Journal of Pharmaceutical and Biomedical Analysis 52 (2010) 9–18

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

# Preparation and solid-state characterization of bupivacaine hydrochloride cyclodextrin complexes aimed for buccal delivery

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ARTICLE INFO

*Article history:*
Received 29 September 2009
Received in revised form
11 November 2009
Accepted 12 November 2009
Available online 18 November 2009

*Keywords:*
Bupivacaine hydrochloride
β-cyclodextrin
Soluble β-cyclodextrin-epichlorohydrin polymer
Solid-state analysis
Buccal delivery

ABSTRACT

Binary products of bupivacaine hydrochloride (BVP HCl), an amide type local anesthetic, with parent β-cyclodextrin (β-CD) and its soluble β-cyclodextrin-epichlorohydrin polymer (EPI-β-CD) were prepared and evaluated as a first phase in the development of a novel mucoadhesive formulation aimed for buccal delivery of this drug. The solid products were obtained by physical mixing, ball milling in high-energy mills, co-evaporation and lyophilisation, in order to rationally select the most effective preparation technique. The solid products obtained were carefully characterised by differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), Fourier transform infrared spectroscopy (FTIR) and environmental scanning electron microscopy (ESEM). The impact of the preparation techniques on the physicochemical properties of plain drug was also studied. Results of solid-state analysis revealed more intense interactions of BVP HCl with EPI-β-CD than with native β-CD, accompanied by stronger reduction of drug crystallinity in the samples, probably favoured by the amorphous nature of the polymeric carrier. While summarising the results of DSC and XRPD analyses, it seems that ball milling of drug/cyclodextrin binary mixtures was particularly efficient in inducing solid-state interaction between the components and it can be considered as the method of choice for preparation of complexes of BVP HCl with β-CD and EPI-β-CD. *In vitro* dissolution properties in artificial saliva of ball-milled BVP HCl and corresponding CD complexes were investigated by simulating the conditions present at the surface of the buccal mucosa. The obtained results confirmed that complexation of BVP HCl with β-CD and EPI-β-CD is a suitable tool for properly tailoring the dissolution properties of the drug and it can be favourably exploited for the development of an effective buccal drug delivery system.



## 1. Introduction

Bupivacaine hydrochloride is an amide type local anaesthetic (LA) that is widely used in management of the pain during and after dental and oral surgery procedures. The drug is commonly administered as injectable solution containing a vasoconstrictor, such as epinephrine [1,2]. The onset of action following dental injections is usually 2–10 min and anesthesia may last two or three times longer than lidocaine and mepivacaine [3]. However, there is a need to further improve the effectiveness of regional administration of LA in the oral cavity and to find suitable alternative formulations to the current mode of drug administration. This would highly increase the patient comfort, especially in needle sensitive persons and children. Moreover, such novel formulations may be very useful in treatment of radiation-induced oral mucositis that occurs during radiation therapy of the carcinoma in the oral cavity or as side-effect of cytostatics administration. This condition is very painful and has a significant impact on the quality of the patients' life [4].

A possible approach to achieve the above stated goals may be the development of a buccal mucoadhesive drug formulation that will ensure the regional delivery of LA to the oral cavity [5,6]. The drug release from such a formulation has to be properly modulated in order to obtain fast onset and prolonged duration of pharmacological action of the drug, which requires the use of suitable carriers [7]. Cyclodextrins (CDs), cyclic oligosaccharides consisting of 6, 7 or 8 α-1,4 linked glucopiranose units are suitable candidates for such role, because they are capable to favourably modify undesired biopharmaceutical properties of the drug, such as low chemical stability and limited aqueous solubility by inclusion complex formation [8]. In particular, β-CD complexation of a typical LA such as benzocaine allowed a marked improvement of its solubility and a significant reduction of its toxicity [9]. Moreover, CDs are able to control drug release from polymeric matrices [10], overcoming obstacles such as the limited amount of dissolution medium in the mouth and the barrier properties of the oral mucosa [11,12]. Furthermore, they

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doi:10.1016/j.jpba.2009.11.013

are biocompatible molecules and in low concentration they do not cause buccal tissue irritation [13].

Therefore, we prepared a series of solid binary systems of BVP HCl with selected CDs, as a first phase in the development of a novel mucoadhesive formulation aimed for buccal delivery of this drug. Preliminary studies indicated that water soluble β-cyclodextrin-epichlorohydrin polymer (EPI-β-CD) can be a suitable carrier for BVP HCl, since it showed particularly good complexing and solubilizing properties towards the drug [14]. The favourable effect of this β-cyclodextrin polymer on solubility and bioavailability of several drugs has been demonstrated [15], and confirmed also in human volunteers [16]. Moreover, although there are not specific toxicological investigations about the toxicity of this β-cyclodextrin polymer on the buccal mucosa, it is plausible that soluble polymeric cyclodextrins, due to their high molecular mass and high hydrophilicity, are not adsorbed and merely serve as temporary carriers [15,16]. Native β-cyclodextrin (β-CD) was used as a reference.

A careful analytical characterization of drug-CD solid systems is an essential step in the development of an effective pharmaceutical formulation [17,18]. In particular, since the effectiveness of CDs can be strongly affected by the technique used for the complex preparation [19,20], drug-CD binary systems were prepared by various methods (i.e. ball milling in a high-energy mill, co-evaporation and lyophilisation), and characterised by differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), and infrared spectroscopy (FTIR), in order to detect the most suitable preparation technique. The influence of the different techniques used for the binary systems preparation on the physicochemical properties of the plain drug was also evaluated. Furthermore, *in vitro* dissolution studies of the best systems were performed using dissolution tests that would allow the simulation of the conditions present in the buccal mucosa, according to guidelines published by Azarmi et al. [21].

## 2. Materials and methods

### 2.1. Materials

Bupivacaine hydrochloride (BVP HCl) was kindly donated by S.I.M.S. (Italy). The cyclodextrins included in this study comprised β-cyclodextrin (β-CD; Kleptose 4PC, Roquette, France) and soluble β-cyclodextrin-epichlorohydrin polymer (EPI-β-CD; Cyclolab R&D Ltd, Hungary). All others chemicals and solvents used in this study were of analytical reagent grade.

### 2.2. Methods

#### 2.2.1. Preparation of solid binary products

Solid binary products of BVP HCl with β-CD or EPI-β-CD were prepared in equimolar drug:cyclodextrin ratio, according to the results of previous phase solubility studies [14] and results published by Dollo et al. [22], using different techniques. Physical mixtures (PM), of BVP HCl with selected cyclodextrins were prepared by gentle mixing of the accurately weighed components using pestle and mortar. Co-evaporated products (COE) were obtained by separately dissolving the drug and the corresponding amount of cyclodextrin in ethanol and water, respectively. The obtained solutions were mixed together and the solvent was removed using a rotary evaporator (Laborota 4000, Heidolph Instruments GmbH, Germany). The ethanol was added with the aim to reduce the amount of the solvent necessary to dissolve the drug and to decrease the boiling point of the solvent, thus facilitating its removal. Ball-milled products (BM) were prepared by co-grinding equimolar drug/cyclodextrin mixtures in a high-energy vibration mill (Retsch, GmbH, Germany) at 24 Hz for different times (30, 45 and 60 min). Each time, the degree of drug residual crystallinity was checked by DSC analysis as described in the following section. Lyophilised products (LYO) were prepared by adding the equimolar amount of the drug into the aqueous solution of each cyclodextrin tested. The sample was stirred until complete dissolution of the drug. The obtained solution was frozen and the solvent was removed using a Lyovac GT2 freeze-dryer (SRK System Technik GmbH, Germany).

To evaluate the effect of the applied techniques for the binary systems preparation on the physicochemical characteristics of the drug, samples of BVP HCl have been treated according to the same procedures, omitting the cyclodextrin from the preparation. All samples were kept in desiccator until further analysis.

#### 2.2.2. Differential scanning calorimetry (DSC)

DSC thermal curves of the solid products were recorded using a Mettler TA 4000 Star$^e$ apparatus equipped with a DSC 25 cell (Mettler Toledo, Switzerland). The instrument was calibrated with indium and zinc prior to analysis of samples under static air atmosphere unless other stated. Accurately weighed samples (2–5 mg, Mettler M3 Microbalance) were placed in sealed aluminium pans with pierced lid and scanned at a heating rate of 10 °C min$^{-1}$ over the temperature range of 30–300 °C. The relative degree of the drug crystallinity (*RDC*) in the samples was calculated according to Eq. (1):

$$RDC = \frac{\Delta H_{sample}}{\Delta H_{drug}} \times 100\% \quad (1)$$

where $\Delta H_{sample}$ and $\Delta H_{drug}$ are the measured heat of the fusion of the sample and of the crystalline drug, respectively, normalised to the drug content in the sample.

#### 2.2.3. X-ray powder diffractometry (XRPD)

The XRPD spectra of the samples were obtained at ambient temperature with a Bruker D8 apparatus ($\Theta/\Theta$ geometry) using a Cu Kα radiation and a graphite monochromator. The samples were analysed in the 2.5–30 $2\Theta$ range, with a scan rate of 0.03 s$^{-1}$.

#### 2.2.4. Fourier transformed infrared spectroscopy (FTIR)

The FTIR spectra of all solid products were recorded by PerkinElmer Model 1600 spectrometer (Wellesley, USA). The samples were prepared by the potassium bromide disc method (3 mg sample in 297 mg KBr) and scanned in the range of 4000–400 cm$^{-1}$ at 2 cm$^{-1}$ resolution.

#### 2.2.5. Environmental scanning electron microscopy (ESEM)

The samples were fixed on a brass stub using a double-sided adhesive tape and observed using an environmental scanning electron microscope XL 30 ESEM FEG (Philips, Netherlands).

#### 2.2.6. In vitro dissolution test

To determine the *in vitro* dissolution properties of the solid products in simulated saliva, two different techniques were used. The aim was to mimic the conditions that are present on the surface of the buccal mucosa, according to the guidelines described by Azarmi et al. [21]. Simulated saliva, consisting of 2.38 g $Na_2HPO_4$, 0.19 g $KH_2PO_4$, 8.00 g NaCl in 1000 mL of distilled water with pH value adjusted to 6.75 by the use of orthophosphoric acid, was used as dissolution medium [23].

The first method used was the dispersed amount technique modified according to Mohamed and Khedr [24] and it was referred in the text as "MDA technique". The dissolution properties of BVP HCl and its binary systems with β-CD and EPI-β-CD were determined by introducing the solid product equivalent to 30 mg of

drug in separate beakers containing 10 mL of simulated saliva solution thermostated at 37 °C. The dissolution medium was gently stirred (50 rpm) by a magnetic stirrer. At predetermined time intervals, the samples were withdrawn and immediately replaced with an equal volume of fresh dissolution medium. Drug concentration in the withdrawn samples was analysed by UV spectroscopy ($\lambda$ = 262.8 nm) after filtration (0.45 μm Millipore membrane filter) and suitable dilution of the filtrate with simulated saliva solution. Preliminary experiments showed that the presence of cyclodextrins and simulated saliva solution had no influence on the drug absorbance at 262.8 nm. A correction was applied for the cumulative dilution caused by replacement of the sample with an equal volume of fresh medium. Each test was repeated four times (coefficient of variation C.V. <2.5%).

The second method involved the use of Franz diffusion cells with a compartment volume of 7 mL and orifice diameter of 10 mm (Rofarma Italia S.r.l.), according to the method described by Sandri et al. [25]. This method was referred in the text as "FDC technique". The receptor compartment was filled with simulated saliva solution, thermostated at 37 °C and the receptor medium was magnetically stirred at 600 rpm. The donor and receptor chambers of the Franz diffusion cells were separated by a cellulose acetate membrane with pore size of 0.65 μm (Albet-Hahnemuehle S.L., Spain). The membrane was wetted with 100 μL of simulated saliva solution. After complete hydration of the membrane, the sample (drug or drug-CD system equivalent to 30 mg of BVP HCl) was evenly applied over the membrane surface. The donor compartment was closed with the screw cap, to ensure the formation of a humid environment, similar to those encountered at the surface of the buccal mucosa. At predetermined time intervals, aliquots of the receptor medium were removed and replaced with fresh dissolution medium. The withdrawn samples were spectrophotometrically analysed for drug content after suitable dilution with the simulated saliva solution as described above. A correction was applied for the cumulative dilution caused by replacement of the sample with an equal volume of fresh medium. Each test was repeated four times (coefficient of variation C.V. <2.5%).

The experimental data obtained by the FDC technique were fitted according to first-order [2] and Higuchi [3] kinetic models, defined by the following equations:

$$Q_t = Q_\infty(1 - e^{-kt}) \quad (2)$$

$$Q_t = k_H t^{1/2} \quad (3)$$

where $Q_\infty$ is the initial amount of the drug, $Q_t$ is the amount of the drug dissolved at time $t$, $k$ and $k_H$ are the first-order and Higuchi rate constants, respectively.

## 3. Results and discussion

### 3.1. Effect of the sample preparation technique on the BVP HCl properties

Equimolar solid systems of the drug with each examined CD were prepared by different techniques (ball milling, co-evaporation, and lyophilisation), in order to investigate the influence of the preparation method on the physicochemical properties of the end product and select the most effective system.

As a first step, to investigate the influence of the techniques used for preparation of the binary systems on the properties of the drug, BVP HCl was treated by the same methods, omitting the CD from the protocol. The obtained samples were analysed by the use of thermal (DSC) and spectral (FTIR and XRPD) methods and the results were compared to those obtained with untreated BVC HCl.

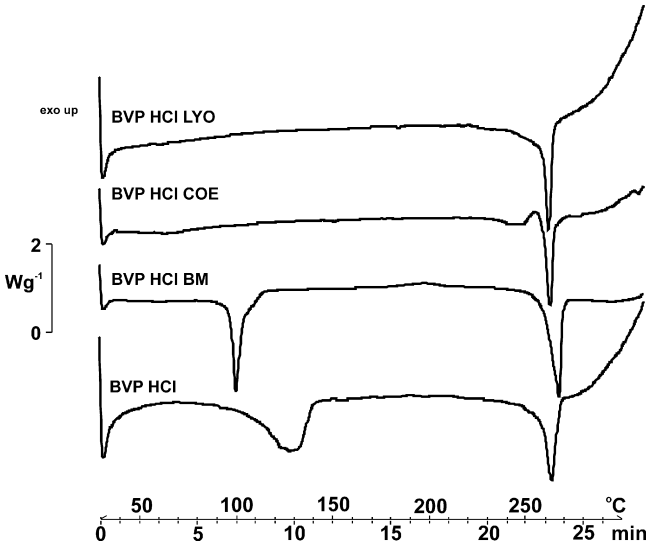


**Fig. 1.** DSC curves of BVP HCl treated with the different techniques used for preparation of solid drug-CD binary systems: ball milling (BM), solvent co-evaporation (COE) and lyophilisation (LYO).

The thermal curve of BVP HCl (Fig. 1) exhibited a broad endothermic transition peak with onset at 112.33 °C and peak temperature at 128.19 °C ($\Delta H$ = 93.4 J/g) followed by a sharp endothermic peak at higher temperature. The commercially available BVP HCl is a monohydrate form of the racemic drug mixture [26]. Therefore, the first peak in the DSC curve of BVP HCl may be attributed to the transition of the monohydrate to the anhydrous form, while the sharp endothermic peak at 253.48 °C with enthalpy of 80.22 J/g is due to the melting of the anhydrous drug form [26,27], and it is followed by the thermally induced drug decomposition.

In DSC curve of ball-milled BVP HCl, the peak corresponding to the dehydration process appeared as a sharp one with peak temperature of 97.00 °C. The shift of the dehydration peak to lower temperature, as well as the reduction of the related transition enthalpy ($\Delta H$ = 57.07 J/g) compared to untreated BVP HCl may be attributed to the decreased particle size upon ball milling. This increased the overall surface of the sample, allowing the dehydration process to occur faster at lower temperature. Temperature and enthalpy values of the melting peak of the anhydrous drug form did not change significantly ($T_{peak}$ = 256.26 °C, $\Delta H$ = 77.93 J/g), indicating the absence of amorphization phenomena.

The peak corresponding to the dehydration process was completely absent in the thermal curve of BVP HCl obtained by solvent evaporation method, as well as in case of lyophilised BVP HCl. In both cases, sharp endothermic peaks corresponding to the drug melting appeared at 252.18 °C with fusion enthalpies of 63.02 and 51.76 J/g for evaporated and lyophilised BVP HCl, respectively. It may be deducted that the solvent evaporation as well as lyophilisation processes caused the formation of the drug anhydrous form, as well as some loss of crystallinity. The relative drug crystallinity degree of samples prepared by lyophilisation and solvent evaporation was 64.52 and 78.56%, respectively. The lowest reduction of drug crystallinity was caused by ball milling procedure, which resulted in only 2.85% of drug amorphization.

To fully explain the results obtained by thermal analysis, the XRPD diffractograms of BVP HCl samples were recorded and the results are presented in Fig. 2. The diffraction pattern of untreated BVP HCl is characterised by several intense and sharp peaks, confirming the crystalline nature of the drug. The observed diffraction pattern is characteristic for the monohydrate form of the racemic

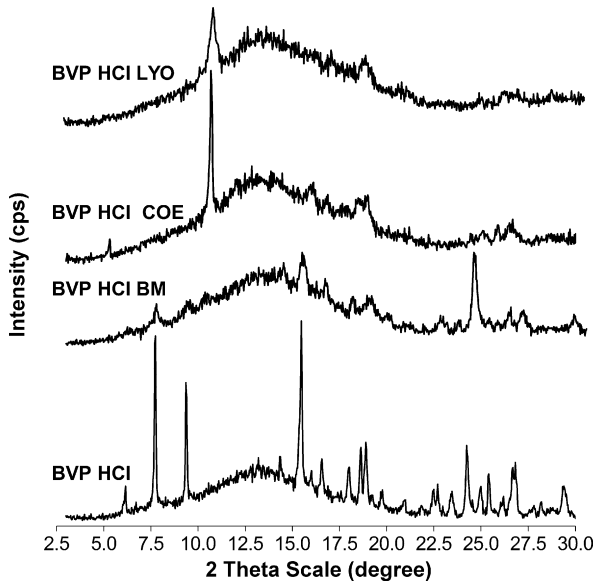


**Fig. 2.** The XRPD patterns of BVP HCl treated with the different techniques used for preparation of solid drug-CD binary systems: ball milling (BM), solvent co-evaporation (COE) and lyophilisation (LYO).

drug mixture [26]. All characteristic peaks of BVP HCl monohydrate are still present in the diffraction pattern of ball-milled BVP HCl, indicating that the ball milling procedure did not change the crystal structure of the drug, thus confirming the conclusion based on DSC experiments. The reduction of the peaks intensity may be explained by the decrease of the drug particle size caused by ball milling. The diffraction patterns of BVP HCl prepared by solvent evaporation and by lyophilisation indicated the existence of a new solid phase. The comparison of our results with those published by Giron et al. [26] showed that this new solid phase may be attributed to the anhydrous form of BVP HCl, thus confirming the conclusions previously made. The slight differences between diffraction patterns of BVP HCl prepared by lyophilisation and solvent evaporation may be explained by the different degree of drug amorphization in the samples, as shown by DSC analysis.

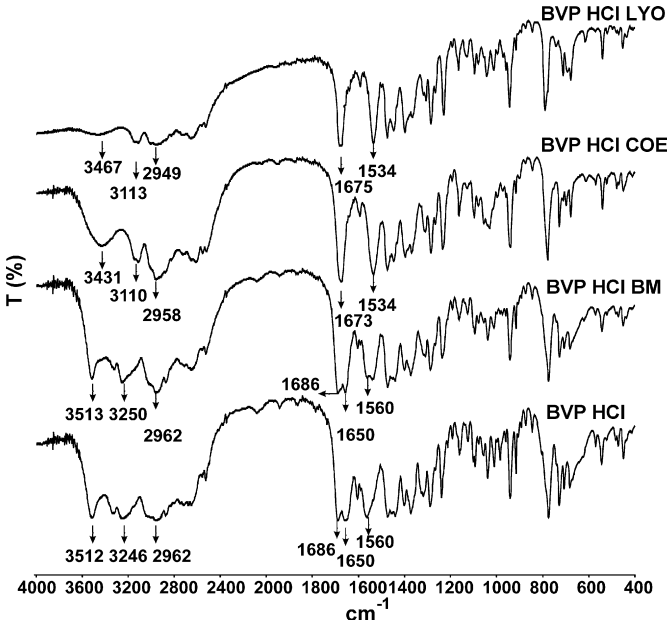


**Fig. 3.** The FTIR spectra of BVP HCl treated with the different techniques used for preparation of solid drug-CD binary systems: ball milling (BM), solvent co-evaporation (COE) and lyophilisation (LYO).

The FTIR spectra of the different samples of BVP HCl are presented in Fig. 3. The FTIR spectrum of untreated BVP HCl exhibited a broad absorption band in the range of the O-H stretching, peaked at 3512 $cm^{-1}$ which confirms the presence of the monohydrate drug form [28]. Also, the shift of the absorption band at 3246 $cm^{-1}$ corresponding to the stretching vibration of the hydrogen bonded N–H group to higher wavenumbers is characteristic for the monohydrate form. The absorption band at 2962 $cm^{-1}$ corresponds to the C–H stretching vibration of the alkyl chain. The amide carbonyl (amide band I) stretching band occurred as a doublet, suggesting the simultaneous presence of different stretching modes: the first peak at 1686 $cm^{-1}$ corresponds to the intermolecularly hydrogen bonded carbonyl groups, while the second peak at 1650 $cm^{-1}$ may be explained by the presence of free carbonyl groups in the crystal lattice of BVP HCl monohydrate. The band at 1560 $cm^{-1}$ represents the characteristic amide II vibration.

The FTIR spectrum of ball-milled BVP HCl, was practically superimposable to that of untreated BVP HCl, indicating that the ball milling procedure has no effect on the crystalline state of the drug, thus confirming previously made conclusions. In the spectrum of evaporated BVP HCl, the O–H stretching band was shifted to lower wavenumbers, while in case of lyophilised BVP HCl it was almost completely absent. In both cases, the absorption band of N–H stretching vibration occurred at lower wavenumbers, compared to the monohydrate form. Also, the C–H stretching band was shifted to lower wavenumbers, which is an indication of the change of pseudo-polymorphic drug form [27,28]. In the same time, the carbonyl stretching band in both samples appeared as a single peak, indicating the reorganisation of the crystal lattice upon the lyophilisation/evaporation process. The marked reduction of O–H absorption band in case of lyophilised BVP HCl further supports the formation of the anhydrous drug form. The differences in O–H region of FTIR spectra of lyophilised and evaporated BVP HCl may probably be attributed to the higher moisture content of evaporated BVP HCl, considering that there are no significant differences in their DCS thermal curves and XRPD diffraction patterns. According to Giron et al. [26], the ethanol solvate of BVP HCl has the same DSC curve and XRPD diffraction pattern as the monohydrate form. Thus, the formation of ethanol solvate in case of evaporated BVP HCl may be excluded.

In order to characterise the morphology of raw drug and samples treated with the different preparation techniques, environmental scanning electron microscopy (ESEM) was used and micrographs obtained are presented in Fig. 4. The raw BVP HCl appeared as large plate crystals (Fig. 4$A_1$ and $A_2$). Ball-milled BVP HCl retained the same morphology (Fig. 4$B_1$ and $B_2$), but the particle size was significantly reduced, as a consequence of the milling procedure, thus confirming the conclusions made on the bases of DSC and XRPD results. On the contrary, the morphology of co-evaporated and lyophilised drug differed significantly compared to raw BVP HCl. A clear loss of crystallinity was observed for both samples, which appeared, respectively, as irregular aggregates of needle-like particles (Fig. 4$C_1$ and $C_2$), or as fluffy flat scraps (Fig. 4$D_1$ and $D_2$). These changes of morphology may be attributed to the transformation to the anhydrous drug form, and, in the same time, to the preparation techniques used. In particular, the aggregation of co-evaporated BVP HCl was a direct consequence of the drug attaching on the wall of the round-bottomed flask upon evaporation of the solvent.

Summarising all results, it may be concluded that ball milling has no influence on the crystalline properties of BVP HCl, while lyophilisation and solvent evaporation procedures gave rise to a reduction of drug crystallinity accompanied by the formation of anhydrous form of BVP HCl.

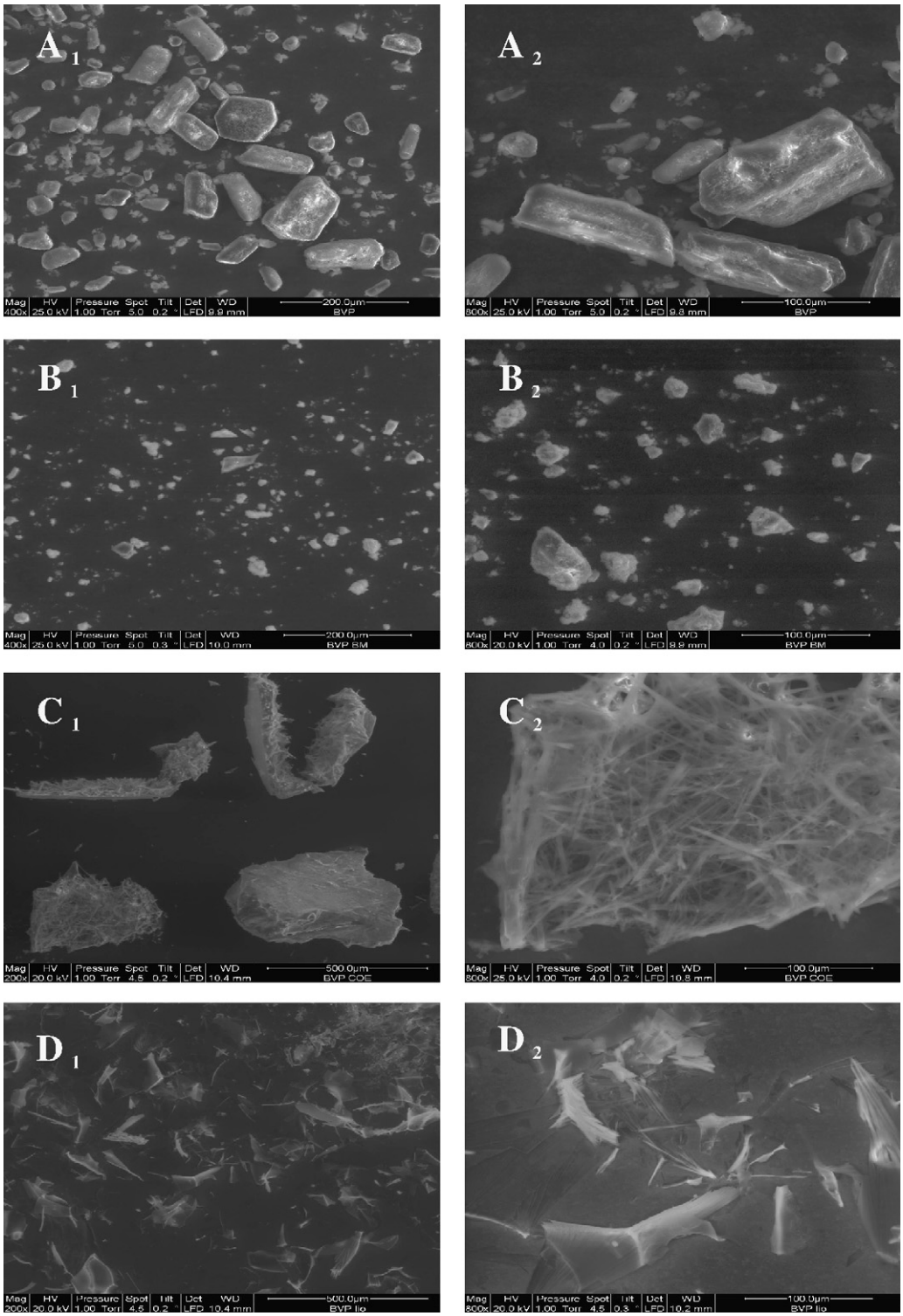


**Fig. 4.** ESEM pictures of raw BVP HCl ($A_1$ and $A_2$), ball milled ($B_1$ and $B_2$), co-evaporated ($C_1$ and $C_2$) and lyophilised drug ($D_1$ and $D_2$) presented at different magnification (200× and 800×, respectively).

### *3.2. Preparation and characterization of the BVP HCl/cyclodextrin inclusion complexes in solid state*

Equimolar binary systems of BVP HCl were prepared using β-CD and EPI-β-CD. EPI-β-CD was selected due to its high affinity for complexation with BVP HCl [14], while β-CD was used as a reference, to evaluate how the different cyclodextrin structure can affect the properties of the binary solid products. To determine the most effective preparation procedure, different techniques were used, including physical mixing, ball milling, solvent evaporation and lyophilisation. In case of ball milling, different treatment times were evaluated, to also investigate the influence of this parameter. Complexes prepared by solvent evaporation were prepared using a 1:1 (v/v) water:ethanol mixture. The obtained products were analysed by DSC, XRPD and FTIR analysis and the results were compared with those of

plain drug as well as with drug treated with the same techniques.

The DSC curves of the different solid binary systems are presented in Fig. 5. The thermal curve of pure β-CD and EPI-β-CD showed a wide endothermic band corresponding to the water evaporation.

The DSC curve of drug/β-CD physical mixture (Fig. 5) showed the broad endothermic band corresponding to the cyclodextrin dehydration, followed by a less intense peak due to the dehydration of the drug molecule. Several intense endothermic and exothermic peaks in the area between 230 and 270 °C may be ascribed to the thermally induced interaction between the components. The experiment was repeated under nitrogen purge (25 mL/min), but the thermal behaviour remained the same as that recorded under static air atmosphere (data not shown). Because of that, the determination of the relative drug crystallinity degree by thermal analysis was not possible in the case of binary systems prepared with β-CD.

To find the best conditions for the ball milling procedure, the milling was performed for 30, 45 and 60 min and the samples were consecutively analysed by DSC. Although the thermally induced interaction between BVP HCl and β-CD prevented the monitoring of the drug crystallinity degree in these samples, a significant reduction of the endo and exothermic peaks intensity in the area between 230 and 270 °C was observed after 30 min of ball milling. Further reduction was observed for the sample milled for 45 min, while in the sample treated for 60 min the peaks were almost completely absent. This thermal behaviour could indirectly point to the formation of an inclusion complex between the components that reduced the intensity of thermally induced interaction between components. A similar conclusion could be made by observing the thermal curves of systems prepared by lyophilisation and solvent evaporation techniques. However, in case of these samples, the intensity of the residuals peaks is somewhat more pronounced than in thermal curve of 60 min ball-milled product, which may lead to the conclusion that lyophilisation and solvent evaporation techniques are less effective preparation techniques.

The DSC curve of BVP/EPI-β-CD physical mixture (Fig. 5), showed in addition to the broad endothermic band corresponding to the cyclodextrin and drug dehydration, an endotermic peak at 236.08 °C ($\Delta H$ = 34.44 J/g) due to the melting of the drug molecule, while other peaks due to thermally induced interactions between the components were absent. The different thermal behaviour of physical mixtures with β-CD and EPI-β-CD may be ascribed to the different chemical structure of these cyclodextrins. The drug crystallinity degree in the sample was 42.93%, which suggest rather strong affinity of the drug for interaction with EPI-β-CD even in solid state.

In the thermal curves of BVP HCl-EPI-β-CD ball-milled systems, a residual endothermic drug fusion peak was still detectable at 231.10 °C and a slight reduction of its intensity was observed with increasing the milling time. The calculated relative degrees of drug crystallinity after 30, 45 and 60 min of treatment were 10.92, 9.10 and 6.83%, respectively. The endothermic drug fusion peak in the thermal curve of BVP HCL/EPI-β-CD co-evaporated sample was observed at 235.41 °C, and the relative degree of drug crystallinity was 22.03%. The DSC curve of BVP HCl/EPI-β-CD lyophilised system showed an endothermic fusion peak corresponding to the drug melting at 233.62 °C, and the relative degree of drug crystallinity was determined 5.62, indicating the high suitability of this technique in establishing strong host-guest solid-state interactions.

It is important to emphasize, that the reduction of the relative drug crystallinity was always significantly higher for the different BVP HCl/EPI-β-CD binary products than for the drug alone treated with the same techniques. This and the changed thermal properties of the other samples compared to the corresponding

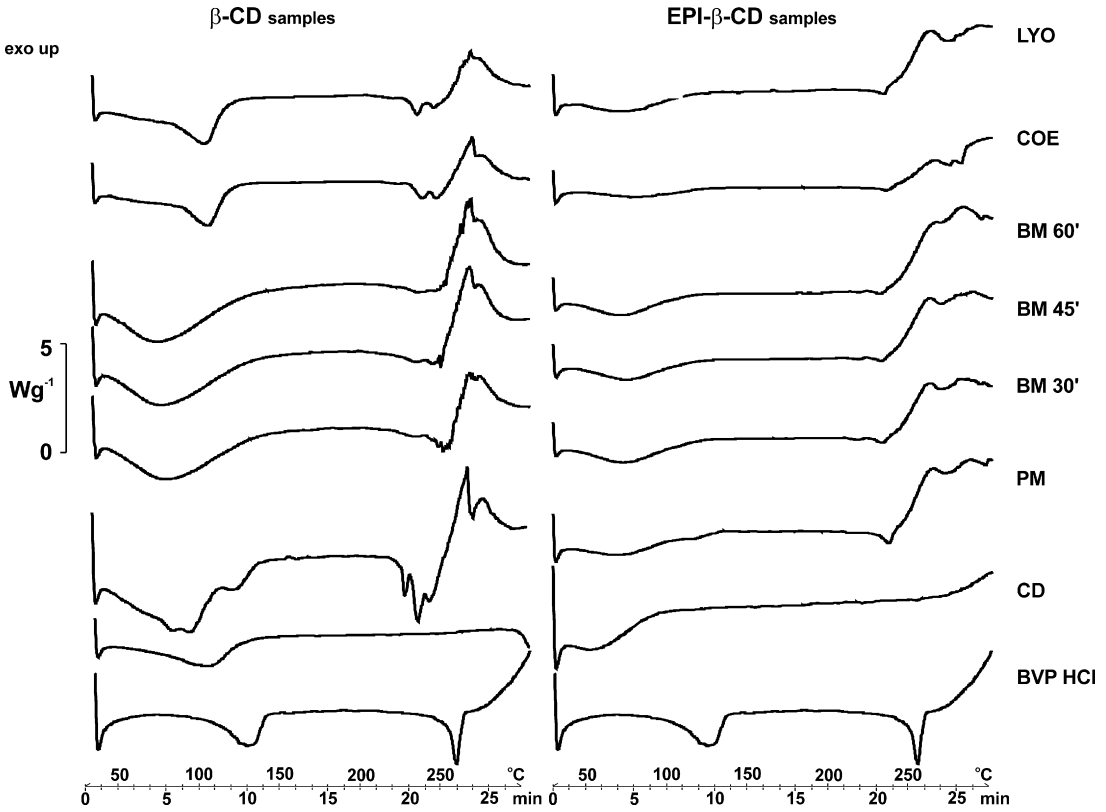


**Fig. 5.** DSC curves of the plain drug (BVP HCl), cyclodextrins used (β-CD and EPI-β-CD) and equimolar binary systems prepared by different techniques: physical mixing (PM), ball milling for 30, 45 and 60 min (BM 30′, BM 45′ and BM 60′), solvent co-evaporation (COE) and lyophilisation (LYO).

drug/cyclodextrin physical mixture clearly points to the formation of complexes in solid state.

Further informations about the interaction between BVP HCl and selected cyclodextrins were obtained by the use of XRPD. The diffraction patterns of BVP HCl and prepared binary systems with β-CD and EPI-β-CD are presented in Fig. 6. The diffraction pattern of β-CD shows several sharp peaks, characteristics for a crystalline compound, while, in case of EPI-β-CD, it is completely diffuse, pointing to the amorphous nature of this cyclodextrin derivate. The diffraction patterns of physical mixtures between BPV HCl and both cyclodextrins represent the overlapping of the diffraction patterns of each component, showing the presence of the crystalline drug in the sample and the absence of interaction between the components. In case of ball-milled binary systems with both cyclodextrins, the obtained diffraction patterns are completely diffuse, indicating the amorphization of the drug in the sample. It is important to mention that the diffraction patterns of the drug treated with the same technique still show some characteristics peaks (Fig. 2). Thus, observed amorphization of products prepared by ball milling may be attributed to the interaction between the components and possible inclusion complex formation, confirming the conclusions made on the basis of DSC experiments. In case of co-evaporated and lyophilised BVP HCl/β-CD samples, peaks corresponding to the anhydrous form of the drug and to β-CD could be observed, indicating only partial interaction between the components. On the contrary, the diffraction patterns of lyophilised and co-evaporated BVP HCl/EPI-β-CD binary systems are completely diffuse, indicating the complete amorphization of the drug. The different amorphization degree of BVP HCl observed in the co-evaporated and lyophilised samples with β-CD and EPI-β-CD may be related to different physicochemical of these cyclodextrins. β-CD is a crystalline compound with limited aqueous solubility, while EPI-β-CD is an amorphous compound with considerably higher solubility in water. Moreover, they have demonstrated different solubilizing power against BVP HCl as shown by phase solubility studies [14]. This reflects a different affinity for the interaction with the drug and may significantly affect the quality of the product obtained by lyophilisation or evaporation of the solution containing their inclusion complexes with BVP HCL. During freezing of BVP HCl/β-CD sample prior to lyophilisation it is possible that reduced aqueous solubility of β-CD may decrease its complexation efficiency and thus give rise to the precipitation of the compounds. Similarly, the evaporation of the solvent during preparation of the binary sample by co-evaporation method resulted in formation of a supersaturated drug-cyclodextrin solution. The evaporation rate of the solvent was slow enough to allow the precipitation of the crystalline compounds. On the contrary, the EPI-β-CD, as an amorphous compound with high aqueous solubility and considerably higher solubilising power towards BVP HCl, may prevent precipitation during freezing or co-evaporation of the sample, resulting in formation of amorphous products.

While summarising the results of DSC and XRPD analysis, it seems that the ball milling can be considered as the method of choice for promoting effective solid-state interactions between the drug and the selected CDs. The other advantages of this method include its short duration, the absence of solvents and the minimal influence on the solid state of the plain drug, as shown previously.

The FTIR spectra of prepared binary systems of BVP HCl with β-CD and EPI-β-CD are presented in Fig. 7. Since the position and shape of the drug absorption bands corresponding to the carbonyl group are highly influenced by the evaporation and lyophilisation processes (as shown in Fig. 3), they cannot be used to monitor the complex formation in the binary systems prepared by different methods. According to the structure of BVP/β-CD inclusion complex proposed by Pinto et al. [29], the complexation in solution occurred by the insertion of the methylated phenyl group of the drug into the central cavity of cyclodextrin molecule. Therefore, its absorption bands should be the most influenced by the complexation and can be used to assess the inclusion complex formation in the solid state. The most prominent and most informative bands in spectra of aromatic compounds occur in the low-frequency range of FTIR spectra, between 900 and 675 $cm^{-1}$. Thus, low-frequency

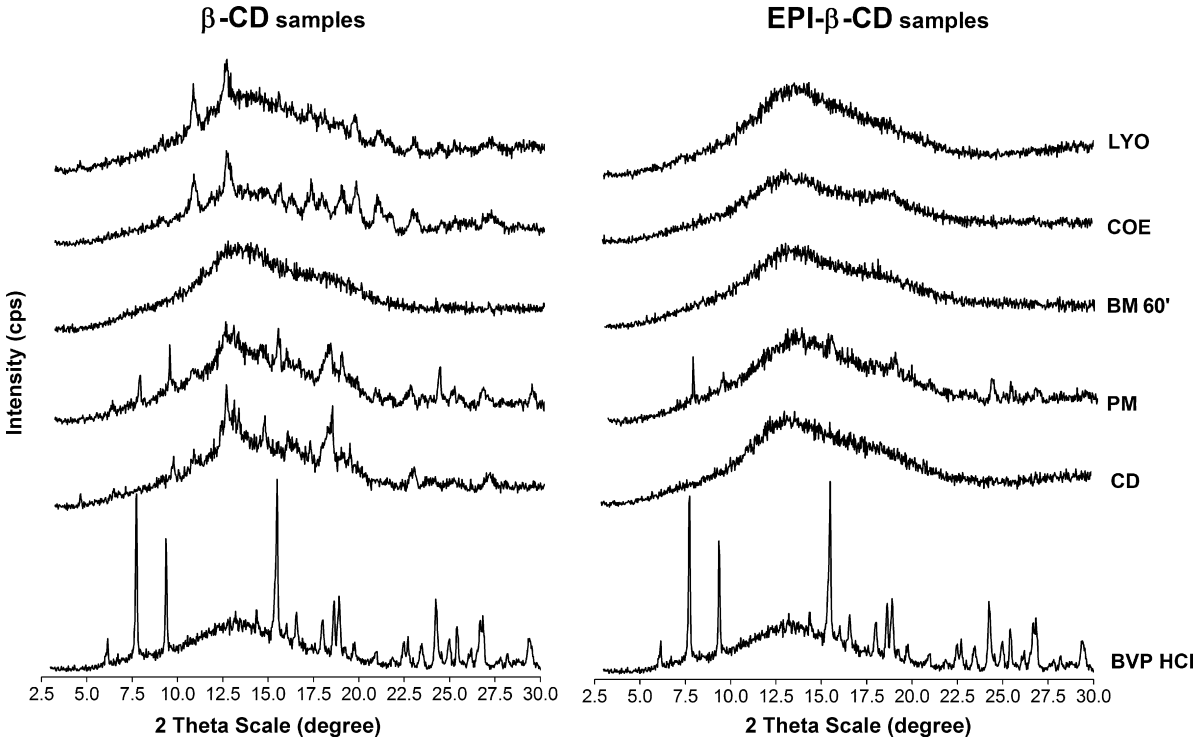


**Fig. 6.** XRPD spectra of the plain drug (BVP HCl), cyclodextrins used (β-CD and EPI-β-CD) and equimolar binary systems prepared by different techniques: physical mixing (PM), ball milling for 60 min (BM 60′), solvent co-evaporation (COE) and lyophilisation (LYO).

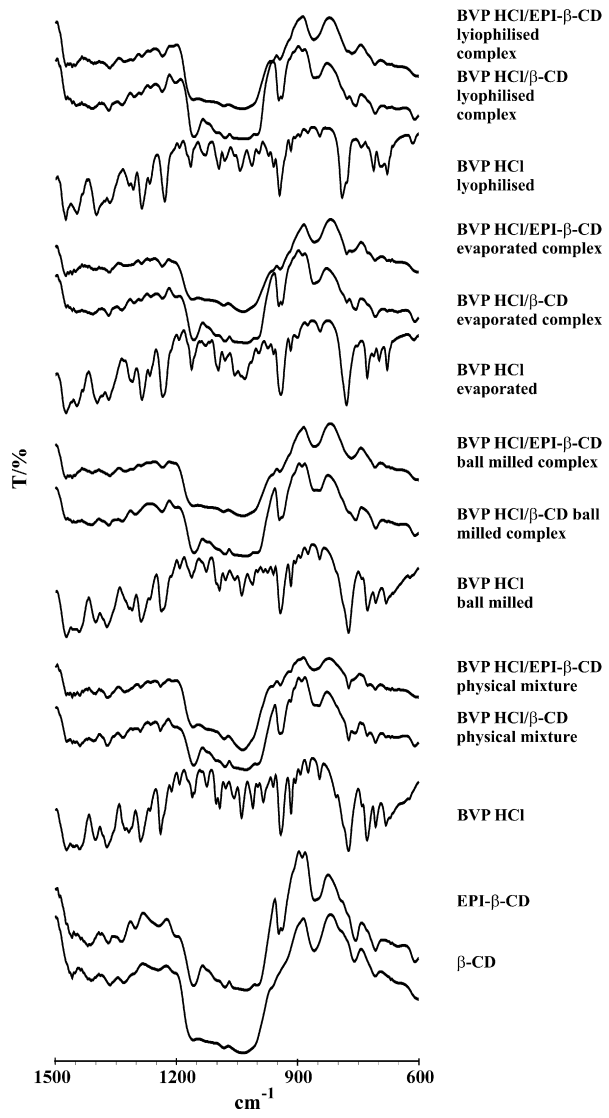


**Fig. 7.** FTIR spectra in the 1500–600 $cm^{-1}$ wavenumber range of the drug and drug/CDs binary systems prepared by different techniques: physical mixing (PM), ball milling (BM), solvent co-evaporation (COE) and lyophilisation (LYO).

range of FTIR spectra and bands corresponding to skeletal vibrations (C–C stretching) in the 1500–1400 $cm^{-1}$ range may be of use in monitoring the solid-state interactions between BVP HCl and selected CDs. It is worth to mention that neither β-CD nor EPI-β-CD showed any intense peaks in the above-mentioned area.

In FTIR spectrum of untreated BVP HCl, the absorption bands corresponding to the C–C stretching of the aromatic ring appeared as a doublet at 1471 and 1440 $cm^{-1}$, while out of plane =C–H bending appeared as a single peak at 774 $cm^{-1}$. In the spectra of physical mixtures of the drug with both CDs, the bands are still present at the same wavenumbers, but their intensity is reduced. This is indicative of the presence of only weak interactions between the components, as shown also by DSC and XRPD analysis. In FTIR spectra of ball-milled BVP HCl, the absorption bands corresponding to the C–C stretching of aromatic ring appeared at 1473 and 1446 $cm^{-1}$ and out of plane =C–H bending appeared as single peak at 774 $cm^{-1}$, while in case of ball-milled systems with cyclodextrins they are shifted to lower wavenumbers (755and 766 $cm^{-1}$ for samples with β-CD and EPI-β-CD, respectively) and reduced in intensity. In case of evaporated and lyophilised BVP HCl, the wavenumbers corresponding to the above-mentioned absorption bands are 1473, 1445, 779 and 1473, 1441, 789 $cm^{-1}$, respectively, while they are significantly reduced in intensity in FTIR spectra of evaporated and lyophilised systems of the drug with both CDs. The shift of the absorption bands and/or its significant reduction may be attributed to more intense interaction between the components with respect to the corresponding physical mixtures, since the insertion of the methylated phenyl group of the drug molecule into cyclodextrin cavity would reduce its vibrational motions, resulting in decrease and/or completely disappearance of the corresponding absorption bands. Thus, the observed changes in FTIR spectra indirectly confirmed the formation of the inclusion complexes in solid state. In all samples, the changes in FTIR spectra are more pronounced for samples with EPI-β-CD, confirming stronger interaction of this cyclodextrin with BVP HCl, compared to β-CD.

While confronting the results obtained by DSC, XRPD and FTIR analysis, it may be deducted that thermal analysis is more sensitive for detection of the degree of crystallinity of the sample compared to XRPD. In the same time its effectiveness can be seriously hampered in the case of the thermally induced interaction between the components. Therefore, the DSC followed by XRPD analysis should be a golden standard in the analysis of the drug/cyclodextrin interaction in the solid state, while FTIR is a complementary method which may be useful to fully elaborate the data obtained by DSC and XRPD methods.

### 3.3. *In vitro dissolution studies*

Solid-state analyses revealed that ball milling was the most effective technique for establish solid-state interactions between BVP HCl and β-CD or EPI-β-CD. Therefore, ball-milled binary systems were selected to determine their dissolution properties in comparison with those of ball-milled drug.

Until now, there is no official pharmacopeial method for dissolution studies of drugs aimed for buccal delivery. *In vivo* drug dissolution on the surface of the buccal mucosa is limited by the amount of saliva present within the mouth. Also, the existence of an unstirred water layer on the surface of the buccal mucosa may have a significant impact on the drug dissolution rate. As a result, dissolution tests using standard USP apparatus and large volumes of dissolution media might not give results that reflect the actual *in vivo* dissolution behaviour [21].

In our study we used and compared two different methods: a modified dispersed amount method (MDA technique) and the Franz diffusion cells (FDC technique). In case of the MDA technique, the rapid dissolution of all solid products in simulated saliva solution was observed. In the first 5 min of the experiment, 94.39% of BVP HCl was dissolved, while in case of its inclusion complexes with β-CD and EPI-β-CD, the dissolution was completed in the same period of time (Table 1). These results reflect the increased aqueous solubility of the drug upon complexation with these CDs.

However, the method was poorly discriminating, probably due to the direct stirring to which the sample is subjected, which does not simulate the conditions present at level of the oral cavity.

In the dissolution studies performed by FDC technique, sample and dissolution medium were separated by a cellulose acetate membrane with pore size of 0.65 μm. The membrane was readily wetted with the dissolution medium and the dissolved drug or inclusion complex could pass freely across the membrane to the receptor compartment. The presence of the membrane allowed the formation of the unstirred drug layer and, in the same time, restricted the volume of the simulated saliva available to the wetting of the drug particles. Thus, this model should permit the creation of conditions more similar to those encountered at the surface of the buccal mucosa. The volume of the dissolution medium was similar to that used in MDA technique.

**Table 1**
Dissolution data in simulated saliva of ball-milled BVP HCl and its ball-milled products with β-CD and EPI-β-CD according to the modified dispersed amount (MDA) and the Franz Diffusion cell (FDC) methods: first-order dissolution constant ($k_1$), Higuchi dissolution constant ($k_H$), correlation coefficient ($r^2$) and quantity of dissolved drug at 5 min expressed as percentage ($Q_{5\,min}$).

| Sample | "FDC technique" | | | | "MDA technique" |
|---|---|---|---|---|---|
| | First-order kinetic model | | Higuchi kinetic model | | $Q_{5\,min}$ (%) |
| | $k_1$ ($\times 10^3$ min$^{-1}$) | $r^2$ | $k_H$ (mg min$^{-1/2}$) | $r^2$ | |
| BVP HCl BM | 2.576 ± 0.174 | 0.9689 | 0.902 ± 0.032 | 0.9910 | 94.39 ± 1.97 |
| BVP HCl/β-CD | 1.573 ± 0.075 | 0.9865 | 0.608 ± 0.011 | 0.9979 | 100.39 ± 2.23 |
| BVP HCl/EPI-β-CD | 3.219 ± 0.093 | 0.9934 | 1.032 ± 0.035 | 0.9968 | 99.99 ± 0.41 |

As it could be seen comparing the data presented in Fig. 8 and Table 1, the dissolution behaviour of BVP HCl obtained by FDC technique was completely different compared to that of MDA technique. The observed differences in results obtained by the two techniques may be attributed to the more controlled wetting of the samples in case of experiments performed by the FDC technique. Among many parameters that may have influence on the dissolution rate of a compound, the wetting of the sample particles with the dissolution media and solubility of the sample in the dissolution media are recognized as the most critical ones [30]. Due to restricted amount of fluid available for the wetting of the drug particles, the dissolution rate of BVP HCl was significantly retarded.

In the case of ball-milled plain drug, after the first 5 min, only 2.11% of the initial content was dissolved, while at the end of the experiment, after 1.5 h, the amount of dissolved drug was 23.32%.

Unexpectedly, and in contrast with MDA results, the dissolution behaviour of BVP HCl/β-CD was worse than that of the plain drug: an initial lag time of 5 min was observed, and, after 1.5 h only 14.86% of the initial drug amount was dissolved (Fig. 8). The observed result may be attributed to the increased bulkiness of the product upon the inclusion complex formation. In fact, although the complexation of BVP HCl with β-CD increased its aqueous solubility, it also increased approximately 4.5 times the amount of the sample. The first step in the dissolution process of BVP HCl/β-CD inclusion complex was the wetting of the sample with the dissolution medium. In case of FDC technique, owing to the limited amount of simulated saliva available for the wetting of the sample particles, the reduced wetting rate of the bulky sample became the rate limiting step, reducing the overall dissolution rate of the BVP HCl/β-CD system. Instead in the MDA technique, the amount of simulated saliva available for the wetting of the sample was not restricted. In such conditions, the solubility of the sample was the rate limiting step that controlled the overall dissolution rate, resulting in faster dissolution of the BVP HCl/β-CD inclusion complex.

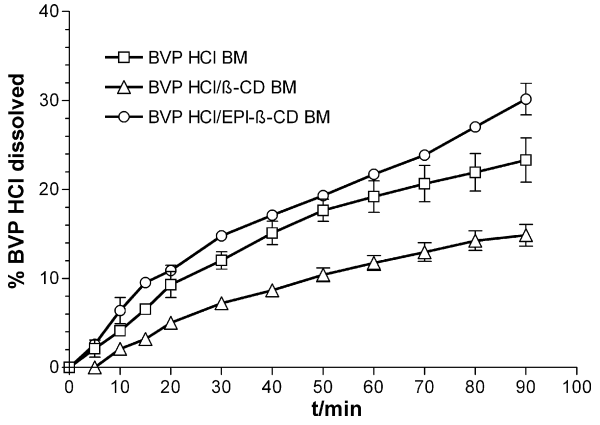


**Fig. 8.** *In vitro* dissolution profiles of ball-milled BVP HCL and drug binary systems with β-CD and EPI-β-CD prepared by ball milling in simulated saliva solution at 37 °C obtained by the FDC technique (mean ± SD, $n = 3$).

Complexation of BVP HCl with EPI-β-CD, in agreement with MDA results, increased the drug dissolution rate, resulting in dissolution of approximately 30% of the initial drug amount after 1.5 h. The observed difference between the dissolution rates of the inclusion complexes with β-CD and EPI-β-CD obtained by FDC technique may be attributed to the different aqueous solubility of these cyclodextrins. In fact, the aqueous solubility of EPI-β-CD is significantly higher compared to β-CD, giving rise to an easier and faster wetting of the sample and then resulting in the faster overall dissolution rate.

To elucidate the mechanisms driving the drug dissolution process, the results obtained by FDC technique were fitted according to first-order and Higuchi kinetic models. The results are presented in Table 1. As it could be seen from the values of the correlation coefficients ($r^2$), the dissolution may be better fitted with the Higuchi kinetic model. In case of BVP HCl, the difference of $r^2$ for both kinetic models is especially pronounced. It is important to emphasize that the characteristic of the cellulose membrane used allowed the free passage of the dissolved drug and inclusion complexes to the receptor compartment. Thus, the observed phenomena may indicate that in all cases the diffusion of the dissolved drug molecules across the unstirred aqueous layer on the membrane surface has a significant influence on the overall dissolution process.

The presented results showed that in the investigation of the dissolution properties of formulations aimed for buccal application, the choice of experimental conditions has a dramatic impact on the observed results. Our opinion is that FDC technique offers a better simulation of the conditions present at the surface of buccal mucosa. By the application of this technique, it has been demonstrated that it is possible to modify the BVP HCl dissolution properties by formation of inclusion complex with different cyclodextrins. In case of inclusion complex with β-CD the drug dissolution rate was reduced, while the complexation of BVP HCl with EPI-β-CD increased the drug dissolution rate. Then it is possible to properly tailor the drug release rate according to the desired effect, i.e., for example, a rapid onset of anesthetic action for simple dental procedures or a prolonged effect in post surgical procedures or in the treatment of oral mucositis.

## Acknowledgments

Financial support from MIUR and a personal post-doctoral grant to M. Jug from the National Foundation for Science, Higher Education and Technological Development of the Republic of Croatia are greatly acknowledged.

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