SURFACE-MODIFIED BIODEGRADABLE NANOPARTICLES FOR TARGETED DRUG DELIVERY

SEDYAKINA Nataliya E.[1], GORSHKOVA Marina Y.[2], MAKSIMENKO Olga O.[1], GELPERINA Svetlana E.[1]

[1] Nanosystem LTD., Moscow, Russian Federation

[2] A.V.Topchiev Institute of Petrochemical Synthesis, RAS, Moscow, Russian Federation

nsedyakina@mail.ru

Abstract

Targeted drug delivery is suggests an affinity-based interaction of drug carriers with specific cells or tissues. Such drug delivery systems must be functionalized with a ligand that will bind specifically to receptors. In this work the density of carboxyl groups on the surface of biodegradable nanoparticles was regulated to attach a model proteinacious ligand.

PLGA/polyacid nanoparticles were prepared using two different methods: nanoprecipitation and high pressure emulsification - solvent evaporation technique. DIVEMA (copolymer of maleic anhydride and divinyl ether) was used as the polyacid. The effect of molecular weight of DIVEMA and the ratio of DIVEMA to PLGA (w/w) on the density of the carboxylic groups on the surface of the PLGA-DIVEMA nanoparticles was investigated. It was shown that the increase of the DIVEMA/PLGA (w/w) ratio from 1:10 to 1:1 led to the increase of the content of carboxylic groups from 1.5 to 10.8 mmol/g PLGA (nanoprecipitation).

The model protein (insulin or human serum albumin [HSA]) was conjugated to carboxylic groups on the nanoparticles surface through a carbodiimide-meditated coupling reaction. The number of the functional groups on the nanoparticle surface was evaluated after each reaction step. The nanoparticles size, polydispersity (PD), and surface charge were measured using Zetasizer NanoZS (Malvern, GB).

Keywords: nanoparticle, poly(lactide-co-glycolide), DIVEMA, conjugation, targeted drug delivery

1. INTRODUTION

Polylactic-co-glycolic acid (PLGA) nanoparticles (NPs) are one of the most successful and promising a polymeric carrier for drug delivery. However, PLGA can not be used for targeted delivery of therapeutics because of its surface properties. Targeted drug delivery is suggests an affinity-based interaction of drug carriers with specific cells or tissues. PLGA does not possess any functional groups for the interaction with cells. PLGA drug delivery systems must be functionalized with a ligand that will bind specifically to receptors [1]. To improve the efficiency of such functionalization is necessary to increase the amount of carboxyl groups on the surface of the PLGA nanoparticles. One way to increase the density of carboxyl groups on the surface of the NPs is the introduction of a polyacid in the polymer matrix of the nanoparticles. In this work binding of a model proteinacious ligand to the surface of biodegradable PLGA nanoparticles was accomplished using a polyanionic copolymer of divinyl ether with maleic anhydride (DIVEMA).

2 EXPERIMETAL

2.1 Preparation of PLGA-DIVEMA nanoparticles

The DIVEMA copolymer was synthezed by radical copolymerization of divinil ether with maleic anhydride. The PLGA polymer (Resomer 502H, 50/50) was obtained from Boehringer Ingelheim (Germany). Other materials were from Sigma (USA).

PLGA-DIVEMA nanoparticles were prepared using two different methods: nanoprecipitation and high pressure emulsification - solvent evaporation technique. The molecular weight of DIVEMA and the ratio of PLGA to DIVEMA (w/w) were varied. In the first method, Poly(lactide-co-glycolide) with acid end groups and DIVEMA at different mass ratios were dissolved in mixtures 1.5 of ml acetone and 3.0 ml of acetonitrile. The organic phase was added drop by drop into 0.5% solution of PVA under stirring at 1000 rpm. The nanosuspension was left stirred for 2 hours at the room temperature at 1000 rpm and then for 1 hour at the 60°C at 450 rpm. The obtained nanosupension was filtered through a glass-sintered filter, purified by 3 times and concentrated using a stirred cell (Amicon, 50 ml, ultrafiltration discs Biomax 100 and 300 kDa, polyethersulfone (PES)) under 10 psi pressure and freeze-dried after addition of 1% w/v of mannitol as a cryoprotector. In the emulsification - solvent evaporation method, PLGA was dissolved in 5 ml of dichloromethane; DIVEMA was dissolved in 1 ml of acetone. These solutions were combined and poured into an 1 % aqueous solution of polyvinylalcohol, and the mixture was emulsified using a multi-step homogenization procedure (high pressure laboratory homogenizer Panda, GEA Niro Soavi, USA). After evaporation of dichloromethane, the resulting nanosuspension was processed as described earlier.

2.2 Characterization of the nanoparticles

The particle size and polydispersity index (PDI) were measured by photon correlation spectroscopy using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at 25°C after dilution (1:50) of the nanoparticle (NP) suspension with Milli-Q water. The zeta potentials (ZP) were measured by micro-electrophoresis in a folder capillary cell (DTS 1060) using a Zetasizer Nano ZS. The residual amount of PVA was determined using a colorimetric method [2]. The amount of the carboxylic groups on the surface of the nanoparticles was determined by potentiometric titration.

2.3 Peripheral attachment of the model protein

The nanoparticles were activated with N-hydroxysuccinimide (NHS) using a water-soluble N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC). NPs were resuspended in MES buffer at pH 5.2. EDC and NHS were dissolved into the nanoparticulate suspension, using a molar ratio of NP-COOH:EDC:NHS 1:5.5:2.85. Samples were then ultracentrifuged (microfilters Microcon 100kDa), resuspended with water, dispersed using ultrasonic generator (Bandeline, Germany) and freeze-dried after addition of 1% of mannitol. The amount of NHS groups present in the NP-NHS samples was determined by the hydroxamate assay according to procedure reported by Strehin et al. [3].

To conjugate the model protein to PLGA/DIVEMA nanoparticles, NPs-NHS were resuspended in 0.5 M N-methylmorpholine. HSA or insulin solutions was added to suspension at a different molar ratios of protein:NP-COOH. Samples were incubated overnight at 4°C, centrifuged at 10°C and washed three times with PBS (pH 7.4) to remove any unbound protein. Control samples were prepared by the same procedure, but using NP without carbodiimide activation. Protein binding was quantified using the Bradford method. Quantification was performed on three independently prepared samples.

3. RESULTS AND DISCUSSION

The effect of the molecular mass (MM) of DIVEMA on the characteristics of the PLGA/DIVEMA nanoparticles was investigated using DIVEMA with MM ranging from 15 to 250 kDa. The results are shown in Table 1 and Fig. 1. In the case of nanoprecipitation, the average particle size varied from 223 nm to 575 nm, while in the high pressure emulsification - solvent evaporation technique it ranged from 179 nm to 232 nm. There was no discernable effect of the MM of DIVEMA on the average size of the NP prepared by the nanoprecipitation. The larger mean sizes of the NPs produced by nanoprecipitation might be explained by the fact that these NPs were not stable enough. Formation of the particle aggregates was observed during hydrolysis and purification procedures. The NPs prepared using the high-pressure homogenization showed a good stability. The particle size of these NPs decreased with decreasing MM of DIVEMA. For all methods, increasing the MM of DIVEMA resulted in a slight decrease of the amount of the carboxylic groups on the surface of the NP

(Fig. 1). This might be explained by the fact that shorter chains of low molecular weight DIVEMA contain more carboxylic acid end groups.

Table 1. Influence of molecular mass of DIVEMA on average sizes and zeta potential of nanoparticles (DIVEMA/PLGA ratio 1:5, w/w).

Method	Molecular mass of DIVEMA, kDa	Z-Average, nm	ZP, mV
Nanoprecipitation	15	512.7±31.0	-29.0±0,9
	20	223±2.134	-27.6±1.5
	40	467.8±17.7	-31.2±2.1
	250	575±9.488	-33.6±2.1
High pressure emulsification - solvent evaporation	15	179.6±0.14	-17.3±1.1
	20	188.2±44.8	-19.2±1.1
	40	229.6±6.9	-21.6±0.2
	250	232.4±0.7	-26.9±0.9

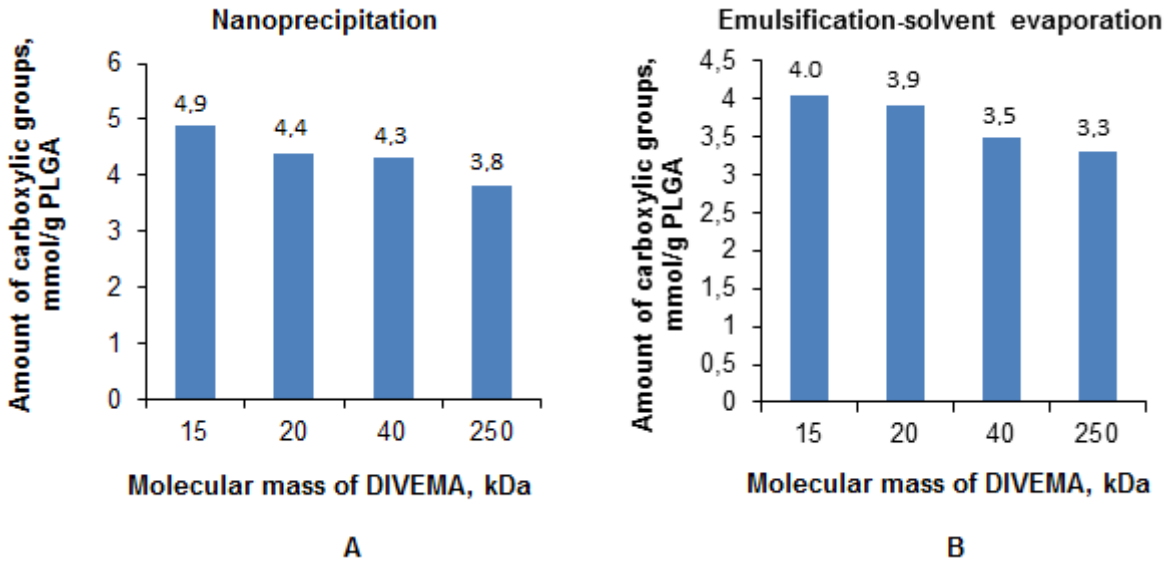

Fig. 1. Influence of DIVEMA molecular mass on the amount of the carboxylic groups on the surface of the nanoparticles prepared by nanoprecipitation (A) and high pressure emulsification – solvent evaporation technique (B)

The average sizes of nanoparticles at different DIVEMA/PLGA (w/w) ratios are summarized in Table 2. The average sizes of the NPs prepared by nanoprecipitation or high pressure homogenization were considerably different and ranged from 223 nm to 549 nm and 129 nm to 188 nm, respectively. In the case of high pressure homogenization, the particle size tended to decrease with decreasing of the DIVEMA/PLGA w/w ratios. The increase of the weight ratio of DIVEMA/PLGA from 1:10 to 1:1 (nanoprecipitation) and from 1:10 to 1:5 (emulsification - solvent evaporation technique) led to the increase of the content of carboxylic groups from 1.5 to 10.8 mmol/g PLGA and from 2.0 to 3.9 mmol/g PLGA, respectively (Fig. 2).

Table 2. Influence of DIVEMA/PLGA ratio (w/w) on average sizes and zeta potential of nanoparticles (MM of DIVEMA 20 kDa)

Method	DIVEMA : Resomer 502H, w/w	Z-Average, nm	ZP, mV
Nanoprecipitation	1:1	302.5±2.18	-18.4±1.6
	1:3	548.8±23.3	-27.8±3.2
	1:5	223±2.134	-27.6±1.5
	1:8	327.4±8.04	-34.0±0.9
	1:10	447.7±12.5	-30.3±3.3
High pressure emulsification - solvent evaporation	1:5	188.2±44.8	-19.2±1.1
	1:7	144.6±2.92	-17.3±0.8
	1:8	129.1±2.87	-15.1±1.4
	1:10	186.4±2.47	-25.1±0.1

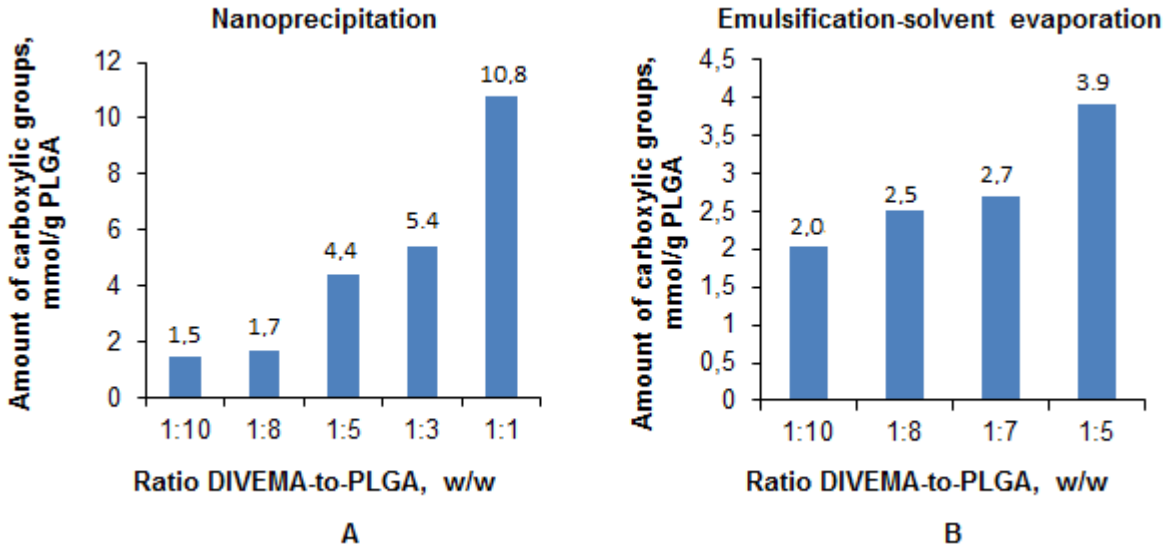

Fig. 2. Influence of DIVEMA/PLGA ratio (w/w) on the amount of the carboxylic groups on the surface of the nanoparticles prepared by nanoprecipitation (A) and high pressure emulsification - solvent evaporation technique (B)

The model protein (insulin or human serum albumin (HSA)) was conjugated to carboxylic groups on the nanoparticles surface through a carbodiimide-meditated coupling reaction. The number of the functional groups on the nanoparticle surface was evaluated after each reaction step. Two NPs samples prepared with different DIVEMA/PLGA weight ratios (1:3 and 1:5) were activated with NHS using a water-soluble EDC. The amount of NHS groups for prepared NPs-NHS is shown in the Table 3. Then the protein was conjugated to

carboxylic functional groups activated with NHS. The amount of protein coupled to the surface of the NPs-NHS was 2.7 μg/mg and 4.9 μg/mg PLGA for insulin and HSA, respectively (Table 3).

Table 3. The amount of the NHS groups and the conjugated protein on the PLGA/DIVEMA NPs surface after each reaction step.

Method	DIVEMA molecular mass, kDa	DIVEMA /PLGA ratio, w/w	Amount of –COOH groups on the surface of NPs, mmol/g PLGA	Amount of attached NHS groups on the surface of NPs, mg/mg PLGA	Protein	Molar ratio of protein: NP-COOH	Amount of conjugated protein, μg/mg PLGA
Nano-precipitation	20	1:3	5.4±0.4	0.43±0.06	Insulin	1:10	2.7±0.3
High pressure emulsification - solvent evaporation	20	1:5	3.9±0.2	0.33±0.012	HSA	1:1	4.9±0.5

CONCLUSIONS

The results showed that the DIVEMA/PLGA ratio significantly influences at the amount of the carboxylic groups on the surface of the nanoparticles. Thus, the DIVEMA/PLGA ratio is an effective tool to regulate the density of carboxyl groups on the surface of biodegradable nanoparticles for the NPs functionalization with a proteinacious ligand.

LITERATURE

[1] McCARRON, P. A., MAROUF W. M., DONNELLY R. F., SCOTT C. J Biomed Mat Res Part A, 2008, 87A (4), 873 – 884.

[2] SAHOO SK, PANYAM J, PRABHA S, LABHASETWAR V. J Control Rel., 2002, 82, 105–114

[3] STREHIN I., NAHAS Z., ARORA K., NGUYEN T., ELISSEEFF J. Biomaterials, 2010, 31(10), 2788–2797.