phosphoric acid R, 30 volumes of methanol R and 60 volumes of water R;
- Mobile phase B. Mix 10 volumes of a 68 g/l solution of potassium dihydrogen phosphate R adjusted to pH 3.5 with a 500 g/l solution of dilute phosphoric acid R, 35 volumes of water R and 55 volumes of methanol R;
- Mobile phase ratio A:B of 70:30;
- flow: 1 ml/min;
- injected volume 20 µl;
- detection wavelength: 254 nm.

Results

Method performance check

Since the used method is a European Pharmacopoeia method a complete validation is not necessary for accepting the obtained results. However, in order to ensure an optimal functioning of the system, linearity and specificity of the method were checked.

A 1–125 µg/ml concentration range was used for the calibration curve. The range was chosen in order to ensure a quantification of at least 1% of the starting concentration. This way the concentration of the active substance can be measured until at least 99% is decomposed; this can be considered a complete decomposition in this case.

Good specificity and linearity (coefficient of correlation R >0.999, residuals with values lower than 10%, no correlation between residuals and concentration) were obtained for both tested substances.

Changes of benzylpenicillin stability in the presence of auxiliary substances

Both tested cyclodextrins significantly increased the stability of BP in acidic environment. Figure 1 shows the effect of cyclodextrin addition on the decomposition curve of BP. It can be seen that stability increases with the increase of BP:β-cyclodextrin mole ratio. Similar type of effect but with lower intensity is obtained by adding 2-HP-β-cyclodextrin to BP solutions.

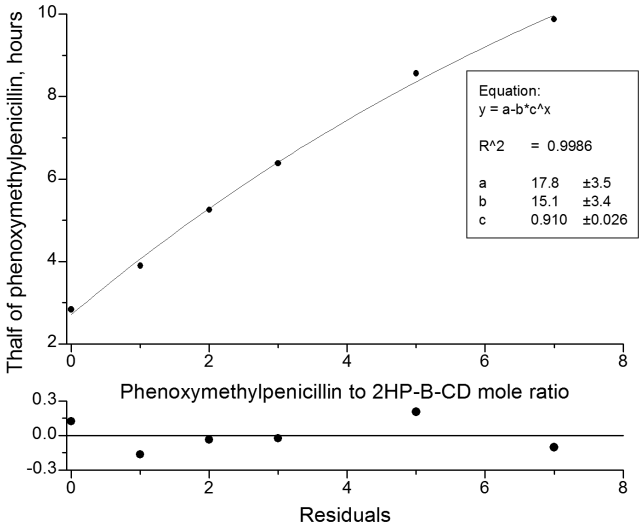

Fig. 5. Asymptotic response between thalf of PMP and PMP:2-HP-β-cyclodextrin mole ratio; residuals plotted against PMP:2-HP-β-cyclodextrin mole ratio

Decomposition of BP was found to follow a first order kinetic (coefficients of correlation R usually >0.98 with residuals usually lower than 10%; residuals were not found to correlate with time) and for calculation of the decomposition rate constant (kd) the following equation was used: $y = a \bullet e^{bx}$, where y is the percent of undecomposed BP, a is the time zero concentration, b is the decomposition rate constant (kd), and x is the time when concentration was sampled. Thalf can be obtained by using the equation: thalf = ln2/kd.

In order to quantify the stability increase induced by cyclodextrin addition thalf and decomposition rate constants (kd) were calculated for BP in the presence and absence of the cyclodextrins (Table I) and plotted against BP:cyclodextrin mole ratio (Figure 2).

A good linear correlation was found between β-cyclodextrin to BP mole ratio and the thalf of BP. Coefficient of correlation is 0.9994 and residuals with very low values do not correlate with β-cyclodextrin to BP mole ratio. This linear correlation suggests that there is a possibility to further increase BP stability by adding larger quantities of cyclodextrin to the solution. Unfortunately, it is difficult to predict to which extent the stability will rise with cyclodextrin concentration because at some point the linear correlation will shift to an asymptotic one. This will be due to the limitations in cyclodextrin solubility or equilibrium processes between formation and decomposition of the BP-cyclodextrin complex.

The same type of linear correlation was obtained for 2-HP-β-CD.

Absolutely no effect on the stability of BP was obtained when magnesium salts of aspartic acid or glutamic acid were added to the solution.

Changes of phenoxymethylpenicillin stability in the presence of auxiliary substances

Both tested cyclodextrins significantly increased the stability of PMP in acidic environment. Figure 3 shows the effect of cyclodextrin addition on the decomposition curve of PMP. It can be seen that stability increases with the increase of PMP:β-cyclodextrin mole ratio. A similar type of effect but with lower intensity is obtained by adding 2-HP-β-cyclodextrin to PMP solutions.

Exactly as in the case of BP, thalfs were calculated for PMP and PMP with cyclodextrins (Table II). It can be seen that in this case too, β-CD had higher protective effect than 2-HP-β-CD did.

When thalf of PMP is plotted against the PMP:cyclodextrin mole ratio in both cases a linear fitting can be done, however fitting parameters are better when an exponential asymptotic fitting is tried for 2-HP-β-CD (Figure 4 and 5).

Absolutely no effect on the stability of PMP was obtained when magnesium salts of aspartic acid or glutamic acid were added to the solution.

Discussions

Results presented in this paper clearly show that a great increase of stability in acidic environment can be obtained in the case of BP and PMP when cyclodextrins are added to the penicillin solution. Results are in agreement with published data regarding other types of penicillins [4–8]. It is interesting to note that the two cyclodextrins had similar effects on both penicillins regarding the magnification factor of the thalfs measured in acidic environment. In most cases excellent linear correlation was found between the concentration of cyclodextrin and the stability of penicillin, however, this is not a true linear correlation because stability increase will cease after a certain amount of cyclodextrin added to the penicillin solution. This will be probably due to the saturation of solution in cyclodextrin molecules.

In the case of PMP the stability increase can be clinically significant (from about 3 to about 14 hours) leading to a better bioavailability, decreased interindividual variations and better prediction of dose plasma concentration correlation. In vivo experiments should be made in order to confirm these claims.

In the case of BP it is hard to tell from these in vivo data if there is any possibility of obtaining oral administration forms for this penicillin. The extremely low half life of BP (about 5 minutes) even if it is significantly increased by cyclodextrins in high molar ratio (1:7) to about 25 minutes is still not enough for an oral administration. In similar conditions the half life of oxacillin (penicillin with a bioavailability of only 30% when orally administered because of the fast stomach decomposition) was 31 minutes. Calculating from the equation presented in Figure 2 results that in order to obtain a thalf similar to that of oxacillin a BP:β-cyclodextrin mole ration of 10 should be used. This, however, will lead to a low bioavailability product for oral administration.

Decomposition of BP and PMP is due to the opening of the β-lactam ring. A protective effect of cyclodextrins can be explained by an inclusion of the penam ring in the cavity of cyclodextrins. This finding is suggested by literature data too.

Interestingly the magnesium salts of amino acids, that were effective in the case of other penicillins [5,8], did not have any effect in the case of BP and PMP.

Changes in the type of the decomposition kinetics obtained in similar studies in the presence of cyclodextrins [5] were not observed in this case.

Conclusions

Significant improvement of BP and PMP can be attained when associated with cyclodextrins.

A promising association is that of PMP with β-cyclodextrin and 2-HP-β-cyclodextrin. The association of BP with these cyclodextrins increases several times the thlaf of BP but the obtained value is still too low for an oral administration.

Further in vivo experiments are necessary for assessing the clinical significance of these findings.

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