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Controlled release of drugs from multi-component biomaterials

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Abstract

In order to control their release, drugs are encapsulated into systems which are expected to provide a certain site with a predetermined amount of drug over a well-defined period of time. Here we report on a multi-component drug delivery biomaterial that consists of a hydrogel matrix in which drug-loaded biodegradable microcarriers are dispersed, and whose potential applications could be found in the design of implantable devices with long-term activity, as required by contraceptive and hormone replacement treatments. The release profile of the drug can actually be tuned by the complex interplay of several release mechanisms, including the permeability and eventually the degradation rate of the microcarriers and the diffusion through the hydrogel. The hydrogel consisted of 2-hydroxyethyl methacrylate cross-linked by ethylene glycol dimethacrylate. The microcarriers were biodegradable poly-ε-caprolactone (PCL) microspheres in which active molecules, such as levonorgestrel (LNG), were encapsulated. The hydrogels were characterized by water swelling, thermal properties, LNG diffusion through drug-free and drug-depleted hydrogel membranes and LNG release from devices with drug dispersed in the hydrogel. The PCL microspheres were observed by scanning electron microscopy; their size distribution, LNG loading and release were also investigated. The hydrogel-microsphere assemblies were characterized in terms of the distribution of the microspheres within the hydrogel, water swelling and the release of the encapsulated molecules. The developed device, due to its composite structure, has the ability to combine several release mechanisms, leading to drug release obeying zero-order kinetics for most of the time.

Keywords: drug delivery; controlled release ; hydrogel ; poly(2-hydroxyethyl methacrylate) ; microspheres

1. Introduction

Theoretically, an ideal drug delivery system should deliver a drug to a specific site in a specific time and release pattern. After more than 40 years of research and development of a large number of systems, polymeric supports are integral to the design and preparation of controlled delivery formulations. In fact, the great versatility of polymers from a structural point of view, together with the possibility of combining hydrophilic and hydrophobic components, as well as polymer-polymer macromolecules, polymer-drug, polymer-solvent or polymer-physiological medium interactions, offers huge possibilities for the design and preparation of formulations with specific properties and functions.

Hydrogels are cross-linked polymer networks that are insoluble, but able to swell in water. Due to their close resemblance to natural tissues, they have been frequently used for tissue engineering and drug delivery [1-5]. Due to their biocompatibility, hydrogels based on poly(2-hydroxyethyl methacrylate) (pHEMA) are commercially employed as soft contact lenses and intraocular lenses, and have been investigated for several biomedical applications, such as substrates for cellular and tissue engineering [6,7] and drug delivery devices [8-11]. The release of the drug from hydrogel controlled release systems is affected by the rate of water diffusion into the polymer, which in turn depends on the chemical structure of the polymer (polarity, glass transition temperature, flexibility of the polymer backbone) and on the cross-link density and inter-chain interactions [3,11,12].

Poly-ε-caprolactone (PCL) is a biodegradable and biocompatible aliphatic polyester with a semicrystalline structure and a very low glass transition temperature. Due to its slow degradation [13-15], high permeability to many drugs and low toxicity [16], PCL microcarriers consisting of a drug dispersed in spherical polymer matrix have been extensively evaluated for the administration of active compounds over long periods [17-20]. In these systems, the drug release is governed by factors depending on the polymer (molecular weight, crystallinity), the drug (solubility in water/biological fluids) and their interactions, as well as on the microcarrier's characteristics (drug loading, particle size, porosity) [21]. Previous studies have indicated that PCL could retain its integrity in

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the body over long periods before being metabolized and completely excreted [22,23]. The fact that the degradation rate of PCL is relatively slower than those of other known biodegradable polymers makes it suitable as a long-term device. By embedding PCL microspheres containing drugs into hydrogels, two different release mechanisms can be combined: diffusion through the polymeric matrix for the microcarriers and diffusion through the hydrophilic matrix for the hydrogel. With the aid of the different parameters mentioned above, the release profile of drugs encapsulated into PCL microspheres, which in turn are embedded in pHEMA-based hydrogels, can be tuned to an apparent zero-order and maintained over a long period. Low-dose drug delivery devices releasing active principles over a long period could find their application in the field of contraceptives and hormone replacement treatments. Intrauterine devices and intrauterine drug delivery devices act locally, avoiding systemic effects, and are therefore particularly attractive for contraception or hormone replacement therapy in post-menopausal women [24,25].

2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma Aldrich and distilled under vacuum or used as received. Freshly prepared aqueous solutions of potassium persulfate, $K_2S_2O_8$ (Sigma Aldrich) and sodium disulfite dry $Na_2S_2O_5$ (Merck) were used together as redox initiators.

Levonorgestrel (LNG) was purchased from Industriale Chimica (Saronno, Italy). PCL (mol. wt. = 50,000) was received as a free sample from Solvay. Poly(vinylalcohol) (PVA) (Mowiol 3-83, mol. wt. = 18,000 g/mol) was supplied from Calbiochem, and the Carbomer 980 was from Certa (Belgium). All other chemicals were analytical grade and were used as received.

2.2. Preparation of multi-biomaterial delivery systems

2.2.1. Microspheres

PCL microspheres, blank and loaded with drug, were prepared by the oil in water (o/w) emulsion-solvent evaporation method, a technique commonly used for the microencapsulation of water-insoluble drugs within hydrophobic polymers [26,27]. PCL and LNG were dissolved in 5 ml dichloromethane, forming the oil phase. This organic phase was gradually added into a 250 ml beaker containing 125 ml of 0.27% PVA aqueous solution, maintained at room temperature and stirred at 300 rpm to form an o/w emulsion. The emulsion system was continuously stirred at room temperature and ambient pressure to allow the evaporation of dichloromethane and to let the droplets harden into microspheres. The microspheres were collected by filtration on a filter paper, washed three times with distilled water and freeze-dried to obtain free flowing PCL microspheres.

2.2.2. Hydrogels

Dissolved oxygen was removed from monomer mixtures composed of HEMA with 0-0.5 vol.% EGDMA by nitrogen bubbling for 5 min. For the preparation of drug-loaded substrates, either appropriate amounts of drug were dissolved or appropriate amounts of PCL microspheres encapsulating drug were dispersed in the monomer mixture. Subsequently, the organic phase was mixed in reagent glasses in a volume ratio of 3:1 with aqueous solutions of redox initiators (6.4 mg of $K_2S_2O_8$ and 3.2 mg of $Na_2S_2O_5$ per ml of water). The nitrogen bubbling continued for another 15 min, then the reagent glasses were left at room temperature under the nitrogen blanket until the gelation of the reaction mass was visible. Finally, the viscous liquid was stirred to achieve a uniform composition and was then either aspirated into 1 ml one-way plastic syringes or poured between two glass plates kept approximately 1 mm apart by the use of a rubber spacer and left to polymerize overnight at room temperature. A series of pHEMA rods embedding increasing amounts of blank PCL microspheres was also prepared.

The pHEMA-based rods and membranes synthesized as above were washed for 5 days with distilled water, with daily water changes, for the removal of unreacted monomer (the residual HEMA was less than 5 ppm after 5 days' washing as determined by high-performance liquid chromatography, HPLC), and then stored either freeze-dried (rods) or in distilled water (membranes).

pHEMA-based drug-depleted membranes, used for drug diffusion experiments, were prepared in the presence of LNG (at an LNG concentration of 0.01 wt.% in the hydrogel mixture) and subsequently washed for 20 days with a 3:1 (v/v) ethanol:water mixture, with daily changes, to ensure the complete extraction of the drug. The drug-depleted and drug-free hydrogel membranes were then equilibrate in water for another 10 days before the diffusion experiments started. The absence of extractable drug was verified by HPLC measurements.

2.3. Morphology

2.3.1. Visual

Hydrogel rods embedding different amounts of blank PCL microspheres were removed from the water, blotted dry with paper towels and aligned on a grey background. Pictures were taken with a Minolta DiMAGE F300 digital camera.

2.3.2. SEM

PCL microspheres were mounted on aluminium stubs using double-sided tape, sputtered with palladium under vacuum and visualized using a JEOL JSM 840A scanning electron microscope.

Hydrogel rod samples were partially air-dried, cut with a razor blade or with a microtome, dried completely under vacuum at room temperature and then prepared for SEM as the microspheres.

2.4. Size distribution

Microspheres geometrical diameter was determined using the laser diffractometry principle of Mastersizer® 2000-Scirocco® 2000 (Malvern Instruments, Malvern, UK), which applies the Mie scattering measurement principle. A sample of 100 mg lyophilized microspheres was placed into the tray in the Scirocco feeder. The microspheres were continuously supported by a vacuum stream from the tray to the measuring window where the sample diffracts the laser beam. Sample dispersion was achieved by accelerating particles within the vacuum stream. The geometrical diameter was expressed in terms of volume median diameter, $D(v,0.5)$, as well as $D(v,0.1)$ and $D(v,0.9)$.

2.5. Drug loading

The drug amount present in the microspheres was determined by dissolving 10 mg of drug-loaded microspheres in 3 ml dichloromethane with vigorous stirring to dissolve the polymer. A volume of 10 ml ethanol was added, leading to polymer precipitation, then the dichloromethane was evaporated under vacuum. A 1:1 ethanol:water mixture was added to the solution containing the drug, which was subsequently filtered using a 0.1 μm Millipore filter assembly. After a suitable dilution, the drug content of the sample was determined by HPLC assay. Each measurement was conducted in triplicate.

2.6. Swelling behaviour

Freeze-dried samples of pHEMA-based hydrogels with embedded blank PCL microspheres were used as the starting materials. Dry hydrogel rods were immersed in distilled water at room temperature. At certain time intervals, the hydrogel pieces were extracted from water, blotted dry with a paper towel and weighed.

2.7. Differential scanning calorimetry (DSC)

The melting temperature (T_m) and glass transition temperature (T_g) were investigated by DSC (DSC Q100, TA Instruments). Small fragments of dried pHEMA hydrogel samples embedding blank PCL microspheres, cut from different parts of the same sample, were weighed in aluminium pans in triplicate, closed with appropriate aluminium lids and subjected to a heating program of 10.0 °C min^{-1} from 25.0 to 150.0 °C. T_m and T_g were determined from the second run.

2.8. In vitro kinetics

2.8.1. Drug release kinetics from microspheres

In vitro LNG release experiments from the microspheres were conducted in triplicate using a USP Apparatus 4 (Sotax CE7smart and CP 7-35 pump, Sotax, Basel, Switzerland). This method has the advantage of ensuring constant optimal sink conditions due to the continuous flow of the solvent [28,29]. Approximately 10 mg of microspheres was accurately weighed and placed in the flow-through cells. The study was performed in pH 7.4 phosphate buffer containing sodium azide (0.025 wt.%) as an antimicrobial agent. The medium was circulated through the cells at a constant flow rate of 16 ml min^{-1}. Samples were withdrawn at specific time points and analysed by HPLC. After the withdrawal of each sample, fresh buffer was added to the cells to ensure the sink condition of the test.

2.8.2. Drug release kinetics from the device

Release of drugs from the device was assayed in triplicate under sink conditions. In order to perform the release kinetics, devices consisting of microspheres with encapsulated drug, embedded in hydrogel, were placed in glass bottles containing 250 ml of phosphate buffer solution (pH 7.4) containing 0.025 wt.% sodium azide. The bottles were placed in a shaking water bath (SW 22, Julabo, Seelback, Germany) and shaken at 37 °C. Samples were collected at specific time intervals, the withdrawn aliquots being replaced immediately with fresh medium. The

amount of released drug was determined by HPLC.

2.8.3. Drug diffusion through hydrogel membranes

Hydrogel membranes, cut in appropriate forms, were fixed between the donor and the acceptor chamber of Franz cells [30], a Teflon ring ensuring a constant membrane area in contact with the two chambers. The donor chambers were filled with a suspension prepared from an aqueous gel of Carbomer 980 (1% in water) and LNG (15 wt.% in the suspension). As the receptor medium, water with 0.1% parabens was used. Throughout the experiment the receptor medium was stirred at 650 rpm and a temperature of 37 °C was maintained using a water thermostat. Samples of 1 ml were withdrawn at specific time points and their LNG content was measured by HPLC. The withdrawn volumes were subsequently replaced by fresh receptor medium.

2.9. Drug assay

2.9.1. LNG assay

The HPLC system consisted of a model 1100 LC system with vacuum degasser, quaternary pump, thermostatted automatic sample injector, thermostatted column compartment and diode-array detector from Agilent Technologies. Fifty microliter samples were injected on a Lichrocart column (250 x 4 mm) prepared with a Lichrospher 100 RP-18 5 µm phase (Merck®). The temperature of the column was maintained at 30 °C. The mobile phase, composed of 60/40 mixture of acetonitrile and water, had a flow rate of l.0 ml min^{-1}. All samples were assayed at 245 nm. This method was successfully validated and showed a good linearity, reproducibility and accuracy from 30.0 to 800.0 ng ml^{-1}.

3. Results and discussion

3.1. Micro spheres

3.1.1. Size distribution and drug loading

The experimental conditions and corresponding values of particle size distribution, drug loading and entrapment efficiencies (DEE) for each sample are summarized in Table 1. The entrapment efficiency of drug in microspheres was determined using the following relation:

$$\text{Drug entrapment efficiency} = \frac{\text{Real drug loading}}{\text{Theoretical loading}} \times 100 \qquad (1)$$

Drug loading values of PCL microspheres containing LNG were found to be 8.2 ±0.2% and 12.4 ±3.6%, respectively for the two samples. The DEE for the batches A and B were 79.5 ± 3.2% and 62.2 ± 4.8%, respectively.

The relatively low DEE suggests the low solubility of the LNG within PCL, a behaviour already shown for other steroid drug (progesterone) in biodegradable polymers [31].

The viscosity of PCL solutions depends directly on polymer concentration, organic solvent and temperature. Accordingly, an increase in the polymer concentration produced a significant increase in the viscosity, thus leading to an increase in the emulsion droplet size and finally to a higher PCL microsphere size. Moreover, the high concentration of polymer in the emulsion droplets led to an enhancement of the encapsulation efficiency, because the high viscosity of the organic phase tends to restrict the drug partitioning into the external aqueous phase [32]. The viscosity of the organic phase presumably increased even more upon addition of LNG, the drug-loaded microspheres (sample B) having a larger particle size than the blank ones (sample C).

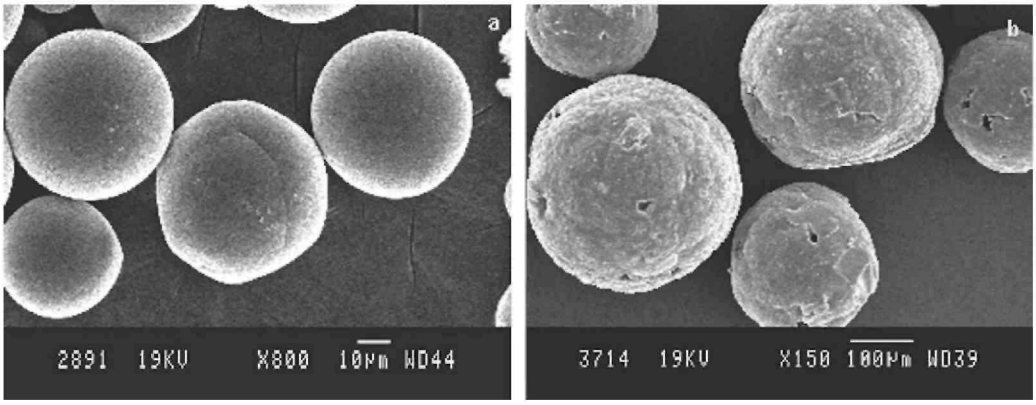
Table 1: *Effect of the processing parameters on the size, drug loading and encapsulation efficiency of PCL microspheres produced by the emulsion evaporation method*

Sample	LNG (mg)	PCL (mg)	PCL concentration in CH_2Cl_2 solution (%)	Particle diameter (µm)			Drug loading (%)	Drug entrapment efficiency (%)
				D(*v*,0.1)	*D*(*v*,0.5)	*D*(*v*,0.9)		
A	50	500	0.100	250.0 ± 19.5	345.8 ± 16.0	456.6 ± 39.1	8.2 ± 0.2	79.5 ± 3.2
B	100	500	0.025	117.9 ±29.1	223.2 ± 22.5	392.4 ± 91.4	12.7 ± 1.6	62.2 ± 4.8
C	-	500	0.025	48.4 ± 1.8	93.2 ±2.5	165.6 ± 5.2	-	-

3.1.2. Morphology

SEM micrographs of the blank microspheres showed quite smooth surfaces with a few pores, probably due to the evaporation of dichloromethane during the emulsion-evaporation process. The particles appeared to be homogeneously distributed, without evidence of collapsed particles. The drug-loaded microspheres showed a certain surface roughness (Fig. lb) and a larger particle size than the blank microspheres.

Fig. 1: *SEM micrographs of PCL microspheres: (a) blank and (b) encapsulating LNG.*



3.1.3. Drug release

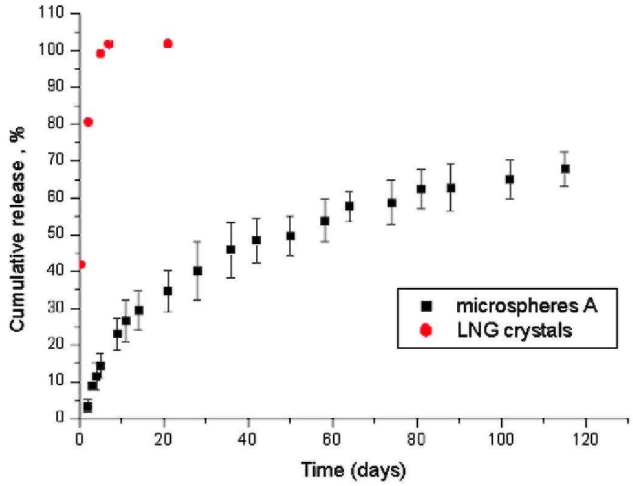
The drug release kinetics of LNG from PCL microspheres were analysed by plotting the cumulative release data of LNG against time. The release behaviour of LNG from PCL microspheres in comparison with the release of LNG crystals is illustrated in Fig. 2, which indicates the continuous release of PCL from microspheres for more than 4 months.

At the initial stage of microsphere release, the burst effect related to the drug entrapped near the surface of the microspheres was small and followed by a slow release phase. The low permeability of PCL with regard to water caused the delay of water penetration in the microspheres, leading to the low burst effect [13,15]. At the later stage, the drug was released more slowly, with a rate determined by the diffusion of the drug through the polymer matrix. For the characterization of the release mechanism of LNG from PCL microspheres we have applied a mathematical model of the release profile, using Eq. (2), proposed by Sinclair and Peppas [33] for the diffusional release from a spherical matrix

$$\frac{M_t}{M_\infty} = kt^n \qquad (2)$$

where M_t/M_∞ is the fractional drug release at the time t, k is a constant characteristic of the polymer-drug interaction and n is the diffusional exponent (0.432 for Fickian diffusional release from a spherical matrix). The experimental values fitted the theoretical curve, built with an n value of 0.432 ($R^2 = 0.97$). Consequently, the main mechanism leading to the release of the drug from the microspheres is diffusion through the polymeric matrix, the erosion of the polymer playing an insignificant role at this stage.

Fig. 2: *In vitro release profiles from LNG crystals and PCL microspheres encapsulating LNG.*



3.2. Hydro gels

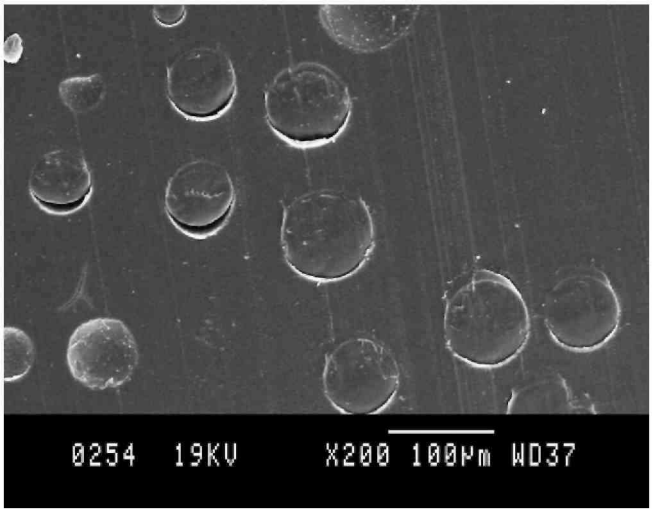
pHEMA hydrogels prepared with different percentages of cross-linker, such as 0.10%, 0.25% and 0.50% EGDMA, did not show any significant differences in their aspect and investigated properties (such as water uptake and water swelling kinetics, glass temperature transition, drug diffusion experiments), typical for this range of relatively low cross-linker concentrations [12]. The hydrophobicity increase due to the cross-linker is too low to bring significant changes to the water diffusion into the hydrogel, and the decrease of the network mesh is not restrictive enough for the diffusion of small molecules. Similarly, the changes in the thermal properties are too small to be detected by DSC. Therefore, the results of the present study will concern only pHEMA hydrogels with 0.1% EGDMA.

3.2.1. Morphology

3.2.1.1. Visual. The diameter of hydrogel rods remained constant after swelling (4.8 mm), regardless of the amount of PCL microspheres embedded into the hydrogels in the range between 0 and 100 mg per ml of hydrogel. With increasing microsphere content, the hydrogel rods, which were still transparent up to a content of approximately 30 mg per ml of hydrogel, lost their transparency.

3.2.1.2. SEM. The composite rods with PCL microspheres showed good incorporation of the microspheres into the hydrogel bulk, with uniform distribution of the microspheres at the cut level (Fig. 3). Moreover, the microsphere integrity was fully preserved, proving the physical embedding of the microspheres into the hydrogel.

Fig. 3: *SEM picture of a pHEMA hydrogel containing 100 mg of blank PCL microspheres per ml of hydrogel mixture.*



3.2.2. Water uptake

pHEMA hydrogels are known to exhibit a narrow range of swelling at equilibrium in water, ranging from 39% to 42% water for the fully swollen hydrogels [34,35], regardless of the dilution of the starting monomer solution and its content of cross-linking agent [36]. The same behaviour was seen for the samples prepared in the framework of the present study. Moreover, for the preparations embedding microspheres, the presence of the microspheres seemed not to have any major influence on the swelling behaviour of the pHEMA hydrogels containing PCL microspheres.

The description of water diffusion into hydrogels is based on Fick's hypothesis [12,37], which assumes proportionality between the flux and the concentration gradient of the physical quantity diffused. A direct consequence of this is that the diffusion along a concentration gradient is expected to scale as $t^{1/2}$ in homogeneous and isotropic systems. Fickian diffusion occurs when the diffusion rate of water into the hydrogel is much slower than the relaxation rate of the polymer chains of hydrogel.

The water uptake (S_w) of hydrogel rods embedding blank PCL microspheres at the time t was calculated according to Eq. (3), where S_w is shown as the exponential function of time (t) in the second part of the equation

$$S_w = \frac{m_t - m_d}{m_d} \times 100 = kt^n \qquad (3)$$

where m_t is the weight at the time t, m_d is the dry weight, k is a constant related to the network structure and n is the number indicating the diffusion type.

To evaluate the values of n and k, curves were fitted through the first experimental points on the water uptake curves of pHEMA hydrogels incorporating different amounts of blank PCL microspheres. The corresponding values are listed in Table 2. The values for n are all very close to 0.45 (value calculated for cylindrical geometry), indicating the Fickian behaviour during water swelling of all hydrogels investigated, and the values for k point out the reproducibility of the network structure in the experimental conditions used.

Table 2: *Water uptake parameters for hydrogels embedding PCL microspheres*

Blank PCL microspheres content of pHEMA hydrogel samples (mg per ml of hydrogel)	Water uptake (% of the blank hydrogel water uptake)	*k*	*n*
0	100.00	0.1344	0.4721
5	100.56	0.1376	0.4506
10	102.35	0.1531	0.4244
15	101.06	0.1336	0.4594
20	100.86	0.1384	0.4606
25	100.56	0.1336	0.4595
30	99.33	0.1350	0.4602

3.2.3. Thermal analysis

Fig. 4 shows the thermal characteristics of the multi-material, identified by DSC measurements. pHEMA hydrogel is characterized by the glass transition at temperatures around 115-116 °C, and drug-free PCL microspheres are characterized by the melting temperature around 55 °C. Similar values obtained for the composites suggest the physical entrapment of the microspheres into the hydrogel matrix, without any chemical interactions.

Furthermore, the technique allows the quantitative evaluation of the microsphere loading, the area of the endothermic peak at around 55 °C being proportional to the PCL microsphere loading for the hydrogel samples investigated (Fig. 4). The linear dependence of the thermal effect on the PCL microspheres content is shown in Fig. 5.

Fig. 4: *DSC thermograms for pHEMA hydrogels containing different amounts of PCL microspheres.*

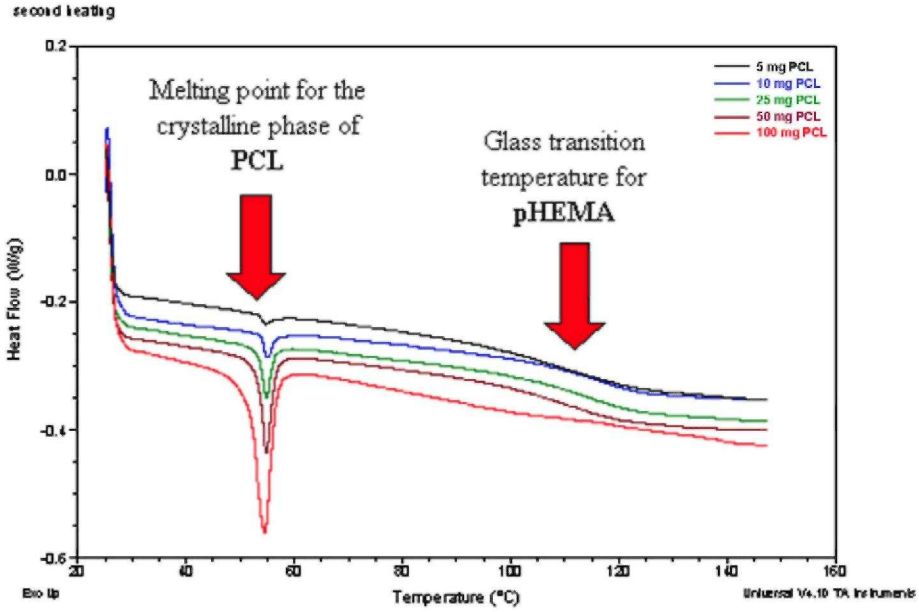
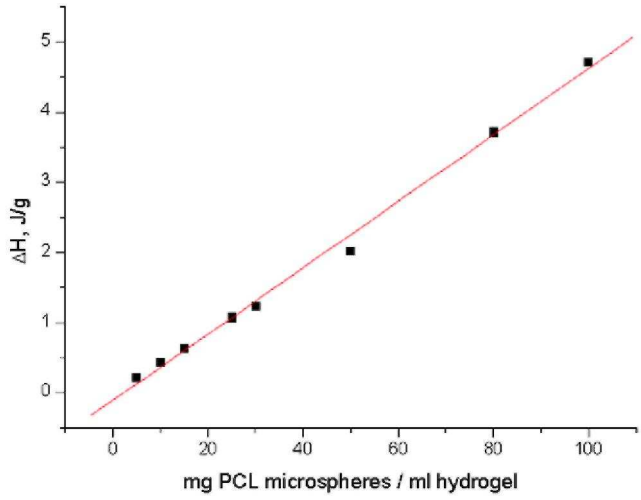


Fig. 5: *Melting enthalpy of pHEMA-based multi-biomaterials at around 55 °C as a function of the PCL microsphere loading.*



3.2.4. In vitro drug diffusion and release

The probe solute size and shape, its hydrophilic and hydrophobic character, and the availability of "free" water molecules to hydrate and dissolve the solute molecules are important factors governing the solute permeation through any particular hydrogel [2]. The drug can be loaded into the hydrogel by either equilibrium partitioning or cross-linking of the matrix in the presence of the drug. Variations in the release rate of the drug can be achieved by changing the nature, type and structure of the substrate. By employing several synthetic methodologies, the density of the matrix and also the solubility of the migrating substance in the polymeric network could be finely tuned.

Because, in our case, different release mechanisms contributed to the final release rate of the drugs, the influence of the individual components of the device on the release rate of LNG was investigated.

3.2.4.1. Drug diffusion through hydrogel membranes. The results of permeation experiments were analysed using the time lag model (Eq. (4)), which allowed the calculation of the diffusion coefficient of LNG through pHEMA membranes from the time-axis intercept of the plot of cumulative weight of diffused drug per unit area ($\mu g\ cm^{-2}$) as a function of time (s) [38,39]

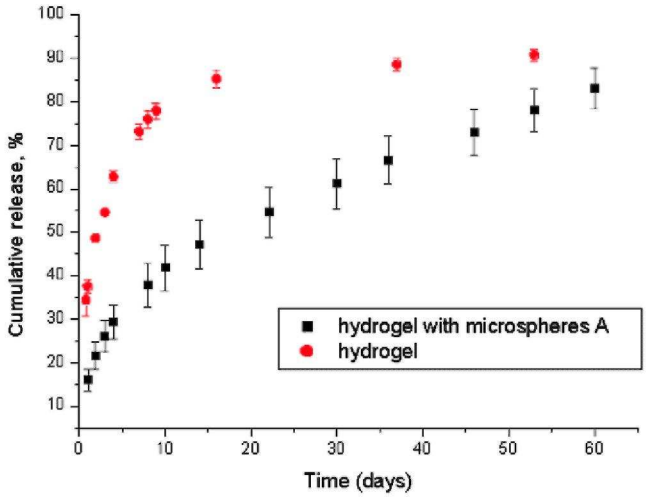
$$D = \frac{l^2}{6t_{lag}} \qquad (4)$$

where D is the diffusion coefficient, l is the thickness of the membrane and t_{lag} is the time-axis intercept of the cumulative release plot.

The value obtained for the diffusion coefficient of LNG through blank pHEMA membranes was (1.58 ± 0.24) x 10^{-8} cm^2 s^{-1}, a typical value of the diffusion coefficient in swollen hydrogels [39,40]. The diffusion experiment using drug-depleted membranes led to a value of the diffusion coefficient of (2.83 ± 0.09) x 10^{-8} cm^2 s^{-1}, which suggests either a partial molecular imprinting of the hydrogel (unlikely when the synthesis is performed at low percentages of cross-linker) or a slight widening of the hydrogel network due to the possible extraction of low molecular weight species (oligomers) during the washing step with the water/ ethanol mixtures.

3.2.4.2. Drug release. Fig. 6 illustrates the release profile of LNG from different devices. The first device type consisted of hydrogel rods containing the drug directly dispersed in hydrogel; the second device contained polymeric microspheres encapsulating the drug, dispersed in the hydrogel. The hydrogel alone was unable to achieve a sustained release of the drug. After 10 days, the LNG-hydrogel device had released almost 80% of the total dispersed drug.

Fig. 6: *Release profiles of LNG from hydrogel rods following: (a) LNG encapsulation in microspheres and embedding of microspheres in hydrogel or (b) LNG dispersion in hydrogel.*



The significant change in the slope of the release profile in the second release stage suggested an incomplete release, because the hydrophobic drug may associate with the hydrophobic domains of the hydrogel [39].

The multi-component biomaterial including microspheres in the hydrogel matrix showed continuous LNG release for more than 2 months. During the first release stage, the device including microspheres only released 42% of the total LNG in a "burst", followed by a constant release characterized by zero-order kinetics (R^2 = 0.997).

The release rate was surprisingly high compared with the release rate of the microspheres encapsulating LNG. This behaviour can be explained by the different conditions in which the release experiments for the different materials were performed, as well as by the markedly hydrophobic character of LNG and its consequently higher solubility in HEMA monomer than in water (the water solubility of LNG is 1.12 µg ml^{-1} at 37 °C). For high encapsulation yields of LNG in PCL microspheres, crystals of LNG lying on the surface of the microspheres dissolve in the monomer mixture before the gelation starts, leading to the molecular dispersion of part of the drug in the hydrogel matrix and to an increase in the overall porosity of the microspheres.

Molecular dispersions of drugs in cross-linked hydrogels -currently being investigated for the delivery enhancement of poorly water-soluble drugs [41] - could be the reason for the fast release of the LNG in the first stage. The permeability of drug-free and drug-depleted membranes of pHEMA towards steroids has been already investigated, with similar results [42]. On the other hand, microcarriers with higher porosity will have higher release, fact which accounts for higher release rates in the subsequent stage for the microspheres embedded in hydrogel when compared with the microspheres alone.

Certainly there are situations when an initial burst followed by a prolonged release could be advantageous, for example, for wound treatment or targeted delivery [43], but for long-term controlled release devices, the burst is often regarded as a negative aspect. Therefore we started to devise several strategies for decreasing the burst in the system described above. The simplest one consists of washing the microspheres prior to their dispersion in the monomer mixture. Other attempts will deal with the coating of the microspheres containing drug, or with their production using a system that allows better encapsulation of the drug in the bulk of the microspheres.

4. Conclusions

This study was undertaken to confirm the feasibility of a multi-biomaterial system comprising microspheres embedded in a hydrogel matrix. The use of the multi-component drug delivery biomaterial enables the drug release to obey zero-order kinetics for most of the time, and allows different release kinetics for drugs contained in the same device but encapsulated either directly in the hydrogel or in the microcarriers. The hydrogel is therefore not only a physical support for the drug-loaded microspheres, building the body of a device which could be, for instance, easily implanted and/or inserted and removed, but also acts as a hydrophilic matrix for the encapsulation of additional drugs. This ability of the hydrogel can be further developed by performing the co-polymerization of the HEMA with a hydrophobic co-monomer, such as methyl methacrylate. Encapsulation of various drugs, including temperature-and organic solvent-sensitive ones, is possible, as the hydrogel synthesis does not use organic solvents or have high reaction temperatures. Potential applications could be found in the design of implantable devices with long-term activity, such as is required by contraceptive and hormone replacement treatments.

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