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THERMAL CHARACTERIZATION OF POLY(LACTIDE-CO-GLYCOLIDE) MICROSPHERES CONTAINING BUPIVACAINE BASE POLYMORPHS

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Abstract

Solid-state chemistry of bupivacaine base, obtained by precipitation from bupivacaine hydrochloride solutions with ammonia, was investigated.

Two bupivacaine base polymorphs (Form I: T_m=105.6±0.0°C; Form II: T_m=97.6±0.2°C) were isolated depending on the precipitation conditions and characterised by thermal analysis and FTIR spectroscopy. No weight loss was evidenced by TG confirming that no solvate formation had occurred.

Biodegradable poly(lactide-co-glycolides) microspheres containing bupivacaine base were prepared by spray-drying. In the microspheres bupivacaine base was present as the metastable low melting crystal form independently of the bupivacaine base / poly(lactide-co-glycolides) ratio.

Keywords: *bupivacaine base, microspheres, poly(lactide-co-glycolide)*

Introduction

Local anesthetics are usually administered by parenteral route for the regional anaesthesia and regional control of major pain, with a consequent decrease of systemic administration of narcotic drugs. Indeed, the prolonged release of local anesthetics produces local anti-inflammatory actions via suppression of neurogenically mediated inflammation. This provides a rationale for pre-emptive analgesia, whereby the blockage of primary afferent input prior to injury may result in a reduction of post-injury pain [1].

One approach for prolonged blockage of primary afferent input is the use of biodegradable microspheres containing local anesthetics. With this aim biodegradable microspheres made of poly(lactide-co-glycolides) (PLGA) containing bupivacaine (BPV) were proposed by several authors [1, 2].

Bupivacaine is currently used as hydrochloride (BPVH) because of the very low aqueous solubility of the base. Nevertheless, the microencapsulation efficiency of this salt in PLGA is very poor due to the hydrophobicity of the copolymer. Thus, BPV base (BPVB) microencapsulation has been performed [3–5].

Many local anesthetic compounds have been reported to exist in different crystal form [6, 7], nevertheless the characterization of the physicalof BPVB is not yet accomplished. In this paper is characterisation of the physical properties of BPVB is not yet accomplished. In this paper we report results, regarding both BPVB and BPVB in loaded PLGA microspheres, obtained by thermal analysis (DSC and TG) and FT-IR spectroscopy. Moreover, to evaluate the effect of drug/polymer ratio on the solid state of BPVB, three types of microspheres with BPVB contents ranging from 10 to 40% *m*/*m* were prepared by spray-drying and assayed.

Materials and methods

Materials

Poly(D,L lactide-co-glycolide) 50:50, (PLGA) Resomer® RG 503, inherent viscosity 0.39 dL g^{-1}, 34000 Mw, (Boehringer Ingelheim KG, Ingelheim am Rheim, G). Bupivacaine hydrochloride (S.I.M.S, Florence, I). The chemical structures of BPVB and PLGA are shown in Fig. 1.

Bupivacaine base preparation

BVPB was obtained by adding an aqueous solution of 3 or 30% *m*/*m* NH_4OH to an aqueous solution of BVPH under stirring until pH 9 was reached and thus complete precipitation was achieved. A 3% *m*/*m* NH_4OH solution was added dropwise to a saturated BPVH aqueous solution at room temperature until pH

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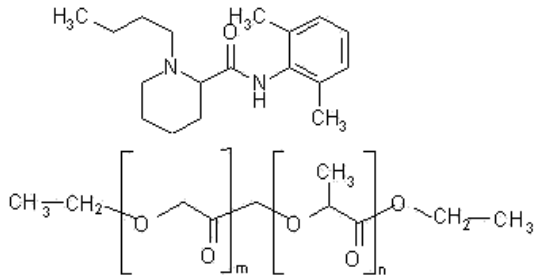

Fig. 1 Bupivacaine and poly(D,L lactide-co-glycolide) chemical structures

9. The 30% *w*/*v* NH_4OH was added to a saturated BPVH solution heated at 45°C dropwise until pH 6 and then rapidly until pH 9; the obtained BPVB suspension was cooled to room temperature.

In both cases, the precipitate was filtered, washed with distilled water until pH 7 and dried in a desiccator under vacuum over silica gel at room temperature until constant weight. BPVB purity was checked and compared to that of BPVH by the HPLC method described in Drug assay.

Microsphere preparation

Microspheres were obtained by spraying (Lab-Plant model SD04 spray-dryer, Lab-Plant LTD, West Yorkshire, UK) 2% *m*/*m* feed solutions of PLGA and BPVB in methylene chloride through a standard nozzle (inner diameter 1 mm). Three microparticulate systems were designed with the following BPVB/PLGA ratios: 10/90 *m*/*m* (formulation **1**); 25/75 *m*/*m* **(**formulation **2**); 40/60 *m*/*m* (formulation **3**). The process parameters were set as follows: inlet temperature: 50°C; outlet temperature: 34–36°C; flow rate: 15 mL min^{-1}.

After preparation the microspheres were stored at 4°C until use.

Drug content

BPVB content of the microspheres was determined as previously described [5]. Briefly, a known amount of BPVB loaded microspheres were dissolved in CH_2Cl_2 (1 mL); the drug was extracted with 5 mL of 1N H_2SO_4. After 2 min stirring and centrifugation for 15 min at 10,000 rpm, 0.50 mL of the aqueous phase were withdrawn and diluted in an appropriate amount of the mobile phase. The samples were assayed by the HPLC method described Drug assay. The method gave 98.5% recovery of the theoretical value (*C.V.*<1%).

Drug assay

The BPV was assayed by an HPLC method described in a previous work [3]. Briefly, the HPLC system was HP1100 Chemstation (Hewlett Packard, USA). Chromatographic conditions: column: Bondclone C18, 10 μm, 300 ×3.9 mm I.D. (Phenomenex, USA); mobile phase: KH_2PO_4 (pH 4.0; 0.01 M) / acetonitrile (70/30 *v*/*v*); flow rate: 1.5 mL/min^{-1}; temperature: 25°C; wavelength set: 205 nm; injection volume: 10 μL. The BPV concentrations were determined from standard curves (5–50 μg mL^{-1}).

Differential Scanning Calorimetry (DSC)

DSC scans were recorded by using a DSC 2010 TA (TA Instruments, USA). Samples (approximately 5 mg, accurately weighed, ± 0.001 mg) were sealed in aluminium pans and heated under nitrogen purging (70 mL min^{-1}). The reference was an empty pan. The equipment was calibrated with an indium sample.

All BPVB samples were scanned at 10 K min^{-1} from 30 to 130°C. Microsphere samples and the corresponding physical mixtures were first scanned at 10 K min^{-1} from 30 to 60°C, cooled down to 0°C at 20 K min^{-1} in order to erase PLGA thermal history and avoid BPVB melting which could affect the glass transition temperature (T_g) of the systems. The samples were re-heated up to 130°C at 10 K min^{-1}.

All determinations were performed in triplicate.

Thermogravimetric analysis

TG was performed by heating the samples from 30 to 130°C at the heating rate of 10 K min^{-1} by using a TGA 2050 TA (TA Instruments, USA).

FT-IR spectroscopy

Fourier-transform infrared (FT-IR) spectra were recorded with a FT-IR spectrometer Paragon 1000 PC (Perkin Elmer, US). 32 scans were collected for each sample with a resolution of 2 cm^{-1} over the 4500–500 cm^{-1} wavenumber region. Samples were prepared as KBr pellets by compaction.

Results

Bupivacaine base characterization

The purity of BPVB loaded in the microspheres was equivalent to that of BPVH, demonstrating that neither the precipitation procedure nor the microsphere preparation process had a significant influence on BPVB chemical stability.

DSC curves of BPVB, obtained according to two different procedures, revealed the presence of two different crystal forms. Form I (T_m=105.6°C) was obtained by addition of a diluted NH_4OH aqueous solution, while Form II (T_m=97.6°C) precipitated when a concentrated NH_4OH solution was used (Fig. 2). According to Bur-

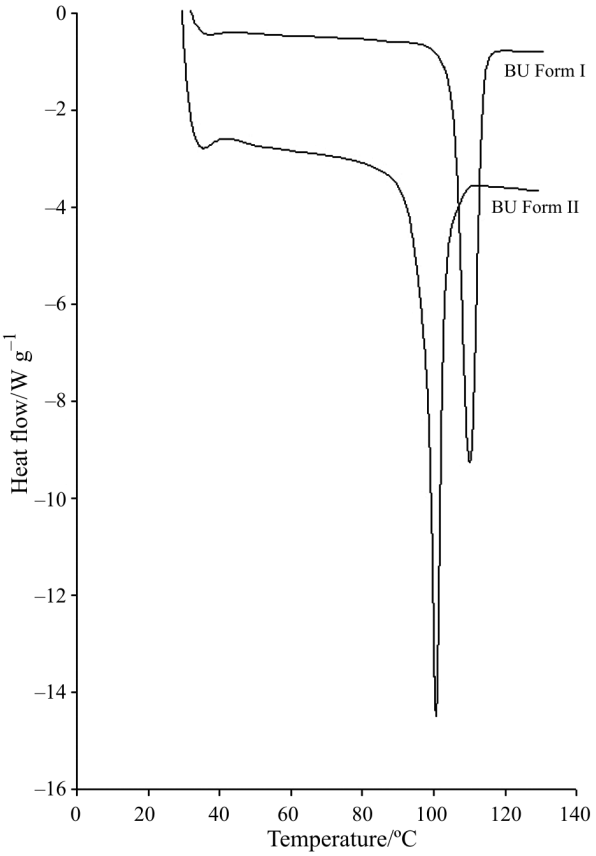

Fig. 2 DSC thermograms of BPVB Form I and Form II

ger's thermodynamic rule for polymorphic transitions [8], BPVB crystal forms represent a monotropic system, since the higher melting form has a fusion enthalpy (ΔH=84±1 J g^{-1}) greater than that of Form II (ΔH=71±1 J g^{-1}). This means that Form II is metastable at all temperatures and that the theoretical solid-solid transition point is located above the fusion temperature of the high melting polymorph.

For both crystal forms, the thermogravimetric analysis evidenced no significant mass loss (below 0.2%) in the same temperature range explored by DSC confirming that the endothermic signals in thermograms were associable to two different polymorphs and not to possible solvates, e.g. with water or NH_4OH.

The FT-IR spectra of the two polymorphs exhibit quite similar patterns. The only difference was detected in the NH deformation region: the band recorded at 1528 cm^{-1} for Form I was shifted to 1534 cm^{-1} for Form II (Fig. 3).

The physical stability of the two BPVB polymorphs was followed up to 12 months. As expected from thermodynamic considerations, Form I resulted stable within the considered period of storage; on the contrary the physical stability of Form II was limited to six months after which slow conversion into the stable form occurred spontaneously (Fig. 4).

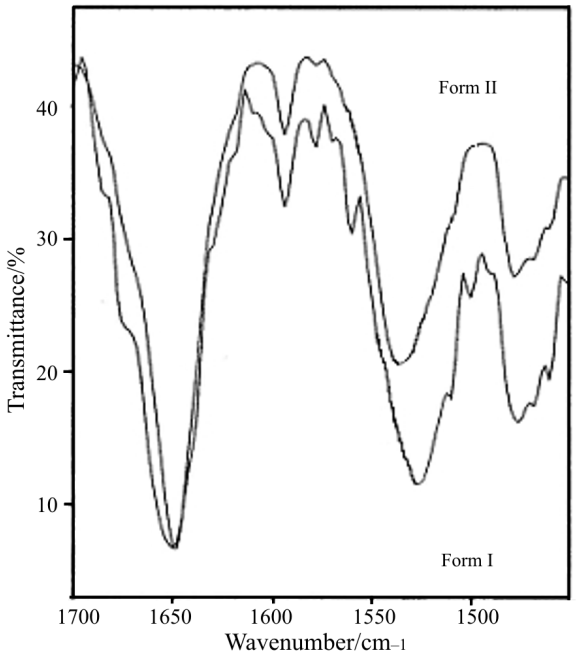

Fig. 3 FTIR spectra of BPVB Form I and Form II

Microsphere characterization

The placebo microspheres exhibited a T_g at 46.8±0.2°C which overlapped that of the raw material (T_g= 46.9±0.4°C) indicating that the production process did not affect the co-polymer structure. The DSC traces of Form I/PLGA and Form II/PLGA physical mixtures prepared with the same ratios of the BPVB microspheres revealed that the thermal pattern of the two components was substantially the same of the two single components and that, in all cases, no interaction had occurred on heating. Figure 5 compares DSC profiles of BPVB/PLGA 40/60 *m*/*m* physical mixtures and the corresponding microspheres.

In DSC curves of microspheres, the endothermic peak corresponds to the melting of the BPV Form II (Table 1 and Fig. 5). The recrystallization of BPVB as Form II could be due to the high rate of solvent evaporation during the spray-drying process. As a matter of fact, the DSC traces regarding films made of polylactide, in which the solvent evaporation process is slower as performed at room condition, showed endothermic peak related to the high melting form or a broad fusion trace according to the drug/polymer ratio [9].

Decreasing BPVB loading, the onset temperature of the endothermic peak tends to decrease with respect to that of pure BPVH. The T_g of the prepared microspheres increased with increasing the drug loading (Table 1). However, it was lower than that of PLGA. The reasons of this pattern are not yet fully understood. It could be attributed to an artefact of the heating process which promoted BPVB crystallization. It is also possible that the slight increase of the

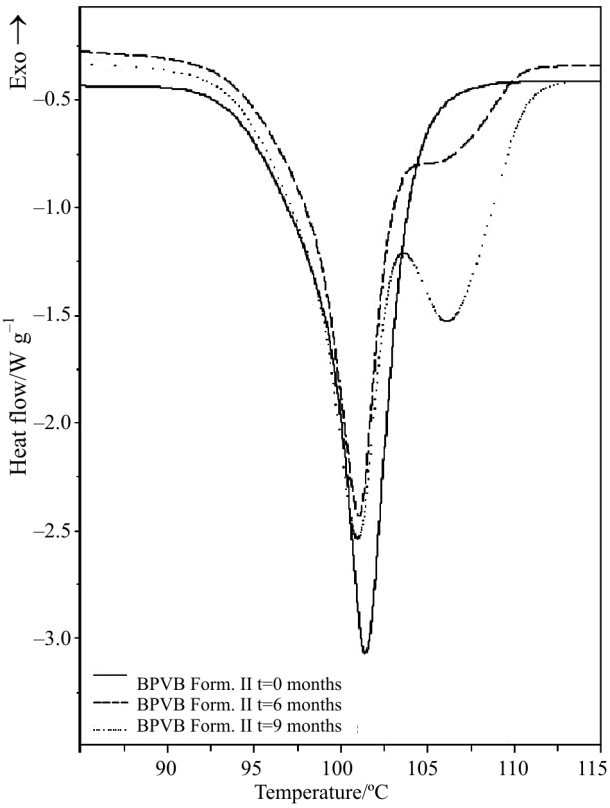

Fig. 4 BPVB Form II DSC traces immediately after preparation and after nine months of storage

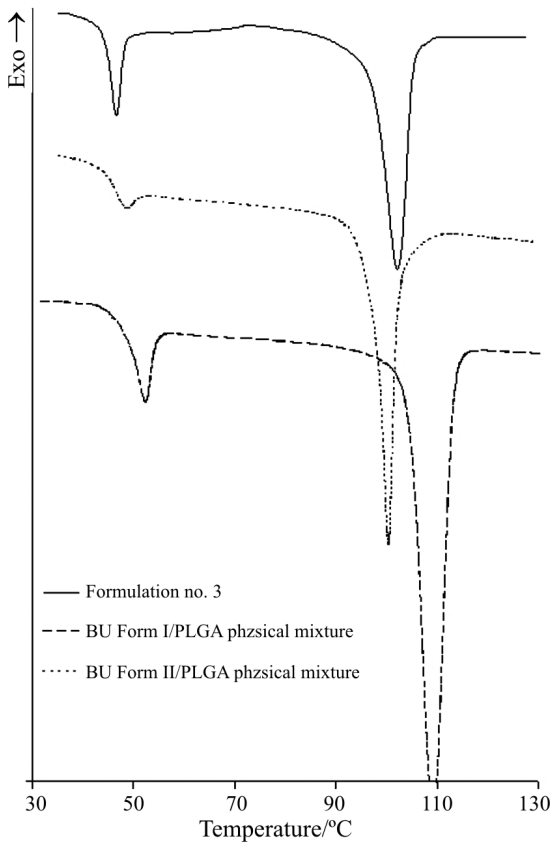

Fig. 5 DSC traces of microspheres (Formulation **3**) and of the corresponding physical mixtures

Table 1 DSC of microspheres

Formulation no.	T_g/ºC	$T_{m,\ BPVB}$/°C	BPVB crystallinity*/%
1	42.76±0.51	94.79±1.09	12.8
2	44.68±0.31	96.05±1.86	33.5
3	45.38±0.29	97.39±2.45	77.1

*calculated on the basis of ΔH of fusion for BPVB Form II

T_g could be related to the plasticizing effect of the non-crystallized BPVB fraction that decreased increasing the drug loading (Table 1).

The DSC patterns recorded on microspheres samples did not change significantly within the considered period of storage.

In all formulations, the FT-IR spectra of BPVB microspheres exhibited a transmission pattern that appeared as the sum of the two components according to their relative abundance indicating that no specific interactions between BPVB and PLGA occurred. Moreover, the NH deformation signal recorded at 1534 cm^{-1} confirmed that BPVB was encapsulated in the PLGA as Form II.

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