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The use of microcalorimetry and HPLC for the determination of degradation kinetics and thermodynamic parameters of Perindopril Erbumine in aqueous solutions

Z. Simončič [a,*], R. Roškar [b], A. Gartner [a], K. Kogej [c], V. Kmetec [b]

[a] *Krka, d.d., Novo mesto, Šmarješka cesta 6, 8501 Novo mesto, Slovenia*
[b] *Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia*
[c] *Faculty of Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva 5, 1000 Ljubljana, Slovenia*

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Abstract

Perindopril Erbumine (PER) is one of the newly used angiotensin-converting enzyme inhibitors (ACE inhibitors) and is used for the treatment of patients with hypertension and symptomatic heart failure. It has two main degradation pathways, i.e. the degradation by hydrolysis and the degradation by cyclization. An isothermal heat conduction microcalorimetry (MC) and high pressure liquid chromatography (HPLC) were used for the characterization of aqueous solutions of PER and its stability properties. The rates of heat evolved during degradation of perindopril were measured by MC as a function of temperature and pH and from these data rate constant and change in enthalpy of the reactions were determined. With the HPLC method the concentration of perindopril and its degradation products were measured as a function of time in aqueous solutions of different pH that were stored at different temperatures. We demonstrated that reactions of degradation of perindopril at observed conditions follow the first order kinetics. The Arrhenius equation for each pH was determined. At pH 6.8 only one degradation pathway is present, i.e. the degradation by hydrolysis. Degradation constants for this pathway calculated from MC data are in good agreement with those obtained from HPLC. MC as a non-specific technique was shown to be useful in studies of PER when one reaction was present in the sample and also when more chemical and physical processes were simultaneously running.

Keywords: Isothermal microcalorimetry; Chromatography; Perindopril; Solution stability; Degradation kinetic

1. Introduction

Stability studies are an integral part of the drug development program and have a very important role in the registration documentation for each individual pharmaceutical product and drug substance. According to International Conference on harmonization (ICH) a note for guidance on stability testing should be followed: stability testing of new drug substances and products (CPMP/ICH/2736/99), long term and accelerated stability studies have to be carried out to prove the stability of the marketed product and to ensure its shelf life.

Finished product formulations are often manufactured using granulation procedure where substance is dissolved in buffer at elevated temperatures and than sprayed to other tablet excipients to produce a granulate. Dissolving can increase the degradation of the substance and knowing the critical factors that can influence the stability of the active substance in solutions, such as temperature and pH can therefore be of high importance in pharmaceutical development.

Perindopril Erbumine (PER), angiotensin-converting enzyme inhibitor (ACE inhibitor) is used in the treatment of hypertension. It is a *tert*-butylamine salt of 1-{(2*S*)-2-[(1*S*)-1-carbethoxybutyl)amino]-1-oxopropyl}-(2*S*,3*aS*,7*aS*)-perhydroindole-2-carboxilic acid.

According to perindopril monographs in European Pharmacopoeia and findings described in this report its main degradation products are perindoprilate (PAT) and diketopiperazine (DKP). The main degradation paths are presented in Fig. 1.

The main aim of this study was to present kinetic and thermodynamic data for the degradation of PER as a new substance

* Corresponding author. Tel.: +386 7 331 2328; fax.: +386 7 332 2742.
E-mail address: zvone.simoncic@krka.biz (Z. Simončič).

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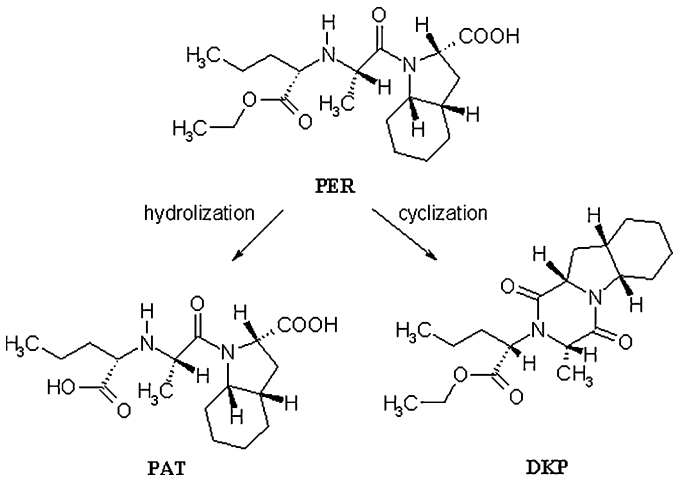

Fig. 1. Degradation of PER through the two main degradation paths.

since no data on kinetics of degradation could be found in the published literature. Our goal was to identify different degradation paths in solutions with different pH values and also to determine rate constants for individual degradation paths. We focused on solutions with pH 2.0 and 6.8 because besides obtaining degradation information in different solution we wanted to mimic in vivo conditions that a drug is exposed to when administered.

Isothermal MC is a non-specific thermo-analytical method that is used by pharmaceutical industry, especially to determine the stability, compatibility and amorphicity properties. Isothermal MC can also be used to determine thermodynamic and kinetic parameters of the long term reactions (Gaisford and Buckton, 2001; Beezer et al., 1999; Buckton, 1995; Wilson et al., 1995a,b; Buckton et al., 1991). Several authors have shown the applicability of this method for studying various aspects of stability (Simončič et al., 2007; Roškar and Kmetec, 2005; Chadha et al., 2003; Schmitt et al., 2001; Zaman et al., 2001a,b; Jakobsen et al., 1997; Wilson et al., 1995a,b; Buckton, 1995; Pikal and Dellerman, 1989). The present study was undertaken to explore the potential of isothermal MC together with the HPLC for the determination of the stability of PER at different pH values under conditions that can arise during production of drug product. The kinetic and thermodynamic parameters of the PER degradation were determined.

2. Materials and methods

2.1. Materials

PER was Ph. Eur. grade, Batch No. 06PR040300, produced by Krka, d.d., Novo mesto.

Studied samples were prepared as aqueous solutions in pH 2.0 and pH 6.8 phosphate buffers. Concentration of samples studied by the HPLC technique was 1 mg of PER/ml (2.26 mmol/l) and for MC studies it was 100 mg of PER/ml (226 mmol/l). Higher concentrations were used in MC studies with the aim to get better responses.

Phosphate buffers with pH 2.0 or 6.8 were prepared by dissolving 136 mg of potassium dihydrogenphosphate in 800 ml of water, adjusting the pH to 2.0 or 6.8 with phosphoric acid and diluting with water to 1000 ml. Buffer capacity was not high enough for the sample with a higher concentration (100 mg of PER/ml; 226 mmol/l) at pH 2.0 and therefore, phosphate buffer with pH 2.0 was prepared at a 1000 times higher concentration.

For HPLC assays the following reagents were used: acetonitrile (Ph. Eur., quality for liquid chromatography) and buffer solution with pH 2.0 (prepared by weighting 0.92 g of sodium heptanesulphonate (p.a.) into 1000 ml volumetric flask with the addition of 1 ml of triethylamine (p.a.), diluting to volume with water and adjusting the pH with perchloric acid (p.a.)).

2.2. Methods

A Micro DSC III (Setaram) calorimeter, operating in the isothermal mode at various temperatures was used together with Hastelloy closed batch vessels. Temperature was maintained with a precision $\pm 1 \times 10^{-4}$ °C. A calorimeter measures the heat conduction out of the sample cell to a heat sink so that the output presents exothermic processes as positive heat flows and endothermic processes as negative heat flows.

The calorimeter was calibrated using the Joule effect method (as described in Micro DSC III User Manual by Setaram) in the range from 20 to 80 °C before experiment set.

For the experiments 850 μl of sample was placed into the sample vessel and the same volume of the buffer was placed into the reference vessel. The thermograms of degradation were monitored at four temperatures (40, 50, 70 and 80 °C) for at least 45 h.

We also monitored the thermograms with the buffer in the sample cell and in the reference cell. The subtraction of this buffer curve from the PER degradation curves was our final thermogram. This was necessary in order to eliminate any instrumental differences arising from the fact that both calorimeter cells are not completely the same. All of the observed effects are thus a consequence solely of the degradation of PER. Studies in the microcalorimeter were performed in three repetitions and the results obtained from separate measurements did not differ significantly. The reported values are average ones.

HPLC area percent method was used for the determination of the contents of PER, PAT and DKP. HPLC instrument (Hewlett Packart 1100 Series) with a variable UV detector and column thermostat was used. Analyses were performed under the following conditions: Hypersil ODS, 5 μm particles, 250 mm × 4 mm i.d. column at temperature 70 °C and mobile phase buffer (pH 2.0) and acetonitrile. The flow was gradient. UV detection was performed at 215 nm.

Studied samples were prepared as solutions of PER in buffers with pH 2.0 or 6.8. Samples were stored at elevated temperatures (40, 50 and 80 °C) for a prescribed time period (1, 2, 4, 8 and 24 h) and afterwards the HPLC was used to determine the content of PER, PAT and DKP present in the sample.

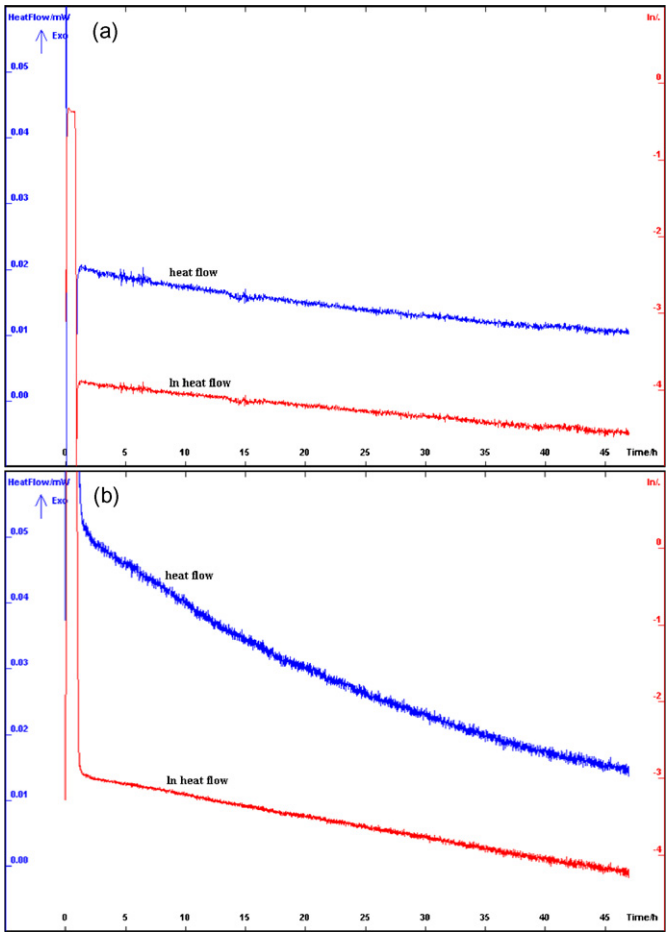

Fig. 2. Thermogram of PER solution at pH 6.8: (a) at 70 °C and (b) at 80 °C. The logarithm of the heat flow (ln(heat flow)) vs. time curve is added to the heat flow curve.

3. Results and discussion

Stability of the PER was first studied with MC technique at 40, 50, 70 and 80 °C. The solvents used were phosphate buffers with pH 2.0 and 6.8.

The thermograms in Fig. 2a and b that represent the degradation of PER in an aqueous solution at pH 6.8 and at 70 and 80 °C

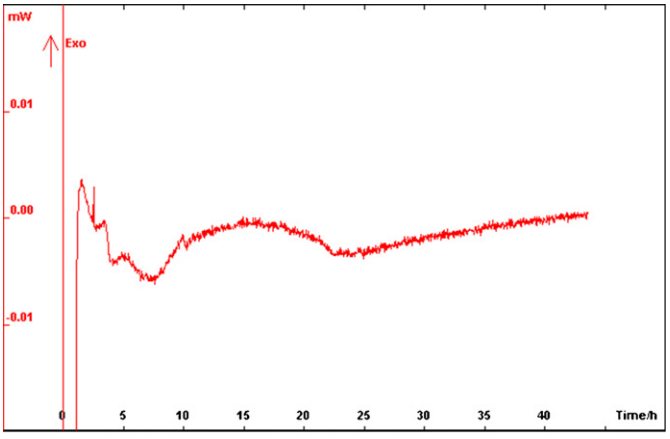

Fig. 3. Thermogram of PER solution at pH 2.0 and at 80 °C.

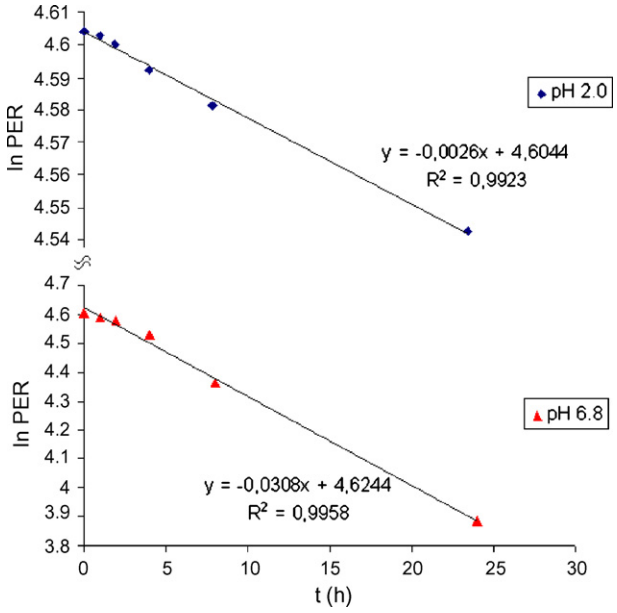

Fig. 4. First order kinetics of PER degradation at 80 °C in aqueous solutions with pH 2.0 and 6.8, respectively.

shows a decreasing heat flow in the sample. On a logarithmic scale that is added to each thermogram the curve is linear showing that the degradation of PER at pH 6.8 follows the first order kinetics. This finding is in accordance with the already published note (Chrzanowski et al., 1991) and was confirmed later by the HPLC, which also showed that the degradation of PER at pH 6.8 proceeds only through the hydrolysis pathway. Consequently, the entire heat flow presented in Fig. 2 is attributed to the degradation of PER to PAT.

The kinetic parameters of degradation by hydrolysis were determined by MC only at 70 and 80 °C. At temperatures 40 and 50 °C we could not determine the kinetic parameters of degradation with MC and therefore we assumed that the kinetics of degradation at these conditions is relatively slow. What we could confirm at all conditions was that at higher temperatures the heat flow increased indicating that more PER was hydrolysed.

In the following treatment it is assumed that the total heat produced (Q_{TOT}) in a given period of time during degradation is proportional to the amount of degraded PER (given in moles, n) and to the enthalpy change (ΔH_{TOT}) (Wilson et al., 1995a):

$$Q_{TOT} = n\,\Delta H_{TOT} \quad (1)$$

For the first order reaction the rate constant is given by Eq. (2) where Φ_0 and Φ_t are heat flows at the initial time ($t = 0$) and at time t, respectively.

$$k = \frac{1}{t}\ln\frac{\Phi_0}{\Phi_t} \quad (2)$$

The initial time for the start of the reaction was taken as the time, when the sample was heated from room temperature to the working temperature in the calorimeter. The first order rate constants that are stated in our work were determined by a graphical method, plotting (ln) heat flow vs. time. The reaction rate constant was then calculated

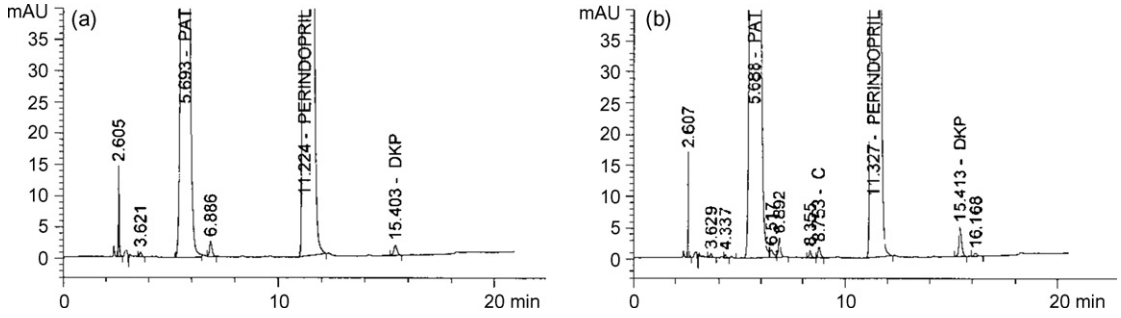

Fig. 5. Typical degradation chromatograms of the PER sample dissolved in a buffer with pH 6.8 and stored at 80 °C for (a) 8 h and (b) 24 h.

from the slope of this line and the change of enthalpy of the reaction (ΔH_{PAT}) from Eq. (1). The calculated values for reaction at 80 °C are $k = 0.0277\,h^{-1}$ ($R^2 = 0.998$) and $\Delta H_{PAT} = -33$ (1 ± 0.05) kJ/mol and at 70 °C they are $k = 0.0144\,h^{-1}$ ($R^2 = 0.992$) and $\Delta H_{PAT} = -26$ (1 ± 0.1) kJ/mol. Hydrolysis of PER is an exothermic reaction.

PER samples that were prepared at pH 2.0 showed that more than one process is running in the sample because different non-linear curves were observed in the thermogram (see Fig. 3). One could expect that in addition to the degradation of PER into PAT also the degradation pathway to DKP takes place. Besides these chemical degradations the heat flow evolved in the vessel could also be a result of some physical changes in the sample. Assuming additivity, the total heat output (Q_{TOT}) can be expressed as the sum of heat outputs of both degradation reactions (Q_{PAT} and Q_{DKP}) and heat output as a result of possible physical changes (Q_{PHYS}):

$$Q_{TOT} = Q_{PAT} + Q_{DKP} + Q_{PHYS} = n_{PAT}\,\Delta H_{PAT} + n_{DKP}\,\Delta H_{DKP} + Q_{PHYS} \quad (3)$$

With the HPLC study presented below we have confirmed that both main degradation paths of PER (to PAT and DKP) are present in the sample at pH 2.0. However, this does not clearly explain the broad peak in the thermogram between 7 and 25 h that points to an exothermic process in the sample. This process could not be described as a chemical degradation. In order to get more information about it we performed a parallel experiment under the same experimental conditions with the sample in a glass flask and noted that during heating some oil droplets were formed at the top of the sample. After shaking the sample, these droplets formed a suspension with the aqueous phase. Knowing the chemical degradation paths and taking into account the lipophilic nature of DKP it was assumed that its concentration was above the solubility limit in a buffer with pH 2.0. As a result a separate oily phase was formed. This physical process (phase separation) resulted in a broad exothermic peak in the thermogram. MC as a non-specific technique cannot distinguish between the individual heat flows and therefore, a quantitative evaluation of various contributions to Q_{TOT} at pH 2.0 was not possible.

Degradation of PER in solutions at two different pH (2.0 and 6.8) and at three temperatures (40, 50 and 80 °C) was studied also by HPLC. The first order degradation kinetics that was demonstrated by MC was confirmed also by HPLC measurements. The first order degradation lines are presented in Fig. 4 for pH values 2.0 and 6.8, both at 80 °C. The kinetic constants for the degradation of PER are reported in Table 1 for all temperatures.

At pH 6.8 PER degraded practically only to PAT, showing that at this pH cyclization reaction hardly appears (approximately 200 times slower kinetics than the degradation to PAT). Typical degradation chromatograms of PER dissolved in a buffer at pH 6.8 and stored for various times at 80 °C are presented in Fig. 5. After 24 h when more than 50% of PER degraded we observed a number of additional degradation products at very low concentrations.

At pH 2.0 both main degradation products were observed but only at 80 °C the concentrations of both were high enough to calculate the kinetic constant of degradation of PER through each degradation path. Typical degradation chromatograms of the sample dissolved in a buffer with pH 2.0 is presented in Fig. 6. Due to relatively high temperature and low pH also some other degradation products could be observed after 24 h but their concentration was not significantly high. Individual partial kinetic

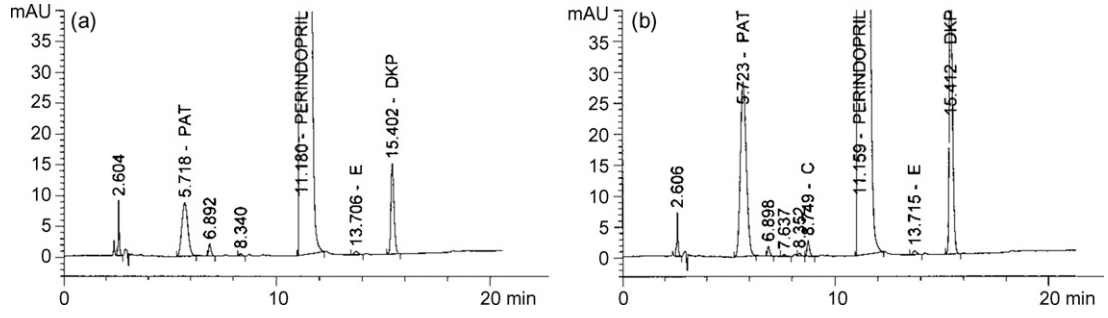

Fig. 6. Typical degradation chromatogram of the PER sample dissolved in a buffer with pH 2.0 and stored at 80 °C for (a) 8 h and (b) 24 h.

Table 1
Data calculated from the HPLC results taking into account the first order kinetics

	pH 2.0	pH 6.8
80 °C	$k_{TOT} = 0.0026\ h^{-1}$, $R^2 = 0.992$; $k_{PAT} = 0.0013\ h^{-1}$, $R^2 = 0.988$; $k_{DKP} = 0.0011\ h^{-1}$, $R^2 = 0.997$	$k_{TOT} = k_{PAT} = 0.0308\ h^{-1}$, $R^2 = 0.996$
50 °C	$k_{TOT} = 0.0003\ h^{-1}$, $R^2 = 0.719$	$k_{TOT} = k_{PAT} = 0.0027\ h^{-1}$, $R^2 = 0.999$
40 °C	$k_{TOT} = 0.0002\ h^{-1}$, $R^2 = 0.840$	$k_{TOT} = k_{PAT} = 0.0008\ h^{-1}$, $R^2 = 0.987$
Arrhenius equation	$\ln k = -7320 \times 1/T + 14.7$, $R^2 = 0.986$	$\ln k = -9910 \times 1/T + 24.6$, $R^2 = 0.996$
E_a (kJ/mol)	60.8	82.4

k_{TOT}: total kinetic constant for the degradation of PER; k_{PAT}: kinetic constant for the degradation of PER through PAT pathway; k_{DKP}: kinetic constant for the degradation of PER through DKP pathway.

constants of degradation of PER into each degradation products (DKP and PAT) were calculated for the degradation at 80 °C in buffer with pH 2.0 (k_{PAT} and k_{DKP}) and are presented in Table 1. Their sum is close to the total degradation constant, which proves that these two degradation paths represent the majority of all the degradation of PER at pH 2.0.

Results in Table 1 indicate that the degradation of PER depends on pH. At pH 2.0, the degradation is approximately 10 times slower than at pH 6.8, which means that PER is more stable at pH 2.0 than at pH 6.8. The cyclization of PER into DKP occurs in 24 h time to a measurable extent only at pH 2.0 and at 80 °C. This is not the case at lower temperatures or at pH 6.8. The hydrolysis of PER to PAT also depends on pH and temperature but with less influence of pH since this degradation is present both at pH 2.0 and at pH 6.8. The rate of the reaction is approximately ten times higher at pH 6.8 and increases faster with increasing temperature than it does at pH 2.0.

When comparing the HPLC results we observed lower values of the determination coefficient (R^2) for kinetic constants at pH 2.0 and at temperatures 40 and 50 °C. This can be explained by low degradation rates of PER under these conditions and therefore greater influence of experimental errors.

Using the results for the degradation constants of PER at different temperatures we determined the Arrhenius equation and compared the activation energy (E_a) for the degradation of PER at each pH. In line with the above results, the activation energy is higher at pH 6.8 (see Table 1).

The constant for the degradation of PER at pH 6.8 and 80 °C calculated based on the HPLC data ($k = 0.0308\ h^{-1}$) is very close to the one obtained from MC ($k = 0.0277\ h^{-1}$). Based on the fact that we used 100 times higher concentration for MC we can conclude that the kinetics of degradation of PER is not concentration dependent. Similarly, the MC result at 70 °C ($k = 0.0144\ h^{-1}$) compares satisfactorily with the HPLC value calculated from the Arrhenius equation ($k = 0.0141\ h^{-1}$). This clearly shows that both techniques give comparable results and can be used for the determination of degradation constants when only one degradation reaction is present in the sample.

4. Conclusion

We have performed an isothermal microcalorimetry (MC) study of the degradation of perindopril (PER) in aqueous solutions at several temperatures (40, 50, 70 and 80 °C) and at two physiologically relevant pH values. The reaction rate constants (k) and enthalpy changes (ΔH_{PAT}) could be determined accurately in solutions with pH 6.8 and at 70 and 80 °C where the only reaction was the degradation of PER to perindoprilate (PAT). At temperatures below 70 °C, the rate of degradation was too low to get reliable results. The values of k and absolute ΔH_{PAT} are higher at 80 °C than at 70 °C. In the ΔH_{PAT} case this means that reaction is less exothermic at 70 °C. Such temperature dependence of ΔH points to a negative heat capacity change for this process. In solutions with pH 2 several processes were found to take place. The two main chemical reactions, which were confirmed by HPLC, are degradations of PER to PAT and diketopiperazine (DKP). In addition to these, a physical process (phase separation of an oily phase) was detected for the first time in the present study. This process was identified by the help of MC because it contributed an important exothermic heat effect to the total heat output after the sample was heated at 80 °C for a period of 7 h.

The MC data were compared also with the HPLC ones that were collected at 40, 50 and 80 °C for both pH values. The HPLC data were used to determine the Arrhenius equation. From this, the rate constant at 70 °C could be calculated. In solutions with pH 6.8, a favorable agreement between rate constants at 70 and 80 °C is obtained with both techniques. This shows that although MC is a non-specific method it can be used in preformulation development of drugs and provide valuable data on stability properties.

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