Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 332 (2007) 98–106
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Comparative effects of some hydrophilic excipients on the rate of gabapentin and baclofen lactamization in lyophilized formulations

Annalisa Cutrignelli, Nunzio Denora, Angela Lopedota, Adriana Trapani, Valentino Laquintana, Andrea Latrofa, Giuseppe Trapani *, Gaetano Liso

Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università degli Studi di Bari, Via Orabona 4, 70125 Bari, Italy

Received 3 May 2006; received in revised form 13 September 2006; accepted 21 September 2006
Available online 5 October 2006

Abstract

The aim of this study was to gain insights into the role played by some excipients on the stability of gabapentin **1** and baclofen **2** which can undergo degradation giving rise to the corresponding lactams 2-azaspiro[4.5]decan-3-one **3** and 4-(4-chlorophenyl)-2-pyrrolidone **4**, respectively. A screening study was carried out on drug and drug-excipient freeze-dried mixtures at 50 °C and under three different humidity values by using a number of commonly available excipients. These include hydroxypropyl-β-(HP-β-CD), sulfobutyl-β-cyclodextrin (SBE-β-CD), lactose, raffinose, trehalose, PVP-K30 and mannitol. For most cases, it was found that the lactam formation can be satisfactory described by an apparent zero-order equation. Excipients shown to negatively impact gabapentin stability are HP-β-CD, SBE-β-CD, lactose and PVP K30 while only this last excipient had a significant effect on the degradation of baclofen. The results can be rationalized in terms of conformational factors favouring the intramolecular dehydration reaction. A positive effect of moisture on the lactamization process was observed under some circumstances. Water may provide a favourable environment for degradation. These findings, taken together, should be considered during the selection of excipients for a possible formulation of gabapentin and baclofen.

Keywords: Gabapentin; Baclofen; Lactamization; Stability; Drug-excipients freeze-dried mixtures

1. Introduction

This paper addresses the lactamization of gabapentin (**1**) and baclofen (**2**) (Fig. 1) in freeze-dried solids in the presence of various excipients. The anticonvulsant drug gabapentin (3,3-pentamethylene-γ-aminobutyric acid) and the centrally acting antispastic agent baclofen (*R*/*S*-4-amino-3-(4-chlorophenyl)butyric acid) are both γ-aminobutyric acid analogues which exhibit high therapeutic activity. Gabapentin shows a pharmacological profile different from that of other marketed anticonvulsants and is used in the treatment of partial seizures with or without secondary generalization (Radulovic et al., 1995). On the other hand, baclofen exhibits high therapeutic value in certain, even severe, spastic disorders. It is approved for the control of spasticity due to multiple sclerosis and spinal cord lesions (Dollery, 1991). Analysis of their commercially available dosage forms revealed that **1** has been included only in peroral hard gelatin capsules, while **2** is formulated both in peroral tablets and parenteral solutions. The development of liquid dosage formulations containing gabapentin are unstable since this drug in aqueous solutions undergoes an intramolecular dehydration reaction giving rise to the lactam **3** (Kearney et al., 1992). Moreover, it was found that the lactamization rate was enhanced in the presence of some cyclodextrins (Kearney et al., 1992). Baclofen, on the other hand, in aqueous formulations stored at 37 °C give rise to little 4-(4-chlorophenyl)-2-pyrrolidone **4** (Estermann et al., 1989; Ruelle et al., 1992; Sitaram et al., 1997). No data has been presented to date on the stability of **1** and **2** in solid formulations. Reported here is the stability of **1** and **2** and their freeze-dried admixtures with a number of hydrophilic excipients, hydroxypropyl-β-(HP-β-CD), sulfobutyl-β-cyclodextrin (SBE-β-CD), lactose, raffinose, trehalose, PVP-K30 and mannitol, and the rate and extent of lactamization evaluated by kinetic experiments performed at 50 °C both in absence and presence of moisture.

* Corresponding author. Tel.: +39 080 5442764; fax: +39 0805442754.
E-mail address: trapani@farmchim.uniba.it (G. Trapani).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.09.053

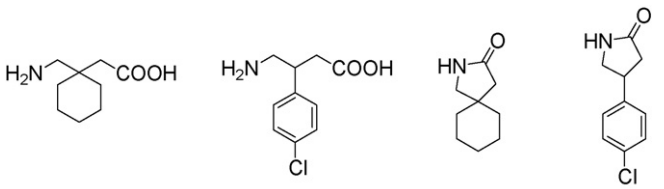


Fig. 1. Chemical structures of compounds **1**–**4**.

2. Materials and methods

2.1. Chemicals

Gabapentin was extracted from a commercial formulation (Neurontin® 400) purchased from a local drugstore as follows. The content of six Neurontin® 400 capsules was collected and the powder extracted with hot ethanol (100 ml) and evaporated. Pure gabapentin was obtained by crystallization from ethanol. Identity and purity of gabapentin was checked by means of spectroscopic methods (IR, ^{1}H NMR, and mass spectroscopy). Poly(vinylpyrrolidone) (PVP) K30 and $CaSO_4$ were purchased from Fluka (Milan, Italy). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) was obtained as a gift from Roquette (Cassano Spinola, Italy). Its total degree of substitution (5.88) was determined by means of ^{1}H NMR spectroscopy. Sulfobutylether-β-cyclodextrin sodium salt (SBE-β-CD, Captisol®) was purchased from CyDex, (Overland Park, KS). Mannitol powder 99% was purchased from Polichimica S.r.l. (Bologna, Italy). Baclofen, α-D-lactose monohydrate 97%, D-raffinose pentahydrate 98%, D(+) trehalose dihydrate 99% and $Mg(NO_3)_2$ were purchased from Sigma–Aldrich (Milan, Italy). Reagents used for the preparation of the buffers were of analytical grade. Fresh deionized water from all glass apparatus was used in the preparation of all the solutions.

2.2. High-performance liquid chromatography (HPLC) analysis

HPLC analyses were performed using a Waters Associates Model 1515 pump equipped with a UV–vis detector Waters 2487 and a 20 μl loop injection autosampler Waters 717 plus. Breeze Software was used to analyze the chromatographic data. For analysis, a reversed phase Simmetry C18 (25 cm × 4.6 mm; 5 μm particles) column in conjunction with a precolumn C18 insert was used and the peaks of interest eluted with mixtures of methanol and deionized water 54:46 v:v. The flow rate of 0.7 ml/min was maintained. The column effluent was monitored continuously at 210 nm for gabapentin and at 220 nm for baclofen. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standards chromatographed under the same conditions ($R^2 > 0.999$). Under these conditions, compounds **1**–**4** resulted in retention times 3.5, 4.7, 9.8 and 10.7 min, respectively.

2.3. Preparation of drug-excipient freeze-dried admixtures

With the exception of PVP K30 (where a 6:1 weight ratio drug/polymer was employed), equimolar amounts of gabapentin or baclofen and each excipient were suspended in 50 ml of deionized water and vortexed at room temperature for 5 min. Then 1 ml aliquots of the resulting solution (containing up to 0.8 mg of gabapentin or baclofen) were poured into 30 vials and lyophilized (Cinquepascal, Lio 5P model freeze-drier, Milan, Italy, equipped with a vacuum pump Edwards 12) for 48 h. The vials containing the lyophilized drug and excipient were divided in three thermostated containers maintained at a relative humidity (RH) content of 0%, 45% or 75%, using $CaSO_4$ granules and saturated solutions of $Mg(NO_3)_2$ and NaCl, respectively. At scheduled time intervals (7, 14, 21, 28 days), one vial was withdrawn, then deionized water (1 ml) was added and the amount of the lactam content evaluated by HPLC. All the experiments were performed in triplicate.

2.4. Preparation of lactam *3* and *4* authentic samples

The pure compound **1** (100 mg) was heated at 150 °C under reduced pressure (14 mmHg) for 30 min. After cooling to room temperature the resulting crude product was purified by column chromatography on silica gel (ethyl acetate as eluent) to give the expected lactam **3**, in moderate yield (45%). Similarly, **2** (90 mg) was heated at 200 °C under reduced pressure (14 mmHg) for 30 min. On work as for **1**, **4** (47% yield) was obtained.

2-Azaspiro[4.5]decan-3-one **3**: mp 86–87 °C (dec). IR (KBr) 3425, 3277, and 1641 cm^{-1}; ^{1}H NMR (D_2O), δ: 1.27–1.36 (m, 10H, CH_2), 2.10 (s, 2H, CH_2), 3.07 (s, 2H, CH_2); GC/MS *m/z* 153 (M^+, 100). Anal. ($C_9H_{15}NO$) C, H, N.

4-(4-Chlorophenyl)-2-pyrrolidone **4**: mp 117 °C (dec). IR (KBr) 3198, 3093, and 1690 cm^{-1}; ^{1}H NMR (CDCl3), δ: 2.41–2.50 (m, 1H, CH_2), 2.70–2.79 (m, 1H, CH_2), 3.35–3.41 (m, 1H, CH_2), 3.62–3.72 (m, 1H, CH), 3.76–3.82 (m, 1H, CH_2), 6.44 (br s, 1H, NH), 7.17–7.20 (m, 6H, Arom.); GC/MS *m/z* 195 (M^+). 138 (100%). Anal. ($C_{10}H_{10}C_lNO$) C, H, N.

2.5. Preparation of solid mixtures of *1* and *2* with HP-β-CD

Equimolar amounts of **1** or **2** and HP-β-CD were added to 10 ml of deionized water. The resulting mixtures were stirred at room temperature for 5 days, filtered through a 0.22 μm membrane, and the resulting filtrate was subjected to freeze-drying.

2.6. Thermogravimetric analysis (TGA)

TGA curves were obtained on a Mettler Toledo Star^e apparatus equipped with a TGA/SDTA 851^e cell. Aliquots of about 5 mg of each sample were placed in a 150 μl aluminium pan without any kind of pre-treatment. Thermograms were recorded by heating the sample from 25 to 400 °C at a rate of 10 °C/min, under a nitrogen flow of 60 ml/min.

2.7. Differential scanning calorimetry (DSC)

DSC curves were obtained on a Mettler Toledo DSC 822[e] apparatus. Aliquots of about 5 mg of each sample were placed in an aluminium pan of 40 μl capacity and 0.1 mm thickness. An empty pan sealed in the same way was used as reference. Thermograms were measured by heating the sample from 25 to 250 °C at a rate of 5 °C/min, under nitrogen flow of 50 cm^3/min. Indium was used as standard for calibrating the temperature. Reproducibility was checked running the sample in triplicate.

2.8. NMR spectroscopic studies

^1H and ^{13}C nuclear magnetic resonance (^1H NMR and ^{13}C NMR, respectively) spectra were recorded in D_2O using a Varian (Milan, Italy) Mercury 300 MHz instrument. Chemical shifts are given in δ values downfield from tetramethylsilane. The samples for NMR measurements were prepared by dissolving 100 mg of solid HP-β-CD complexes in 0.75 ml of D_2O in 5 mm sample tubes. D_2O [4.64 ppm from tetramethylsilane (TMS)] was used as solvent and the water signal as an internal reference for ^1H NMR, whereas dimethylsulfoxide (DMSO) [37.994 ppm from tetramethylsilane (TMS)] was used as an external reference for ^{13}C NMR. Chemical shifts were expressed in ppm downfield from the signal (0 ppm) of tetramethylsilane.

Stability constants (K_c, M^{-1}) for the complexes gabapentin/HP-β-CD and baclofen/HP-β-CD in D_2O at 25 °C were determined according to Hanna's equation (Hanna and Ashbaugh, 1964; Fielding, 2000), and tetrasilylpropionic acid sodium salt (TSP) [0.00 ppm from tetramethylsilane(TMS)] was used as an external reference for this determination. The total concentration of gabapentin or baclofen was maintained constant at 1.0 mM while concentrations of HP-β-CD were varied from 8 to 0.5 mM (8, 4, 1, 0.5 mM, respectively).

2.9. Statistical analysis

Statistical comparisons of kinetic rate constants were made by utilizing analysis of variance (ANOVA) followed by the Tukey post hoc tests (GraphPad Prism v. 4 for Windows, GraphPad Software, San Diego, CS). Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

The thermal behaviour of gabapentin **1** and baclofen **2** was examined by thermogravimetric (TGA) and differential scanning calorimetry (DSC) analyses. TGA was used to evaluate the percentage weight loss of a gabapentin sample with increasing temperature. As shown in Fig. 2, for gabapentin itself, no weight loss is seen until about 130 °C, then a weight loss of 16% and 77% was evidenced at 167 and 235 °C, respectively. A final residue of about 6.10% of initial weight of the sample was recovered. The first weight loss occurred at about the gabapentin melting point and can be attributed to a partial intramolecular water loss yielding the lactam **3**. The second and more relevant weight loss occurred beyond the gabapentin melting point and

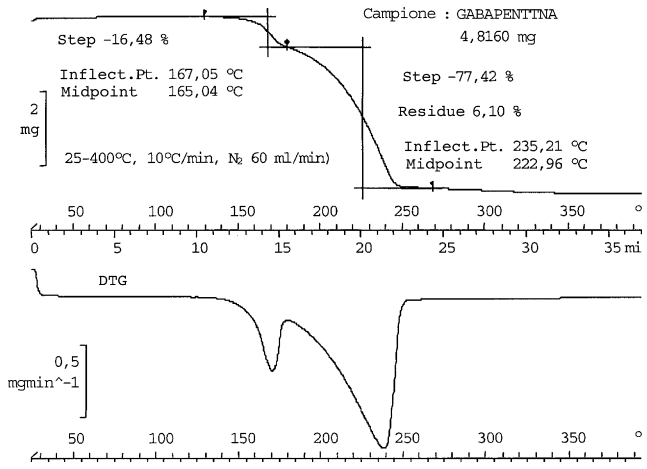


Fig. 2. Thermogravimetric curve of gabapentin **1**.

it can be due to an essentially complete decomposition of the drug. This suggestion is supported by DSC results. As shown in Fig. 3a, indeed, the DSC profile of gabapentin showed a single endothermic peak at about 167 °C corresponding to the apparent melting of the drug. After quenching of a gabapentin sample heated up to 250 °C and reheating, DSC analysis showed a single endothermic peak at about 91 °C, while the peak previously observed at 167 °C disappeared. The peak at 91 °C corresponds to the melting endotherm of lactam **3**, as evidenced by DSC analysis of an authentic sample of lactam **3**. A sample exposed to 350 °C and allowed to cool did not display this behaviour confirming so the complete decomposition of the drug after the heating up to 350 °C.

We next investigated whether the intramolecular lactamization reaction may occur in solid state starting from gabapentin crystals under different conditions, such as by freeze-drying or compression at 10 t. In both cases no evidence of lactam formation was seen.

Fig. 3b shows the DSC profile of a commercially available sample of baclofen **2**. As can be seen, a single endothermic peak at about 209 °C corresponding to the melting of the drug was detected. After cooling of a baclofen sample heated to 250 °C and reheating, DSC analysis showed the lack of the peak at about 209 °C but a single endothermic peak at about 117 °C, corresponding to the melting endotherm of lactam **4** proven by comparison with an authentic sample. Clearly from these observations both gabapentin and baclofen readily undergo lactamizaion on exposure to temperatures above their melting.

The role of excipients in altering drug stability is well known and it has been extensively described (Yoshioka and Stella, 2000). Excipients can interact with drugs leading to degradation of active ingredients, thereby reducing the amount available for therapeutic effect. Understanding how excipients may interact with the active principle allows to avoid undesirable drug development issues. Excipients may simply act as catalysts of degradation or may participate directly in degradation by acting as a reactant (Yoshioka and Stella, 2000). In the present study, gaining insight into the solid state lactamization of gabapentin and baclofen in the presence of commonly employed excipi-

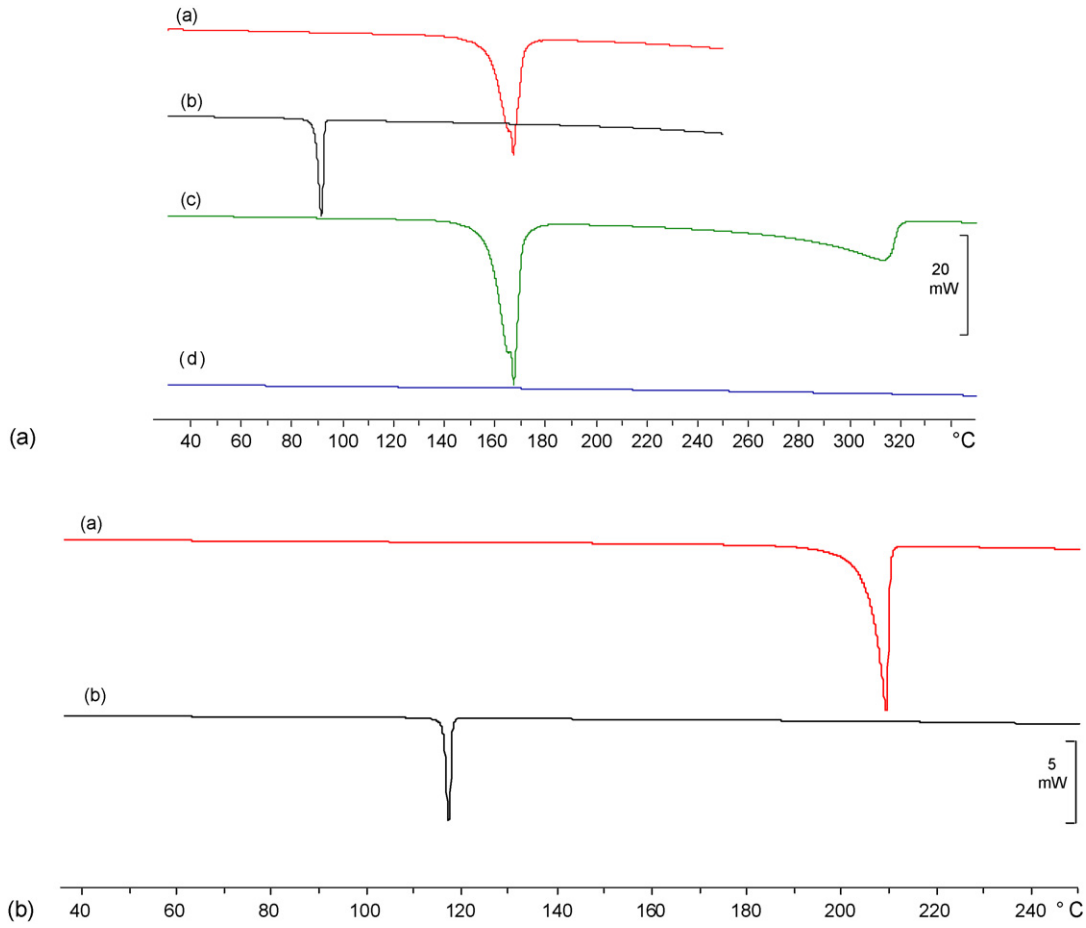


Fig. 3. (A) DSC thermogram of (a) gabapentin heated up to 250 °C; (b) a quenched gabapentin sample heated at 250 °C and reheated; (c) gabapentin heated up to 350 °C; (d) a quenched gabapentin sample heated at 350 °C and reheated. (B) DSC thermogram of (a) baclofen heated up to 250 °C; (b) a quenched baclofen sample heated at 250 °C and reheated.

ents such as HP-β-CD and SBE-β-CD, lactose, raffinose, trehalose, polyvinylpyrrolidone (PVP-K30) and mannitol and in the presence or absence of moisture seemed a worthwhile goal considering the relative ease with which lactamization was seen in solution of gabapentin. Water may participate in the drug degradation by providing a favourable environment for degradation. In particular, the experiments carried out in the present study involved storing lyophilized samples of the pure drugs and their mixture with the above mentioned excipients for 4 weeks at 50 °C in receptacles maintained at RH (0%, or 45%, or 75%). Moreover, the physical states of the freshly prepared freeze-dried samples were also investigated by DSC analyses (data not shown). Essentially amorphous samples occurred when HP-β-CD, SBE-β-CD or PVP-K30 but not lactose, raffinose, trehalose, or mannitol were employed. In these last cases, the samples resulted essentially crystalline. However, it should be taken into account that quantification of low content of amorphous/crystalline phase by DSC or other analytical techniques was proved to be ineffective (Shah et al., 2006). As for the T_g of the amorphous samples, they were found all above 50 °C ranging from 124 to 140 and to 164 °C for SBE-β-CD, HP-β-CD and PVP-K30, respectively. Examination by DSC of the physical states of the samples after 4 week storage at different RH showed that dispersions based on lactose, raffinose, trehalose or mannitol were still crystalline, while the ones involving CDs or PVP appeared sticky or glassy. In these last cases, however, it was not possible to evaluate the exact change of T_g. Moreover, depending on the RH used, the crystalline samples showed very different thermal profiles. Thus, for example, the thermograms of baclofen/trehalose mixture after storage at 45% or 75% RH showed a sharp endotherm at 205 °C corresponding to the melting of the drug together with an endothermic peak at about 95 °C. When the sample was stored at 0% RH, the endotherm at 95 °C does not occur while a peak at 185 °C was detected together with that at 205 °C. The thermal event at 95 °C could be due to dehydration of the carrier (Taylor and York, 1988), while the peak at 185 °C could be assigned to the melting of the anhydrous trehalose.

In most cases, the formation of the expected lactam **3** or **4** could be satisfactory described by zero-order equations and the corresponding apparent rate constants are reported in Table 1. Although pseudo-zero-order kinetics were not always followed, for sake of immediate comparison the relative rates in apparent zero order units are reported here. The effect of the excipients on the lactam **3** formation at 75% RH is shown in Fig. 4. Similar plots were seen at the remaining RH values.

Over 4 week at 50 °C and irrespective of RH, gabapentin undergoes negligible lactamization when not included in

Table 1
Apparent rates of lactam **3** and **4** formation in solid-phase at 50 °C under different relative humidity

Formulations	Relative humidity (%)	k (% lactam/day)	Formulations	Relative humidity (%)	k (% lactam/day)
Gabapentin	0	0.0429 ± 0.0104	Baclofen	0	0.0056 ± 0.0002
	45	0.0341 ± 0.0096		45	0.0020 ± 0.0006
	75	0.0233 ± 0.0081		75	0.0006 ± 0.0004
Gabapentin/HP-β-CD	0	1.127 ± 0.104	Baclofen/HP-β-CD	0	0.2904 ± 0.0165
	45	2.053 ± 0.287		45	0.2299 ± 0.0038
	75	2.433 ± 0.148		75	0.1327 ± 0.0122
Gabapentin/SBE-β-CD	0	0.2506 ± 0.0188	Baclofen/SBE-β-CD	0	0.0194 ± 0.0015
	45	2.047 ± 0.141		45	0.0156 ± 0.0011
	75	2.213 ± 0.091		75	0.0310 ± 0.0004
Gabapentin/lactose	0	0.6119 ± 0.0380	Baclofen/lactose	0	0.0077 ± 0.0029
	45	0.1506 ± 0.0409		45	0.0060 ± 0.0020
	75	0.9601 ± 0.1281		75	0.0005 ± 0.0009
Gabapentin/trehalose	0	0.2600 ± 0.0548	Baclofen/trehalose	0	0.0110 ± 0.0007
	45	0.0447 ± 0.0155		45	0.0033 ± 0.0010
	75	0.0126 ± 0.0111		75	0.0040 ± 0.0023
Gabapentin/raffinose	0	0.2780 ± 0.0316	Baclofen/raffinose	0	0.0080 ± 0.0022
	45	0.0783 ± 0.0089		45	0.0037 ± 0.0016
	75	0.0248 ± 0.0072		75	0.0042 ± 0.0011
Gabapentin/PVP K30	0	1.540 ± 0.442	Baclofen/PVP K30	0	1.1140 ± 0.4139
	45	1.522 ± 0.515		45	0.5700 ± 0.0486
	75	1.571 ± 0.209		75	0.1936 ± 0.0251
Gabapentin/mannitol	0	0.154 ± 0.025	Baclofen/mannitol	0	0.0063 ± 0.0022
	45	0.062 ± 0.007		45	0.0027 ± 0.0011
	75	0.022 ± 0.005		75	0.0034 ± 0.0020

freeze-dried admixtures. Furthermore, it was noted that (i) in the presence of HP-β-CD, intramolecular gabapentin lactamization were the highest observed and positively correlated with increasing RH; (ii) a similar correlation was found with SBE-β-CD; (iii) considerable and statistically significant lactamization was observed both on lyophilizing the aqueous gabapentin/PVP K30 mixture and on storage of the corresponding lyophilized mixture; (iv) lactose, one of the most widely used excipients in tablets, brought about moderate and significant lactam **3** formation and (v) trehalose, raffinose, and mannitol produced no substantial lactam **3** formation.

Baclofen degradation to **4** was noted only when PVP-K30 was used. Fig. 5 shows the dependence of **4** formation at different RH values. As can be seen for PVP K30, the intramolecular lactamization was faster at 0% RH than that at 75% RH.

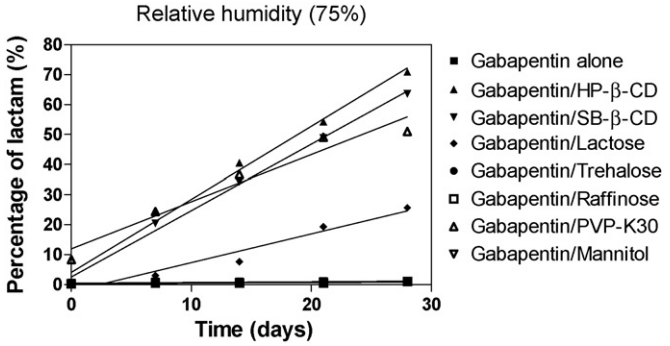


Fig. 4. Effect of the employed excipients on the lactam **3** formation at 75%RH.

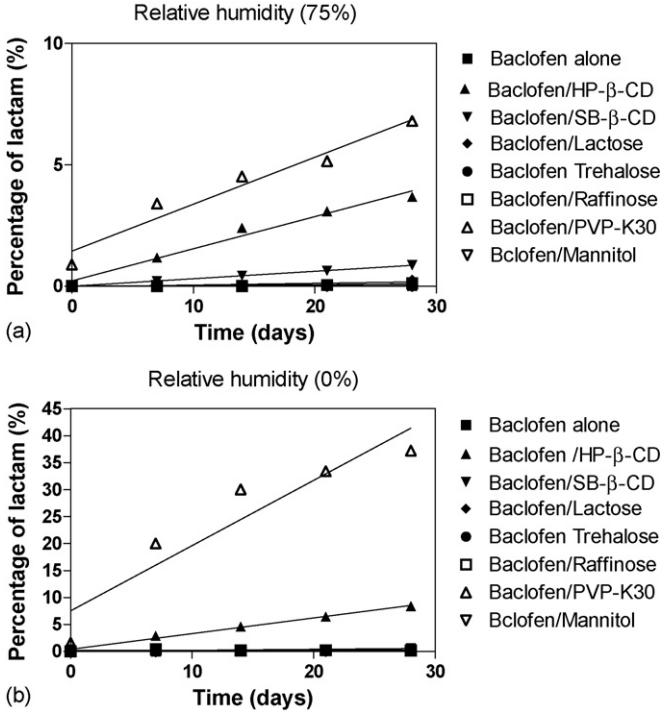
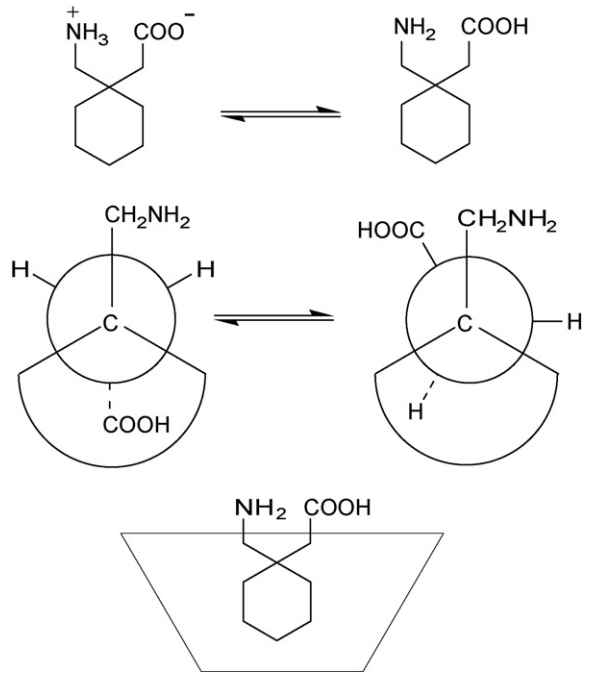


Fig. 5. Effect of the employed excipients on the lactam **4** formation at 75% (a) and 0% (b) RH.

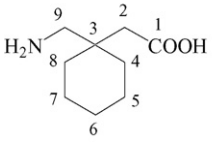
To account for these findings in the presence of the cyclodextrins, the possible interaction of both drugs with the cyclodextrin were considered. It might be hypothesized that the rate enhancements might be attributed to a restricted conformational freedom as a result of the inclusion complexation just as previously suggested in aqueous solution by Kearney et al. (1992). Hence, drug inclusion into the non-polar cavity of the HP-β-CD may cause drug molecule to adopt a geometry that is conducive to intramolecular attach of the amine on the carboxyl group. Gabapentin may exist in the corresponding inclusion complex either as a zwitterion or more probably in its uncharged form as shown in Scheme 1. The non-polar cavity of the cyclodextrins constitutes an unfavourable microenvironment for charge separation. Lactam formation is likely to involve a polar transition state so interaction with the charged SBE-β-CD may be different than with the neutral HP-β-CD resulting is different lactamization rates. However, since a marked difference in the extent of lactamization in the presence of the two cyclodextrins was not observed the inclusion mode shown in Scheme 1 is suggested. The fact that increased humidity accelerates the lactamization of **1** is difficult to interpret. It may be possible that the increased humidity may influence the drug degradation by providing a favourable environment for the inclusion complexation formation. To better understand a study of the **1** and **2** inclusion complexation was carried out. ^{1}H NMR and ^{13}C NMR spectroscopy was used to determine complex formation.

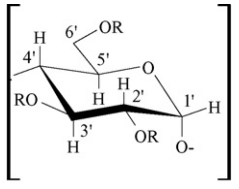
Tables 2 and 3 show ^{1}H-chemical shifts displacements of gabapentin **1** and baclofen **2** in the presence and absence of HP-β-CD, respectively. As can be seen from Table 2, in the case of gabapentin, a significant downfield shift of H-2 and H-9 protons was observed in the presence of HP-β-CD. On the other hand, large ^{13}C-chemical shift changes were observed for C-2, C-3, C-9 and for the cyclohexyl carbons of **1**. As observed by Ikeda



Scheme 1. Acid–base and conformational equilibria, and possible inclusion mode of gabapentin.

Table 2
^{1}H and ^{13}C NMR chemical shifts displacements of gabapentin and HP-β-CD as free molecules and inclusion complexes

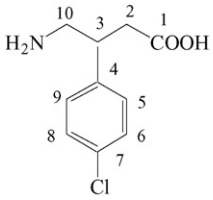


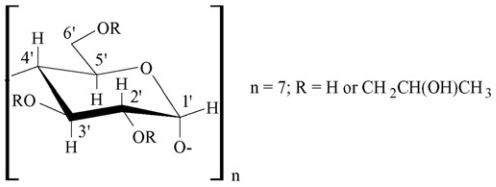


n = 7; R = H or $CH_2CH(OH)CH_3$

Proton chemical shifts	Gabapentin	Gabapentin/HP-β-CD complex	$\Delta\delta$ ppm ($\delta_{complex} - \delta_{free}$)
H-9	2.8280	2.8663	0.0383
H-2	2.2530	2.3398	0.0868
Cyclohexane	1.2691	1.3118	0.0427
Proton chemical shifts	**HP-β-CD**	**Gabapentin/HP-β-CD complex**	**$\Delta\delta$ ppm ($\delta_{complex} - \delta_{free}$)**
H-3′	3.8315	3.8678	0.0363
H-5′	3.4096	3.4312	0.0216
H-6	3.7137	3.6522	−0.0615
CH_3	0.9903	1.0023	0.0120
Carbon chemical shifts	**Gabapentin**	**Gabapentin/HP-β-CD complex**	**$\Delta\delta$ppm ($\delta_{complex} - \delta_{free}$)**
C-5, C-7	18.8436	19.2669	0.4233
C-6	23.2165	23.7309	0.5144
C-4, C-8	31.3170	31.9738	0.6568
C-2	32.1901	32.5375	0.3474
C-3	43.9917	44.6085	0.6168
C-9	46.2426	46.6280	0.3854
C-1	178.5610	177.8838	−0.6772

Table 3
^{1}H and ^{13}C NMR chemical shifts displacements of baclofen and HP-β-CD as free molecules and inclusion complexes





Proton chemical shifts	Baclofen	Baclofen/HP-β-CD complex	$\Delta\delta$ ppm ($\delta_{complex} - \delta_{free}$)
H-5, H-6, H-8, H-9	7.2377	7.2505	0.0128
H-10, H-3	3.1185	3.1795	0.0610
H-2	2.4192	2.4678	0.0486
Proton chemical shifts	**HP-β-CD**	**Baclofen/HP-β-CD complex**	**$\Delta\delta$ ppm ($\delta_{complex} - \delta_{free}$)**
H-3′	3.8315	3.8843	0.0528
H-5′	3.4096	3.4890	0.0794
H-6′	3.7137	3.7486	0.0349
CH_3	0.9903	1.0321	0.0418
Carbon chemical shifts	**Baclofen**	**Baclofen/HP-β-CD complex**	**$\Delta\delta$ppm ($\delta_{complex} - \delta_{free}$)**
C-1	179.3430	178.6509	−0.6921
C-7	138.2220	139.1619	0.9399
C-4	133.0590	132.6629	−0.3961
C-5, C-6, C-8, C-9	129.4960	129.3529	−0.1431
	129.2450	128.9809	−0.2641
C-3	44.1131	44.1263	0.0132
C-10	42.2379	42.4296	0.1917
C-2	40.8144	40.4557	−0.3587

et al. (2002), the H-3′ and H-5′ protons on the inner surface of HP-β-CD shifted upfield, while the H-6′ proton shifted in the opposite direction. These findings indicate, as expected, that only the cyclohexyl portion of a molecule of gabapentin should be located inside the HP-β-CD cavity but not the acetic and the aminomethyl moieties (Scheme 1). As for baclofen **2**, its H-2, H-3, and H-10 protons undergo a significant downfield shift in the presence of HP-β-CD while its H-3′, H-5′, and H-6′ shifted downfield (Table 3). Large ^{13}C-chemical shift changes were also observed for C-7 and C-4 carbons of **2**. These results are in agreement with the suggestion that only the phenyl group but not the γ-aminobutyric acid moiety of baclofen should be located inside the HP-β-CD cavity.

To evaluate the stability constant (K_c) of the complexes and hence the interaction degree between **1** or **2** and CD, the CD concentration dependencies of the chemical shift (observed in aqueous solution) were treated by the Hanna equation (Eq. (1)) derived on the assumption of 1:1 complex formation:

$$\frac{1}{\Delta\text{obs}} = \frac{1}{K_c \Delta c[\text{CD}]} + \frac{1}{\Delta c} \quad (1)$$

where Δobs represents the chemical shift difference of drug in the absence and presence of CD and Δc is chemical shift difference between free and complexed drug.

As shown in Fig. 6a and b, straight lines ($R^2 > 0.93$) are obtained by plotting the chemical shift displacements relative to the methylene protons of the acetate moiety (CH_2COOH) according to Eq. (1). Thus, the stability con-

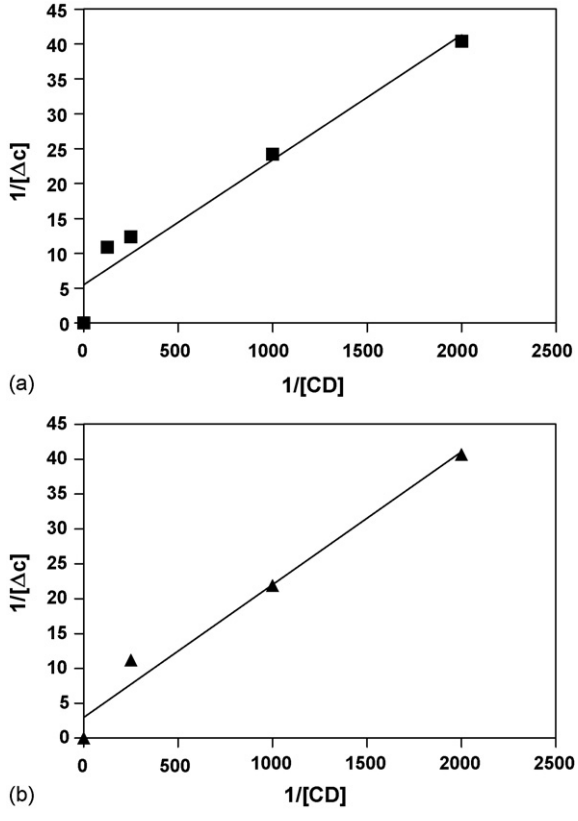


Fig. 6. Plot according to Hanna's equation for gabapentin (a) and baclofen (b).

stants (K_c) of the gabapentin/HP-β-CD and baclofen/HP-β-CD complexes were estimated [318 ± 16 and 154 ± 3 M^{-1} ($n = 2$), respectively].

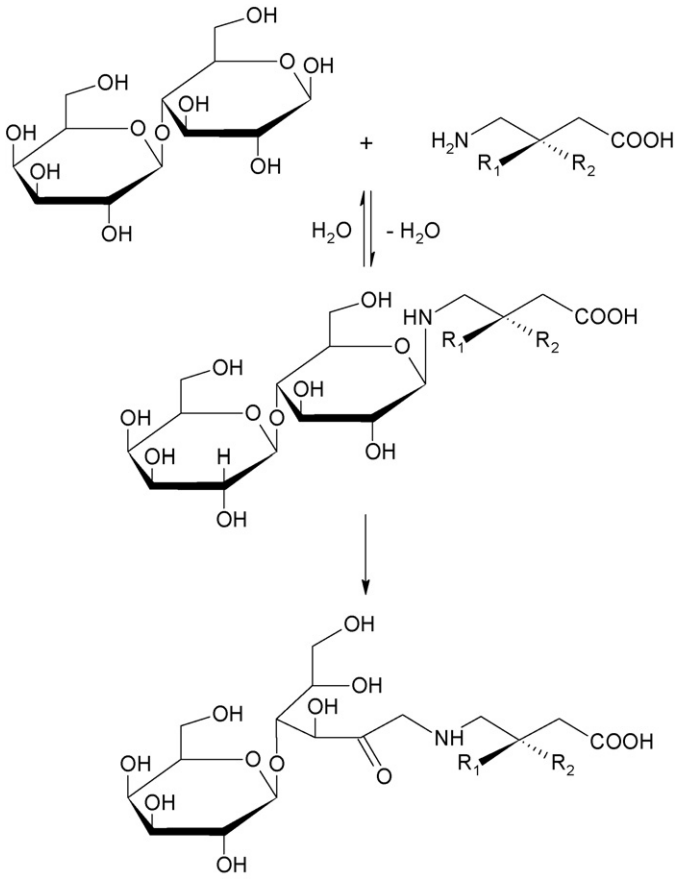
The stability constants were within the range considered by various authors (Blanco et al., 1991; Sinha et al., 2005) as adequate for the formation of an inclusion complex. It should be noted that the value of the stability constant reported for gabapentin with HP-β-CD (Kearney et al., 1992) is more than 10-fold lower than that measured in this work (i.e., 22.4 ± 2.2 versus 318 ± 16). The difference observed may be partially due to the different methods used for investigating the inclusion complexation (i.e., kinetic method versus NMR data, respectively). It is well known that the binding constant value may vary depending on the method used in the determination (Ma et al., 2000).

The negligible lactamization observed for baclofen **2** in the presence of the cyclodextrins can be attributed simply to a greater flexibility of this molecule compared to gabapentin **1** and hence the geometrical requirements for the intramolecular dehydration (i.e., proximity of the reacting groups, the amino and carboxyl groups in a *gauche* or even *eclipsed* conformation) being unaffected by complexation. However, the effect of the cyclodextrins on the lactamization of baclofen **2** was significantly more evident than that caused by lactose, trehalose or mannitol. The behaviour of the lyophilized mixtures containing these last excipients could be explained taking into account that their crystalline states hampered drug lactamization.

As mentioned above, the analysis of the rate constants for drug/lactose interaction again showed a different behaviour for **1** and **2**. A possible gabapentin/lactose interaction resulted in a moderate increase in lactam formation, while this was not observed in the analogous freeze-dried baclofen/lactose system. A possible explanation for this observation can be that the primary amino group of gabapentin and the reducing carbohydrate lactose in solid state interact to give the product of the Maillard reaction that can successively undergo the Amadori rearrangement (Wirth et al., 1998) and that this interaction may in some way favour lactam formation. In Scheme 2 are outlined the Maillard reaction and Amadori rearrangement.

As for the effect of humidity on the gabapentin/lactose interaction, the observed results are difficult to explain. The fact that at highest value of RH (i.e., 75%) the lactamization was accelerated may be due to simply more drug being dissolved thus accelerating the reaction. It is well known (Wirth et al., 1998) that reducing sugars such as glucose, maltose, and lactose are substrates for the Maillard reaction since their cyclic tautomers are in equilibrium with their more reactive aldehyde forms. Non-reducing sugars such as trehalose and raffinose as well as the polyol mannitol are not subjected to Maillard reactions. Hence, the observed lack of intramolecular dehydration when these excipients are used with both **1** and **2** is not surprising but does provide a clue that the aldehyde forms of the reducing sugars are important.

Finally, the behaviour of lyophilized samples of **1** and **2** with PVP K30 can be rationalized taking into account the amorphous nature of this polymer (Yoshioka and Stella, 2000; Crowley and Zografi, 2002) and that the glass transition temperature (T_g) of PVP K30 is high enough (i.e. 164 °C). It is also known to be a nucleation inhibitor of crystal growth (Crowley and Zografi, 2003). Hence, the lyophilized samples of both **1** and **2** may be maintained in an amorphous or glassy state, conditions that might be more favourable for the intramolecular dehydration. The effect of the relative humidity observed in the case of the system gabapentin/PVP K30 can be considered negligible, while in the case of the corresponding system with baclofen a clear increase in lactamization rate was observed under anhydrous conditions. It is difficult to account for this finding and more thorough studies are needed to explain these observations.



Scheme 2. Maillard reaction and Amadori rearrangement involving gabapentin.

4. Conclusions

In this work lyophilized samples of gabapentin and baclofen in the presence of various excipients were shown to undergo intramolecular lactamization with rates strongly dependent on the excipient used. In particular, HP-β-CD, SBE-β-CD, PVP K30 and lactose accelerated the reactivity of gabapentin. Furthermore, PVP K30 also accelerated the degradation of baclofen. The effect of relative humidity on these lactamization reactions was seen and possible explanations for the observations speculated. These findings simply emphasize the need for care in the choice of excipients for the reformulation of gabapentin and baclofen.

Acknowledgments

This work was supported by a grant from Ministero dell'Istruzione dell'Università e della Ricerca (Fondi d'Ateneo). Thanks are due to Prof. V.J. Stella (Department of Pharmaceutical Chemistry, Lawrence, KS) for helpful suggestions. We also thank Dr. Di Masi (Dipartimento Farmaco Chimico, Università di Bari) for technical assistance in recording NMR spectra.

References

Blanco, J., Vila-Jato, J.L., Otero, F.J., Anguiano, S., 1991. Influence of method of preparation on inclusion complexes of naproxen with different cyclodextrins. Drug Dev. Ind. Pharm. 17, 943–945.

Crowley, K.J., Zografi, G., 2002. Water vapor absorption into amorphous hydrophobic drug/poly(vinylpyrrolidone) dispersions. J. Pharm. Sci. 91, 2150–2165.

Crowley, K.J., Zografi, G., 2003. The effect of low concentrations of molecular dispersed poly(vinylpyrrolidone) on indomethacin crystallization from the amorphous state. Pharm. Res. 20, 1417–1422.

Dollery, C. (Ed.), 1991. Baclofen. In Therapeutics Drugs, vol. 1. Churchill Livingstone, London, pp. 83–86.

Estermann, B., Ruelle, P., Ho Nam Tran, Negre, J.C., Kesselring, U.W., 1989. Methodology for studying the thermal stability of baclofen in the presence of Avicel PH 101. Congr. Int. Technol. Pharm. 5th 4, 259–267.

Fielding, L., 2000. Determination of association constants (Ka) from solution NMR data. Tetrahedron 56, 6151–6170.

Hanna, M.W., Ashbaugh, A.L., 1964. Nuclear magnetic resonance study of molecular complexes of 7,7,8,8-tetracyanoquinodimethane and aromatic donors. J. Phys. Chem. 68, 811–816.

Ikeda, Y., Motoune, S., Matsuoka, T., Arima, H., Hirayama, F., Uekama, K., 2002. Inclusion complex formation of captopril with α- and β-cyclodextrins in aqueous solution: NMR spectroscopic and molecular dynamic studies. J. Pharm. Sci. 91, 2390–2398.

Kearney, A.S., Mehta, S.C., Radebaugh, G.W., 1992. The effect of cyclodextrins on the rate of intramolecular lactamization of gabapentin in aqueous solution. Int. J. Pharm. 78, 25–34.

Ma, D.Q., Rajewski, R.A., Velde, D.V., Stella, V.J., 2000. Comparative effects of (SBE)7m-δ-CD on the stability of two anti-neoplastic agents, melphalan and carmustine. J. Pharm. Sci. 89, 275–287.

Radulovic, L.L., Taylor, C.P., Walker, R.M., 1995. The preclinical pharmacology, pharmacokinetics and toxicology of gabapentin. Drugs Today 31, 597–611.

Ruelle, P., Estermann, B., Ho, N.T., Kesselring, U.W., 1992. Adsorbed water as a dominant factor in the thermal cyclodehydration of baclofen in the presence of Fractosil. Congr. Int. Technol. Pharm. 6th 1, 267–279.

Shah, B., Kakumanu, V.K., Bansal, A.K., 2006. Analytical techniques for quantification of amorphous/crystalline phases in pharmaceutical solids. J. Pharm. Sci. 95, 1641–1665.

Sinha, V.R., Anitha, R., Ghosh, S., Nanda, A., Kumria, R., 2005. Complexation of celecoxib with β-cyclodextrin: characterization of the interaction in solution and in solid state. J. Pharm. Sci. 94, 676–687.

Sitaram, B.R., Tsui, M., Rawicki, H.B., Lam, S., Sitaram, M., 1997. Stability and compatibility of intrathecal admixtures containing baclofen and high concentration of morphine. Int. J. Pharm. 153, 13–24.

Taylor, L.S., York, P., 1988. Characterization of the phase transition dehydrate on heating and subsequent dehydration. J. Pharm. Sci. 87, 347–355.

Wirth, D.D., Baertschi, S.W., Johnson, R.A., Maple, S.R., Miller, M.S., Hallenbeck, D.K., 1998. Maillard reaction of lactose and fluoxetine hydrochloride, a secondary amine. J. Pharm. Sci. 87, 31–39.

Yoshioka, S., Stella, V.J., 2000. Stability of Drugs and Dosage Forms. Kluwer Academic/Plenum publishers.