Introduction

Biotechnology has evolved to the point that it is possible to prepare therapeutic genes for most diseases. The use of these new medicines is hampered by the lack of a suitable carrier system that helps the DNA in reaching the target cells and in the intracellular trafficking. Cationic polymers have been proposed as vectors for genetic material since they readily form polyelectrolyte complexes with DNA. A drawback of using basic polymers like poly(L-lactic acid) is that they are able to interact with erythrocytes when they are placed in contact with blood.

The purpose of this work is to synthesise new polymers based on poly-α-L-amino acids that are able to condense and protect DNA, show low cyto and haemotoxicity and enhance transfection efficiency compared to naked DNA.

Results and discussion

Polymers based on poly-L-glutamic acid are prepared via aminolysis of poly-γ-benzyl/trichloromethyl-L-glutamate. In this way polymers were prepared that feature in their side chain tertiary amines in combination with primary amines, imidazole groups, dithiopyridine groups, maleimide groups or hydrazide functions, depending on the future purpose. The molecular weight of these polymers were controlled by the monomer to initiator ratio.

The physico-chemical properties of the new polymers were determined with EtBr fluorescence, photon correlation spectroscopy and titration studies. The interaction with erythrocytes was assessed by monitoring haemolysis and haemagglutination. The MTT assay was used to get more information on the cytotoxicity of the polymers.

Acknowledgements

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HYDROGELS BY STEREO-COMPLEXATION OF WATER-SOLUBLE PLLA-PEO-PLLA AND PDLA-PEO-PDLA TRIBLOCK-COPOLYMERS

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Introduction

This work describes the formation of new hydrogels for use in biomedical applications by the stereo-complexation of triblock-copolymers of PEO and L- or D-lactide from solutions in water.

Poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) are known to form stereo-complexes upon blending or mixing [1,2]. By analogy, similar stereo-complexes are formed upon mixing di- and triblock-copolymers, if the stereo-regular lactide sequences are of sufficient length [3,4]. Water-soluble triblock-copolymers containing poly(lactide) (PLA) blocks can be prepared by initiating lactide polymerizations with polyethylene glycol (PEG) [5].

PLA-PEO-PLA triblock-copolymers that are soluble in water and at the same time are able to form water-insoluble stereo-complexes, offer exciting new possibilities in tissue engineering and in the controlled delivery of sensitive drugs like proteins and peptides.

Materials and Methods

PLA-PEO-PLA triblock-copolymers were prepared in bulk at 130°C by initiating either L-lactide or D-lactide polymerizations with PEG [3]. L- and D-lactide were also polymerised under the same conditions to yield the stereo-regular homopolymers. PEGs of different molecular weights were obtained from Merck (Germany), polymer grade L- and D-lactide monomers from Purac Biochem (The Netherlands) and the catalyst stannous octoate from Sigma (USA). All materials were used without further purification.

The absolute molecular weights of the synthesized (triblock-co)polymers were determined by GPC (Waters system) in CHCl₃, at 25°C, calibrated with narrow polystyrene standards with viscometer- (Viscotec H502) and refractive index (Waters 410) detection. The triblock-copolymer composition and the lactide monomer conversion were characterized by ¹H-NMR (Varian 300 NMR).

The solubility of the synthesized triblock-copolymers in water (1 g per 20 ml) was tested at 22°C.

Polymer films of (triblock-co)polymers were prepared by casting 1:1 mixtures of the L-lactide and D-lactide (triblock-copolymers in CHCl₃, or water. The 1:1 mixtures of L-lactide and D-lactide triblock-copolymers in water (1 g per 20 ml) were also freeze-dried. After removal of the solvent, the solubility in water was again evaluated. DSC (Perkin-Elmer DSC-7) was used to show the formation of stereo-complexes in the obtained materials.
Results and discussion

In the absence of PEG, which acts as an initiator in the ring opening polymerization of lactide, very high molecular weight L- and D-lactide homopolymers with number averaged molecular weights in excess of $100 \times 10^3$ could be synthesized. Therefore, in the PLA-PEG-PLA triblock-copolymer synthesis the molecular weights and the lengths of the lactide blocks could precisely be tuned by adjusting the PEG to lactide ratio in the feed. GPC and NMR confirmed the living character of the PEG-initiated ring opening polymerization of lactide.

The phase diagram of PLLA-PEO-PLLA triblock-copolymers in water is presented in Fig. 1.

As PLA is hydrophobic and PEO is hydrophilic, the ratio of the lengths of the lactide- and PEO blocks will determine the solubility of the triblock-copolymer. As the lactide content in the triblock copolymer increases, its solubility in water decreases. PLLA-PEO-PLLA triblock-copolymers with longer lactide sequences are only soluble in water if the molecular weight of the PEO block is high enough.

Stereo-complexation of the L- and D-lactide segments can only occur provided the length of these sequences is adequate. The water-soluble triblock-copolymers with the highest PEO-block molecular weight of $13 \times 10^3$ were further investigated in their complexation behaviour.

Table 1 shows the formation of stereo-complexes in solvent cast films, as observed by DSC.

Films prepared mixed PLLA and PDLA solutions in chloroform show a large melting endotherm at temperatures much higher than the regular melting temperature of the lactide homopolymers, indicating stereo-complexation. Polylactides melt at temperatures between 180 and 190°C, while the stereo-complex shows a peak melting temperature at approximately 235°C.

Films prepared from mixtures of triblock-copolymers in chloroform and in water, in which the lactide sequences are 13 or 15 lactide units long, also show an endotherm at high temperatures. The melting temperature and the heat of fusion of these stereo-complexes is significantly lower than that of the homopolymers. Nevertheless, even in films prepared from solutions in water, stereo-complexation can be observed. Once the films are formed and stereo-complexation has taken place, these films are not soluble in water anymore.

Freeze-drying was also carried out to remove the water part from these triblock-copolymer solutions in water. The obtained porous structures, with a porosity of approximately 95%, were not very strong, but could nevertheless be handled easily.

Table 1
Stereo-complexation of 1:1 mixtures of lactide homopolymers and triblock-copolymers

<table>
<thead>
<tr>
<th>Complex</th>
<th>Solvent</th>
<th>Melting range (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLLA + PDLA</td>
<td>CHCl$_2$</td>
<td>203–246</td>
</tr>
<tr>
<td>15L-PEO-15L + 15D-PEO-15D</td>
<td>CHCl$_2$</td>
<td>191–242</td>
</tr>
<tr>
<td>13L-PEO-13L + 13D-PEO-13D</td>
<td>CHCl$_2$</td>
<td>183–241</td>
</tr>
<tr>
<td>13L-PEO-13L + 13D-PEO-13D</td>
<td>water</td>
<td>183–241</td>
</tr>
</tbody>
</table>
It is therefore possible to prepare water-insoluble polymeric materials with water as the only solvent. Current research is aimed at optimizing these systems and investigating their suitability for the controlled delivery of proteins and peptides.

Conclusions
PLA-PEO-PLA triblock-copolymers, where the PLA part consists of either L- or D-lactide can form water-insoluble stereo-complexes from solutions in water. The use of these hydrogels offers new perspectives in cell encapsulation and in the preparation of controlled protein and peptide delivery systems.

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References

NEW IONIC AMPHIPHILE BIOVECTOR™ AS CARRIER OF POOR SOLUBILITY DRUGS
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Introduction
Improving formulation capabilities and bioavailability of poorly soluble or lipidic drugs has been one of the most challenging fields in applied pharmaceutical research. A wide variety of approaches has been developed including nanoparticles (1), lipid based colloidal systems (2), liposomes (3), solid lipid nanoparticles (4), lipobeads (5) and polymeric micelles (6). Depending on the route of administration and drug characteristics, each system has its own advantages and limitations. Hydrophobic or amphiphilic systems allow to solubilise and stabilise non-soluble and hydrophobic compounds, but drug loading in the matrix is sometimes a difficult process and may lead to stability problems. Limitations are generally related to drug loading, loading stability and stability of the particulate system in suspension. Surfactants are often necessary to ensure the stability of the particulate system.

Ion exchange gel matrices allow efficient loading of ionic compounds, but they lack stability under saline conditions. We thus deduced that the combination of ionic and hydrophobic properties could be an interesting tool to obtain good solubilisation properties, stability of drug entrapment, stability of the particulate system as well as superior formulation properties.

Experimental
Preparation of cationic amphiphile lipid/polymer matrices.
Cationic hydrogel particles were obtained by cross-linking maltodextrins (Glucidex 2, Roquette Freres, France) with epichlorohydrin (Fluka, Switzerland) and further modification with glycidyl trimethyl ammonium (Fluka, Switzerland). The resulting hydrogel was sized down in a high pressure homogeniser (Minilab H-80, Rannie, Denmark). Pressure was adjusted to obtain microparticles around 10–50 μm and nanoparticles around 80 nm. The particles were further purified by ultrafiltration against pure water (System Minisette equipped with a 30 kD membrane, Pal/Filtron, USA). Nitrogen elementary analysis gave 1.7 mmol/g of charge density.

To prepare the amphiphile matrix, an ethanol solution of dipalmitoyl phosphatidyl glycerol (DPPG, Lipoid, Germany) or sodium palmitate (Sigma, USA) was added to the polysaccharide matrix suspension under stirring, at a given ratio and temperature. The mixture was incubated during 1 h.