

(VTXRPD). DRIFTS spectra were recorded on a Nicolet Nexus 470-FT-IR spectrometer (Thermo Nicolet, USA) over a range of 600–4000 cm^{-1} . Powdered samples were mixed with KBr prior to the measurement. X-ray powder diffraction profiles were obtained with a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target: Cu; voltage: 40 kV; current: 30 mA; divergence slit: 2 mm; anti scatter slit: 0.6 mm; detector slit: 0.2 mm; monochromator; scanning speed: $2^\circ/\text{min}$ (step size: 0.025° ; step time: 1.0 s). The effect of an increase in temperature (variable temperature X-ray powder diffraction, VTXRPD) on the XRPD pattern was investigated with an Anton Paar TTK 450 low-temperature camera, attached to the Bruker D8 Advance diffractometer. A heating rate of $10^\circ\text{C}/\text{min}$ was used during all the measurements. DSC thermograms were recorded with a Shimadzu DSC-50 instrument (Shimadzu, Japan). The measurement conditions were: sample weight: ≈ 2 mg; sample holder: aluminum crimp cell; gas flow: nitrogen at 40 ml/min; heating rate: $10^\circ\text{C}/\text{min}$. Mean volume particle size distributions in suspension were measured with a Galai-Cis-1 particle size analyzer (Israel). Powder dissolution was measured using Method 2, paddle, of the USP 24. The paddle was rotated at 75 rpm and samples were taken at 7.5, 15, 30, 45 and 60 min intervals. The powder sample, 50 mg, was rinsed from the glass weighing boat into a 10 ml test tube with exactly 2 ml of the dissolution solution. Glass beads, 25 mg, with a mean size of 0.1 mm, were added to the suspension and the mixture was agitated for 120 s using a vortex mixer. The contents of each test tube was transferred into the dissolution medium, 1000 ml (0.1 N HCl + 0.1% SLS; 0.1 N HCl; H_2O) and the dissolution rate was measured. The concentration of dissolved powder was calculated from the UV absorbance at 242 nm. Results are the mean of six experiments.

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Use of 1,4-dioxan for preparation of bupivacaine loaded PLGA microspheres with an o/w emulsion extraction process

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The oil-water emulsion extraction process is one of the most popular methods for preparation of microspheres. In this method the organic solvent used for dissolving PLGA is extracted with water what results in polymer precipitation. Benzyl alcohol is the most frequently used solvent in this procedure, however acetone, ethyl-methyl ketone, ethyl formate, ethyl acetate and dimethyl sulphoxide (DMSO) were used in some studies [1–5]. The aim of this study was to prepare microspheres with 1,4-dioxan, to our knowledge not used before for such purpose. According to the pharmaceutical classification, dioxan is a class 2 solvent (methylene chloride, widely used pharmaceutical solvent belongs to the same class) [6], what means that it may be used in technological processes, but its residue in the product must strictly be controlled (LD_{50} after oral delivery is 2 g/kg) [7]. The advantage of this solvent is its good miscibility with water and most organic solvents and a high freezing point (11.8°C), what enables removing the residues during the freeze-drying step in a process of preparation of microspheres.

Microspheres prepared with dioxan (formulation D) were compared with those obtained with benzyl alcohol (formulation BA) and DMSO (formulation DMSO). Bupivacaine was encapsulated in the microspheres in order to study the relationship between the type of solvent and encapsulation and drug release rate from the PLGA matrix.

Solubility of bupivacaine in dioxan and benzyl alcohol was very good (at least 400 mg/ml) while in DMSO the drug was less soluble (50 mg/ml). PLGA dissolves easily in dioxan as well as in the two other solvents. The microspheres were prepared by a standard procedure [1].

The microscopic observation revealed that microspheres prepared with benzyl alcohol were spherical, sizing in range 1–20 μm (80% in the range 1–5 μm) (Table). In contrast to the formulation BA, formulations D and DMSO were porous and larger in size (80% of the particle in the range 1–15 μm). Particles obtained with dioxan were spherical but a significant portion of the particles prepared with DMSO was irregular in shape.

When the ratio of bupivacaine to PLGA was 10/90 all solvents enabled producing microspheres, which were similar in size and shape to the drug-free particles. However, when the amount of the drug was elevated to 25%, microspheres were only produced if dioxan was used as a solvent. Production of spherical microparticles with other solvents was not possible due to fast and uncontrolled pre-

Table: Effect of type of solvent used in the emulsion extraction method on morphology of PLGA microspheres and encapsulation of bupivacaine

		Dioxan	Benzyl alcohol	DMSO
Shape		spherical, porous	spherical, non-porous	spherical or irregular, porous
Size (µm)	range	1–70	1–20	1–60
	80% below	15	5	10
Bupivacaine content (%)	drug/polymer ratio			
	10/90	3.20	0.50	3.16
	25/75	5.02	–	–

cipitation of the drug-polymer mixture. At the bupivacaine theoretical loading 50%, preparation of the microspheres was impossible, even if dioxan was employed.

In contrast to benzyl alcohol dioxan enabled higher loading of bupivacaine, similar to that observed for formulation DMSO (Table). When the amount of bupivacaine was increased to the theoretical loading 25% the concentration of bupivacaine in the microspheres prepared with dioxan elevated to 5%. However, this result demonstrates that the loading of bupivacaine using the proposed method is less efficient than the evaporation technique used by Corre and al. [8], who encapsulated 23% of bupivacaine in PLA microspheres.

The microspheres prepared with dioxan and DMSO, loaded with bupivacaine 32 mg/g, were used for the release studies. The results are presented in the Fig. The *in vitro* release profile was biphasic with a rapid release of 23–35% drug within 24 h, followed by a slower but steady release rate over at least 4 days. The release process in the steady state phase was faster from the microspheres prepared with dioxan than from formulation DMSO: 9.7% and 4.2% of the drug was released per 24 h, respectively. The dioxan residue was analysed in the lyophilized microspheres using gas chromatography and the procedure described in USP 24. The USP limit for dioxan impurity in pharmaceutical preparations is 380 ppm. Four batches were analysed and the residual solvent varied: in two batches it was below the limit and in two others it was higher (600–750 ppm). Since in two of the formulations the amount was below the limit it may be possible to optimize conditions of the lyophilization process to reduce the level of dioxan in microspheres.

Rate of solvent extraction varies depending on solvent type, what can explain the porosity of the resulting microspheres and loading efficacy. The study demonstrates that

dioxan can be used as an alternative solvent in the emulsion extraction method, in comparison to benzyl alcohol providing better loading efficacy for the model drug bupivacaine. It may be concluded, however, that regarding the encapsulation rate, the o/w emulsion extraction method is less suitable for preparation of bupivacaine loaded microspheres than the solvent (methylene chloride) evaporation method.

Experimental

1. Materials

Bupivacaine base was kindly provided by "Polfa" (Warsaw, Poland). Poly(D,L-lactic acid-co-glycolic acid) copolymer 50:50, m.w. 33 000 (PLGA, Resomer RG 503 H) was purchased from Boehringer Ingelheim Pharma (Ingelheim, Germany) and used as received. Polyvinyl alcohol (PVA, m.w. 22 000) and dimethyl sulphoxide were purchased from Fluka (Steinheim, Switzerland), 1,4-dioxan and acetonitrile from Merck (Darmstadt, Germany) and benzyl alcohol, methylene chloride and methanol from POCh (Gliwice, Poland).

2. Preparation of microspheres

The microspheres were prepared by a standard procedure [1]. PLGA, 180 mg, was dissolved in 4.0 ml of benzyl alcohol (formulation BA), dioxan (formulation D) or DMSO (formulation DMSO) and the solution was added dropwise to 8.0 g of 12.5% w/w PVA aqueous solution. The emulsion was obtained at the stirring at 8000 rpm (Ultra-Turrax, Ika – Labor-technik, Staufen, Germany). Extraction of the solvent with water was performed by slow addition of 160 ml water (30 min) at a stirring rate of 400 rpm with a magnetic stirrer. After 2 h the suspension was centrifuged (2000 g for 10 min) and the microspheres were rinsed with 4 portions of water (3 ml) with sonication and centrifugation. Finally, 48 h freeze-drying process was carried out in an Alfa 2–4 lyophilizer (Christ, Osterode, Germany): the drying was performed by gradual increasing the shelf temperature from –40 °C to +30 °C at a pressure 0.08 bar.

Bupivacaine was incorporated by dissolving in a polymer solution in one of the three solvents used. The pH measured in the aqueous phase of the emulsion prepared by dispersing this solution in the PVA solution was 8.7. The theoretical drug content in microspheres was: 10% (20 mg drug and 180 mg PLGA), 25% (50 and 150 mg) and 50% (100 and 100 mg).

Each experiment was performed in at least triplicate.

The morphology of the microspheres was assessed using an optical microscope Studar H (PZO, Warsaw, Poland).

3. Analysis of the encapsulating of bupivacaine

The amount of the encapsulated drug was analysed using an extraction procedure: microspheres (10 mg) were dissolved in 4.0 ml methylene chloride and bupivacaine was extracted 3 times with 5.0 ml of HCl (0.05 mol/l). Concentration of the drug in the extracts was analysed with HPLC using a C18 column (Merck); a mixture of acetonitrile, 0.01 mol/l phosphate buffer pH 7.7 and methanol (40:35:25% v/v) was used as a mobile phase (1 ml/min). Chromatograms were recorded at 220 nm.

4. *In vitro* release studies

Twenty mg of the lyophilized microspheres prepared with dioxan and DMSO (bupivacaine/PLGA ratio 10/90) was introduced to 2.0 ml of water. The tube with the suspension was shaken at 37 °C. In order to study the amount of the drug released, 1.0 ml of the clear supernatant was withdrawn and the drug concentration was analysed using HPLC; 1.0 ml of water was added to the suspension and the release study was continued up to 120 h (formulation D) or 168 h (formulation DMSO).

5. Analysis of dioxan residue in the microspheres

The dioxan residue was analysed in the lyophilized microspheres using gas chromatography and employing the USP 24 method.

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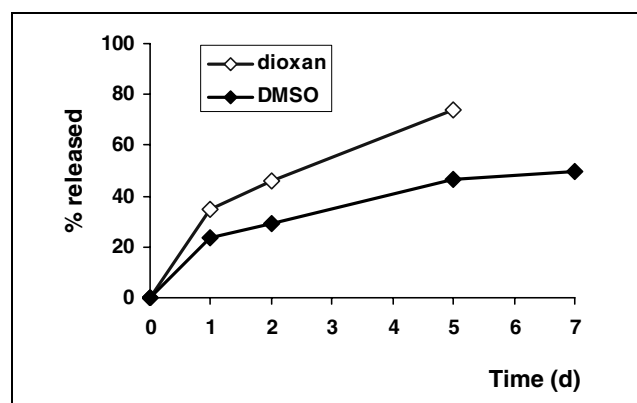


Fig.: *In vitro* release profiles of bupivacaine from PLGA microspheres prepared with aid of dioxan or DMSO