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PREPARATION OF AMINO-PEGYLATED POLY(ANHYDRIDE) NANOPARTICLES APPLYING SOLVENT DISPLACEMENT METHOD

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Summary. Simple solvent displacement technique was applied for the preparation of amino-pegylated poly(methyl vinyl ether-co-maleic anhydride) nanoparticles (PVM/MA-NP). Two amino-functionalized polyethylene glycols were selected, in particular bis-aminoethyl polyethylene glycol (DAE-PEG) and bis-aminopropyl polypropylene glycol–polyethylene glycol-polypropylene glycol (DAP-PEG). Higher pegylation degree was achieved with DAE-PEG probably due to the higher capacity of its amino-groups to interact with the anhydride groups of PVM/MA copolymer. Further, the increasing of the initial concentration of both PEGs increased pegylation degree. However, the higher association of PEGs reflected in larger nanoparticle size and higher polydispersity. Hence, appropriate adjustment of initial concentration of DAE-PEG and DAP-PEG is needed for the achievement of nanoparticles with optimal physico-chemical properties.

Key words: nanoparticles, pegylation, solvent displacement, amino-functionalized PEGs

ПРИГОТВЯНЕ НА АМИНО-ПЕГИЛИРАНИ ПОЛИАНХИД-РИДНИ НАНОЧАСТИЦИ ЧРЕЗ МЕТОД НА ИЗМЕСТВАНЕ НА РАЗТВОРИТЕЛЯ

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Резюме. Лесен и удобен метод, базиран на изместване на разтворителя, е приложен за приготвянето на пегилирани наночастици с носител поли(метил винил етер-ко-малеинов анхидрид) (PVM/MA-NP). Два амино-функционални полиетилен гликола са проучени в изследването – бис-аминоетил полиетилен гликол (DAE-PEG) и бис-аминопропил полипропилен-полиетилен-полипропилен гликол (DAP-PEG). По-висока степен на свързване на полиетилен гликол е постигната с DAE-PEG вероятно поради по-големия капацитет на неговите аминогрупи да взаимодействат с анхидридните групи на полимерния носител. Изследването показва, че с увеличаване на първоначалната концентрация на полиетилен гликолите се повишава степента на пегилиране. По-високата степен на пегилиране от своя страна рефлектира в по-голям размер на наночастиците и по-висока полидисперсност. Подходящо вариране на първоначалната концентрация на DAE-PEG и DAP-PEG би довело до получаването на наночастици с оптимални физикохимични свойства.

Ключови думи: наночастици, пегилиране, изместване на разтворителя, аминофункционални полиетилен гликоли

Introduction

The selection of the method for nanoparticle preparation is an important factor taking in account its influence on the properties of the resulting nanoparticles [4, 6]. One of the most frequently applied methods is the solvent evaporation method [3]. This method includes formation of emulsion consisting of organic solution of the polymer carrier and an aqueous solution of surfactant and drug. In the second stage of the method, the organic solvent is evaporated and the precipitation of the polymer into particles occurs. One of the problems associated with this method is the incomplete solvent elimination. The latter leads to development of new

techniques, particularly those where the preparation conditions are less problematic, e.g. lower solvent toxicity. On the other hand, the pegylation of nanoparticles, which is a new strategy in nanoparticle technology, is associated with some difficulties regarding preparation procedure. The main problems are the need of high temperature copolymerization between polymer carrier and the selected polyethylene glycol, as well as the need of specific conditions and catalysts [1, 2]. In this view, the aim of the present study was to develop a simple method for preparation of pegylated poly(anhydride) nanoparticles.

Materials and methods

Poly(methyl vinyl ether-co-maleic anhydride) (PVM/MA) (Mw of 200 kDa) was a gift from ISP (Barcelona, Spain). O,O' – Bis – (2-aminoethyl) polyethylene glycol 2000 (DAE-PEG) and O,O'–Bis-(2-aminopropyl)–polypropylenglycol-polyethylenglycol-polypropylenglycol 2000 (DAP-PEG) were supplied by Fluka (Switzerland). Micro BCA Protein Assay Reagent Kit was purchased by Pierce (Rockford, USA).

Preparation of poly(anhydride) pegylated nanoparticles

PVM/MA copolymer (100 mg) and various concentrations of DAE-PEG or DAP-PEG were dissolved and stirred in acetone (5 ml) for 1 h. After their incubation, equal volumes of ethanol and water were added subsequently to the organic phase. The solvents were eliminated under reduced pressure (Buchi R-144, Switzerland). The nanoparticles were purified by twice centrifugation at 17000 rpm for 20 min (Sigma 3K30, Germany) and finally lyophilized (Genesis 12EL, Virtis, USA) using sucrose as cryoprotector (5% w/v).

Characterization of nanoparticles

The nanoparticle size and zeta-potential were determined by photon correlation spectroscopy and electrophoretic laser doppler anemometry using a Zetamaster analyzer (Malvern Instruments, UK). Samples were diluted with 0.05M phosphate buffered saline (pH 7.4) and measured at 25°C with a scattering angle of 90°.

The associated amounts of DAE-PEG and DAP-PEG to the nanoparticles were calculated by measurement of their concentrations in the supernatants after centrifugation of the nanoparticles. Micro BCA Protein Assay Reagent Kit (150 μl) was added the same volume of the supernatants (150 μl) and the plates containing the samples were incubated at 37°C for two hours. Further, the absorbance was determined by colorimetry at a wavelength of 570 nm (Labsystems iEMS Reader MF, Finland). The calculations were made using standard curves of DAE-PEG and DAP-PEG prepared under the same conditions at concentration range of 2-10 mg/ml (for DAE-PEG r = 0.9958 and for DAP-PEG r = 0.9993, respectively).

Results and discussion

Poly(methyl vinyl ether – maleic anhydride) copolymer (PVM/MA) could be suitable nanoparticle carrier allowing ligand attachment without need of special chemical reagents because of the ability of anhydride functional groups. The affinity of acid anhydride residues of PVM/MA to amino groups has been previously reported regarding an interaction between PVM/MA and various proteins [5]. In this view, the association of polyethylene glycols under mild conditions was expectable. The reaction is based on the attack of a nucleophile group (such as amino-groups) to the anhydride.

In the present study, a simple solvent displacement method was applied and evaluated for preparation of pegylated PVM/MA-nanoparticles. The first step of the procedure included dissolution and simultaneous incubation of copolymer and polyethylene glycol in organic solvent (acetone). On the second stage, a formation of nanoparticle suspension was performed by addition of appropriate non-solvents, in our study hydroalcoholic mixture. According to this technique, approximately 56% of PVM/MA were encapsulated into nanoparticles modified with DAE-PEG (DAE-PEG-NP), and 65% into nanoparticles modified with DAP-PEG. The percentage of the encapsulated PVM/MA copolymer differed depending on the amount of the associated polyethylene glycol. This fact illustrated that both polyethylene glycols possessed different affinity to PVM/MA copolymer. As shown in Fig. 1, higher pegylation degree was achieved in the case of DAE-PEG, in particular at lower initial concentration. The latter was probably due to higher ability of amino-groups of DAE-PEG to interact with the anhydride residues of PVM/MA copolymer. One of the reasons for the lower capacity of DAP-PEG could be the presence of polypropylene glycol chains in DAP-PEG.

The second important observation was that the pegylation degree increased with the increasing of the initial concentration. However, the higher pegylation changed nanoparticle characteristics (Table 1). It is well known that nanoparticle characteristics (size,

zeta-potential, surface architecture) determine the interactions with the cells [7]. In the present study, the higher association of DAE-PEG and DAP-PEG increased nanoparticle polydispersity and nanoparticle size. This phenomenon was more pronounced in the case of DAE-PEG-NP which could be explained with the higher pegylation. Further, zeta-potential of the pegylated nanoparticles was significantly lower compared to the potential of non-modified nanoparticles. Thus, the reduction of zeta-potential of nanoparticles assumed the location of chains of both polyethylene glycols on the nanoparticle surfaces.

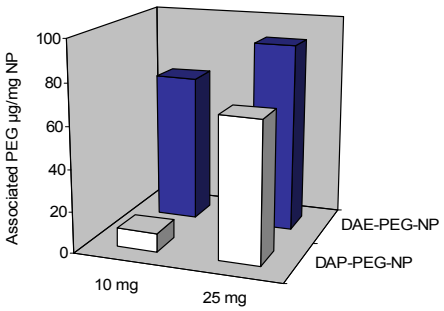

Fig. 1. *Association of amino-functionalized polyethylene glycols (DAE-PEG and DAP-PEG) to nanoparticles by solvent displacement method*

Table 1. *Physico-chemical properties of nanoparticles depending on the initial concentration of DAE-PEG and DAP-PEG*

Nanoparticles	Size (nm)	Polydispersity	Zeta-potential (mV)
NP	289.0 ± 11.4	0,101	- 33,5 ± 6,6
DAE-PEG-NP (10 mg)	387.0 ± 23.1	0,296	- 11,9 ± 3,5
DAE-PEG-NP (25 mg)	505.0 ± 88.1	0,946	- 5,5 ± 1,5
DAP-PEG-NP (10 mg)	335.4 ± 28.1	0,089	- 4,1 ± 1,7
DAP-PEG-NP (25 mg)	356.5 ± 28.4	0,169	- 2,7 ± 0,8

Solvent displacement method appeared to be simple and effective method for preparation of

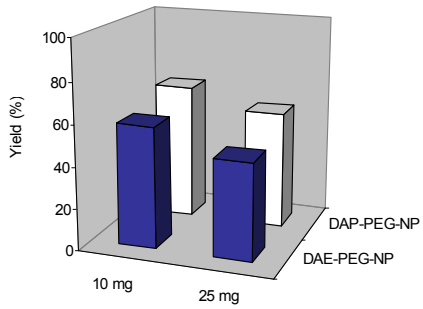

Fig. 2. *Nanoparticle yield depending on the initial concentration of amino-functionalized polyethylene glycols*

amino-pegylated nanoparticles based on PVM/MA copolymer. The nanoparticle yield was relatively high although it decreased with increasing of the initial polyethylene glycol concentration (Fig. 2). However, appropriate variation of the initial concentration of polyethylene glycol could result in optimal physicochemical properties and nanoparticle yield.

References

1. Avgoustakis, K. et al. Effect of copolymer composition on the physicochemical characteristics, in vitro stability, and biodistribution of PLGA–mPEG nanoparticles. – Int. J. Pharm., **259**, 2003, 115-127.
2. Beletsi, A. et al. Effect of preparative variables on the properties of PLGA-mPEG copolymers related to their application in controlled drug delivery. – Int. J. Pharm., **182**, 1999, 187-197.
3. Bodmeier, R. et J. W. McGinity. The preparation and evaluation of drug-containing poly(DL-lactide) microspheres formed by the solvent evaporation method. – Pharm. Res., **4**, 1987, 465-471.
4. Conti, B., I. Genta, T. Modena, et F. Pavanetto. Investigation on process parameters involved in polylactic-co-glycolic microspheres preparation. – Drug Dev. Ind. Pharm., **21**, 1995, 615-622.
5. Isosaki, K., N. Seno, I. Matsumoto, T. Koyama, et S. Moriguchi. Immobilization of protein ligands with methyl vinyl ether-maleic anhydride copolymer. – J. Chromatography, **597**, 1992, 123-128.
6. Jalil, R. et J. R. Nixon. Biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. – J. Microencaps., **7**, 1990, 297-325.
7. Yoncheva, K., S. Gomez, M. A. Campanero, C. Gamazo, et J. M. Irache. Bioadhesive properties of pegylated nanoparticles. – Exp. Opinion Drug Deliv., **2**, 2005, 205-218.

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