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Adhesion of PLGA or Eudragit®/PLGA nanoparticles to *Staphylococcus* and *Pseudomonas*

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Abstract

The aim of present study was to examine whether cationic Eudragit® containing poly(lactide-*co*-glycolide) (PLGA) nanoparticles can adhere to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In order to prepare fluorescent nanoparticles, fluorescein was covalently coupled to PLGA. Fluorescent PLGA and Eudragit®/PLGA nanoparticles were prepared by w/o/w emulsification solvent evaporation. Particle size and zeta potential of the nanoparticles were measured. Nanoparticles were incubated for a short time with *P. aeruginosa* and *S. aureus* followed by measurement of the size of nanoparticles and of *P. aeruginosa* and *S. aureus* with and without adherent nanoparticles. Flow cytometric measurements were performed to detect the attachment of particles to microorganisms. Eudragit® containing nanoparticles possessed a positive zeta potential, while PLGA nanoparticles were negatively charged. Following adsorption of Eudragit® containing nanoparticles, a size increase for *P. aeruginosa* was observed. Flow cytometric analyses confirmed that Eudragit® containing particles showed stronger interactions with the test organisms than PLGA nanoparticles. Adhesion of particles was more pronounced for *P. aeruginosa* than for *S. aureus*. Cationic Eudragit® containing nanoparticles showed better adhesion to microorganisms than anionic PLGA nanoparticles, which is probably due to enhanced electrostatic interactions.

Keywords: Nanoparticles; PLGA; Eudragit®; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; Flow cytometry

1. Introduction

Topical application is the most popular administration route for ocular drugs. However, one of the major problems is their low bioavailability, due to the different defence mechanisms of the eye (Järvinen et al., 1995). The use of colloidal drug delivery systems such as microparticles or nanoparticles has been proposed to improve the bioavailability (Zimmer and Kreuter, 1995; Le Bourlais et al., 1998). They are frequently made of poly(lactic-*co*-glycolic acid) (PLGA) because of its biocompatibility and biodegradability. An additional approach to optimise ocular drug delivery is the so-called mucoadhesive concept, based on entrapment of particles in the ocular mucus layer and interaction of bioadhesive polymer chains with mucins. This induces a significant increase in precorneal residence time of the preparation (Ludwig, 2005). As the mucus layer at the eye surface is negatively charged, cationic polymers might interact with it (Baeyens and Gurny, 1997). Various polymers have been examined in order to prepare mucoadhesive nanoparticles. Pignatello et al. (2002) developed nanoparticles made of Eudragit® RL100 with good ocular tolerance, no inflammation or discomfort in the rabbit's eye. Positively charged nanoparticles can also be prepared when Eudragit® RL100 is combined with PLGA (Dillen et al., 2006).

Most bacteria carry a net negative surface charge, thereby promoting adhesion on positively charged materials (Gottenbos et al., 2001, 2003; Dunne, 2002). *Stenotrophomonas maltophilia* is an exception to this rule, possessing an overall positive surface charge at physiological pH (Jucker et al., 1996). Bacterial cell wall polymers such as teichoic acid (Gram-positive bacteria),

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lipid A (part of the lipopolysaccharide, Gram-negative bacteria), peptidoglycan, and most of the phospholipids are negatively charged (Caroff and Karibian, 2003; Bos and Tommassen, 2004; Fedtke et al., 2004).

The aim of present research is to examine whether nanoparticles made of PLGA or of a mixture of Eudragit® and PLGA can adhere to Gram-positive and Gram-negative microorganisms. This adhesion could provide a sustained antimicrobial activity of the drug incorporated against the target organisms causing eye infections. In order to enable visualisation of nanoparticle adhesion to microorganisms during flow cytometric experiments, fluorescent nanoparticles are required. Fluorescent polystyrene particles are used as a model for the uptake of poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles (Tosi et al., 2005). However, polystyrene nanoparticles are more hydrophobic than PLGA nanoparticles (Panyam et al., 2003). A second possibility is the incorporation of a fluorescent dye, like fluorescein or rhodamin (Torché et al., 2000; White and Errington, 2005). Particles labelled with unbound fluorescein rapidly release the fluorescent dye in the medium (Tosi et al., 2005). Derivatisation of PLGA with covalently bound substances, like fluorescein, and consequent formulation of the resulting fluorescent polymer into nanoparticles, would permit adhesion studies.

In an earlier study, nanoparticles consisting of PLGA or a mixture of PLGA and Eudrgit RL 100 were produced and shown to have a drug loading efficiency of 62% and 68%, respectively. DSC measurements showed that the drug is homogeneously dispersed in the matrix in an amorphous state The release of the drug was tested in vitro, and the model that seemed to fit the release curves best was the Higuchi model, with a rate constant k_H of 4.45 ± 1.01 (% $h^{-1/2}$) for PLGA nanoparticles and a k_H of 4.28 ± 0.56 (% $h^{-1/2}$) for PLGA:Eudragit 3:1 (Dillen et al., 2006).

In present study, blank particles without drug as well as particles loaded with ciprofloxacin HCl are prepared to evaluate adhesion. The model drug ciprofloxacin is one of the most commonly used fluoroquinolones in ophthalmology, due to its broad *in vivo* activity spectrum against both Gram-positive and Gram-negative ocular pathogens (Hwang, 2004). Therefore, the test microorganisms employed in this experiment are *Pseudomonas aeruginosa* and *Staphylococcus aureus*, representing the most common ocular pathogens causing human corneal bacterial infections (Moreau et al., 2002).

2. Materials and methods

2.1. Materials

Poly(lactide-*co*-glycolide) (PLGA) with a lactide:glycolide molar ratio of 52:48 and a molecular weight of 40,000 (Resomer® RG 503 and Resomer® RG 503 H) was obtained from Boehringer Ingelheim (Ingelheim am Rhein, Germany). Resomer RG® 503 H differs from Resomer RG® 503 in that it has free carboxylic end groups in the polymer chain (with an acid number > 3 mg KOH/g). Poly (vinylalcohol) (PVA MW 30,000–70,000) and 5(6)-carboxyfluorescein were purchased from Sigma Chemicals Co. (St. Louis, USA) and Fluka Chemie (Buchs, Switzerland). Eudragit® RL100 was supplied by Röhm (Darmstadt, Germany) and ciprofloxacin HCl by Roig Farma (Barcelona, Spain). Dichloromethane was obtained from Sigma–Aldrich (Steinheim, Germany) and acetonitrile (HPLC grade) from Acros Organics (New Jersey, USA). D-mannitol was supplied by Federa Co. (Braine-l'Alleud, Belgium). Filtered (Porafil 0.20 μm Membranfilter, Düren, Germany) purified Milli Q water (Millipore, Mollsheim, France) was used throughout all experiments. Chemicals for synthesis of the fluorescein–PLGA conjugate were from Acros Organics (New Jersey, USA).

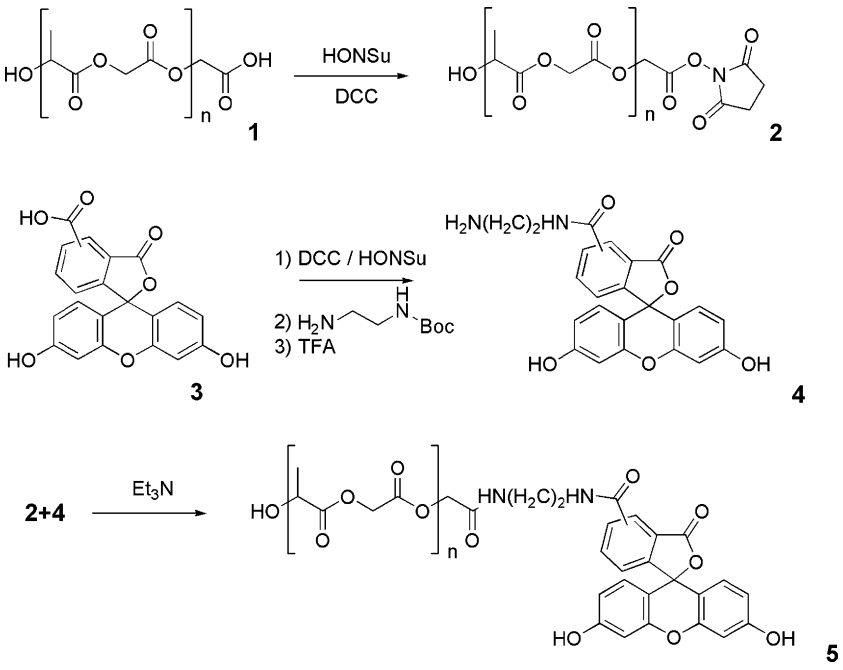
Phosphate Buffered Saline or PBS was prepared at the Laboratory of Microbiology, Parasitology and Hygiene. One liter of PBS contains 200 mg potassium chloride (Merck), 200 mg potassium dihydrogen phosphate (Merck), 8 g sodium chloride (Fisher scientific), and 1.15 g disodium hydrogen phosphate (Merck). The solution was sterilised by autoclaving at 121 °C for 20 min.

2.2. Synthesis of fluorescein–PLGA conjugate (f–PLGA)

The synthesis was carried out following a method developed by Tosi et al. (2005) (Scheme 1). First, PLGA (Resomer® RG 503H) (**1**) was transformed into the corresponding *N*-hydroxysuccinimidate (**2**) using DCC. The second step consisted of the derivatisation of fluorescein with an ethylene diamine spacer arm, allowing coupling to PLGA in order to obtain the fluorescein–PLGA conjugate (f–PLGA) (**5**). To this end, 5(6)-carboxyfluorescein (**3**) was coupled to mono-Boc protected ethylenediamine using EDC. Acidolytic removal of the Boc-protecting group was followed by coupling of derivatised fluorescein (**4**) with the NHS-activated polymer (**2**).

2.3. Preparation of fluorescent nanoparticles

Fluorescent nanoparticles were prepared according a W/O/W emulsification solvent evaporation method previously described for non-fluorescent particles (Dillen et al., 2006). Briefly, an aqueous 2.5% (w/v) ciprofloxacin solution (2.0 ml) was emulsified by means of sonication in a 5 % (w/v) f–PLGA/PLGA Resomer RG® 503 (1:3, w/w) or Eudragit® RL100/f–PLGA/PLGA Resomer RG® 503 (1:1:2, w/w) solution (10.0 ml). The resulting W/O emulsion was dispersed in a 1 % (w/v) PVA stabiliser solution (25 ml), and sonicated in order to obtain a W/O/W emulsion, which was homogenised employing a Microfluidizer M-110L (Microfluidics, Newton, USA). The emulsion was then diluted in the second outer water phase, consisting of an aqueous 0.3% (w/v) PVA solution (120 ml) containing 5% (w/v) mannitol as a cryoprotectant to obtain isotonicity after redispersion. The organic solvent was allowed to evaporate during 4 h at room temperature under agitation. Part of the resulting nanosuspension was subsequently cooled down to −18 °C and freeze-dried (Leybold-Heraeus D8B, GT-2A, Germany). Blank nanoparticles were prepared by replacing the ciprofloxacin HCl solution with filtered (0.2 μm) purified water. Non-fluorescent nanoparticles were prepared by replacing f–PLGA by PLGA Resomer RG® 503.



Scheme 1. Synthesis of f–PLGA.

2.4. Characterisation of fluorescent nanoparticles

Nanoparticle size (Z_{ave}) was determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, Malvern, UK) after appropriate dilution with PBS. At least three replicate measurements were recorded in each case. The zeta potential of the nanoparticles was measured by Laser Doppler Velocimetry using a Zetasizer 3000 (Malvern Instruments, Malvern, UK). The zeta potential measurements were performed ten times for individual samples and zeta potentials of at least three replicate samples were determined.

2.5. P. aeruginosa and S. aureus strains and growth conditions

P. aeruginosa ATCC 9027 and *S. aureus* ATCC 6538 (American Type Culture Collection, Rockville, USA) overnight cultures (37 °C for 24 h) in Tryptone Soy Broth (E&O Laboratories, Bonnybridge, Scotland) were subjected to centrifugation (2500 rpm for 15 min) (Centrifuge SW 9, Froilabo/Firlabo, Meyzieu, France). The supernatant was discarded and the pellet suspended in sterile Phosphate Buffered Saline (PBS). The same procedure was repeated three times and the deposit was finally resuspended to approximately 5×10^7 colony forming units (cfu) ml^{-1} by dilution with PBS.

2.6. Treatment of P. aeruginosa and S. aureus with nanoparticle suspensions

The treatment of bacteria with nanoparticles was performed using a modification of a method described by McCarron et al. (2004). A 2 ml *P. aeruginosa* or *S. aureus* suspension in PBS was added to an equal volume of the nanoparticle suspension – diluted 10 times after solvent evaporation – and mixed gently for 300 s at 100 oscillations min^{-1}. Similar exposure of suspensions of *S. aureus* and *P. aeruginosa* with particle-free, sterile PBS acted as a control. Exposure was terminated by centrifugation at 2500 rpm for 15 min, the supernatant containing unbound particles was removed and the pellet resuspended in PBS. Experiments were repeated three times for each formulation.

2.7. Evaluation of nanoparticle adhesion to P. aeruginosa and S. aureus

Size of *P. aeruginosa* and *S. aureus* before and after exposure to the different nanoparticle formulations was determined by Photon Correlation Spectroscopy using a Zetasizer 3000 (Malvern Instruments, Malvern, UK) as described above.

In addition, flow cytometric analysis was used to visualise nanoparticle adhesion to bacteria. Samples of particles (fluorescent and non-fluorescent), *P. aeruginosa* and *S. aureus* and of these organisms treated with the nanoparticles were analysed. Samples were diluted 1 in 10 in Phosphate Buffered Saline and measured on a FACSCalibur (BD, Erembodegem, Belgium). Ten thousand events were counted and analysis was carried out using side scatter versus fluorescence (SSC/FL1) plots. All experiments were performed in triplicate. Data were analysed using WinMDI software.

3. Results

3.1. Physicochemical characterisation of nanoparticles

The results of the PCS measurements of average particle size of the different nanoparticle formulations made of PLGA alone or blends of PLGA and Eudragit® RL100 are given in

Table 1
Characterisation of nanoparticles (mean ± S.D., $n = 3$)

Nanoparticle formulation		Characterisation	
Polymers	Ciprofloxacin	Particle size (nm)	Zeta potential (mV)
f–PLGA, PLGA	Yes	237.8 ± 0.4	−15.4 ± 0.4
f–PLGA, PLGA, E-RL	Yes	171.4 ± 0.9	+20.9 ± 1.3
f–PLGA, PLGA	No	240.0 ± 3.0	−19.0 ± 0.9
f–PLGA, PLGA, E-RL	No	182.0 ± 7.4	+24.3 ± 1.8
PLGA	Yes	233.7 ± 1.0	−15.9 ± 0.4
PLGA, E-RL	Yes	171.8 ± 2.3	+20.0 ± 2.0
PLGA	No	251.3 ± 9.2	−18.7 ± 1.2
PLGA, E-RL	No	173.6 ± 3.7	+23.6 ± 3.4

Table 1. Size of PLGA nanoparticles after resuspension in PBS ranged from 230 to 250 nm, while sizes found for Eudragit® containing nanoparticles were around 170–180 nm with a narrow polydispersity index of 0.01–0.08 and 0.04–0.07, respectively. Incorporation of ciprofloxacin HCl did not have an influence on particle size and sizes of fluorescent and non-fluorescent particles were comparable as well. In an earlier study, it was shown that the smaller size of Eudragit containing particles could be attributed to a lower viscosity of the organic phase during emulsification (Dillen et al., 2006).

The zeta potential values of the various nanoparticle preparations are listed in Table 1. All PLGA nanoparticles possessed a negative zeta potential between −15 and −19 mV, while Eudragit® containing PLGA nanoparticles were positively charged (+20 to +25 mV). Zeta potential values were not changed by incorporation of the drug, ciprofloxacin HCl, or by the use of f–PLGA instead of PLGA.

3.2. Adhesion between nanoparticles and microorganisms

3.2.1. Size measurements

The size distributions of samples consisting of *P. aeruginosa* or *S. aureus* after treatment with sterile PBS or with the various nanoparticle systems are shown in Fig. 1. The size of *P. aeruginosa* in sterile PBS was observed to be 998 ± 24 nm, while that of *S. aureus* was 1125 ± 17 nm. The average size of *P. aeruginosa* following treatment with fluorescent Eudragit® containing nanoparticles (f–PLGA-E-RL) (sized 184 ± 4 nm) was 1306 ± 105 nm. For *P. aeruginosa* incubated with PLGA nanoparticles (f–PLGA) (sized 240 ± 5 nm), the average size was 668 ± 24 nm. However, as can be seen from Fig. 1A, the size distribution of *P. aeruginosa* with f–PLGA nanoparticles showed two separate peaks. For *S. aureus* (Fig. 1B), incubation with f–PLGA-E-RL nanoparticles did not change the Z_{ave} measured (1103 ± 8 nm) significantly. When incubated with f–PLGA nanoparticles, the Z_{ave} was 779 ± 30 nm. Like for *P. aeruginosa*, two separate peaks were obtained.

3.2.2. Flow cytometry

Fig. 2A and B show densitograms for non-fluorescent and fluorescent nanoparticles, respectively. These were used to set the threshold for fluorescence. Fig. 2C and 2D show the densitograms for *S. aureus* and *P. aeruginosa* in sterile PBS. Densitograms of f–PLGA-E-RL nanoparticles (3A) or f–PLGA nanoparticles (3B) mixed with *S. aureus* and f–PLGA-E-RL (3C) or f–PLGA nanoparticles (3D) mixed with *P. aeruginosa*, after termination of treatment by centrifugation, are shown in Fig. 3. The events in the four quadrants were enumerated and expressed as percentage of the total of 10,000 events recorded for each measurement.

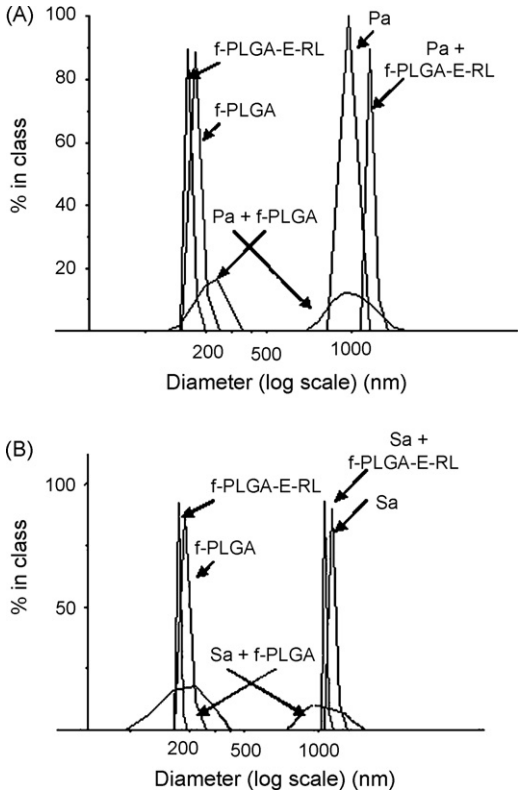


Fig. 1. Size distributions of fluorescent nanoparticles, *P. aeruginosa* and *P. aeruginosa* treated with fluorescent nanoparticles (A), and of fluorescent nanoparticles, *S. aureus* and *S. aureus* treated with fluorescent nanoparticles (B). f–PLGA: f–PLGA nanoparticles/f–PLGA-E-RL: PLGA:Eudragit RL nanoparticles/Pa: *Pseudomonas aeruginosa*/Sa: *Staphylococcus aureus*/Pa + f–PLGA: *Pseudomonas aeruginosa* treated with f–PLGA nanoparticles/Pa + f–PLGA-E-RL: *Pseudomonas aeruginosa* treated with PLGA:Eudragit RL nanoparticles/Sa + f–PLGA: *Staphylococcus aureus* treated with f–PLGA nanoparticles/Sa + f–PLGA-E-RL: *Staphylococcus aureus* treated with f–PLGA:Eudragit RL nanoparticles.

No autofluorescence of neither *P. aeruginosa* nor *S. aureus* was recorded, so any fluorescence detected in the adhesion experiments originated from adsorption of fluorescent nanoparticles to the bacterial surface. The bacteria being larger exhibited higher forward scatter than the particles (see Fig. 2).

4. Discussion

Fluorescent PLGA and Eudragit®/PLGA nanoparticles were prepared in order to evaluate adhesion to *S. aureus* and *P. aeruginosa*. To avoid leakage during experiments, the fluorescent dye fluorescein was covalently coupled to PLGA. Fluorescent particles were prepared by the solvent evaporation method. Polymeric Eudragit® containing particles possessed a small particle

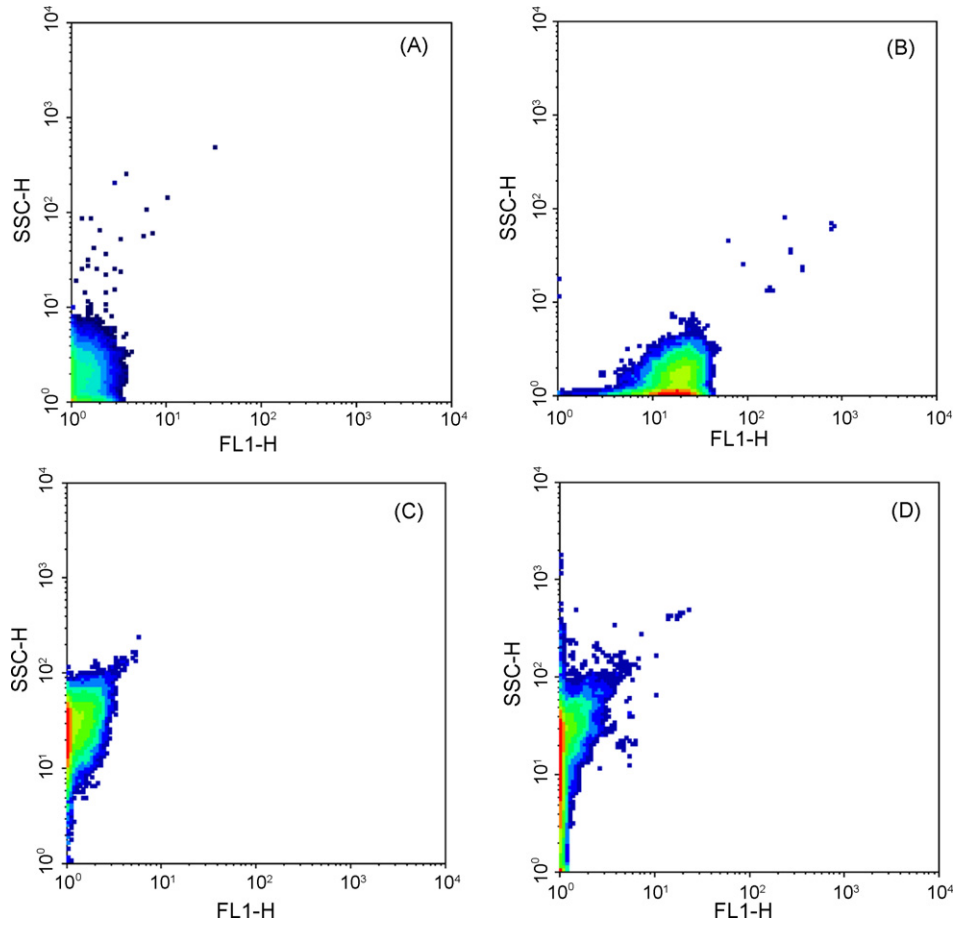


Fig. 2. Flow cytometric densitograms of PLGA or PLGA-E-RL (A) and f–PLGA or f–PLGA-E-RL nanoparticles (B), and of *S. aureus* (C) and *P. aeruginosa* (D) in sterile PBS. Comparing the nanoparticles, a 20-fold higher fluorescence intensity was observed (A vs. B: FL1 axis). Bacteria have the same fluorescence intensity as non-fluorescent particles (A vs. C and D: FL1 axis) but their side scatter is about 30 times higher.

size with narrow size distribution and a positive zeta potential. PLGA nanoparticles on the other hand, also possessed a small average size but were negatively charged. Size of Eudragit® containing nanoparticles was smaller than that of their PLGA counterparts, as already observed in previous nanoparticle optimisation studies (Dillen et al., 2006). The characteristics of ciprofloxacin-loaded nanoparticles were not different from their corresponding blanks. Furthermore, the use of f–PLGA in the formulation did not give rise to alterations in size or zeta potential. This is a desirable property, since changing the properties of the particles might affect the adhesion to microorganisms.

The surface of microbial cells is net negatively charged (McCarron et al., 2004). Several authors published zeta potential values of different *P. aeruginosa* and *S. aureus* strains suspended in PBS. All strains had negative zeta potential values: for *S. aureus*, values between −16 and −40 mV (Jones et al., 1997; Truesdail et al., 1998; Kim et al., 1999; Wang et al., 2004), and for *P. aeruginosa*, values between −13 and −40 mV were reported (Jones et al., 1997; Habash et al., 1997; Bruinsma et al., 2006). Since zeta potential values of Eudragit® containing nanoparticles were positive, adhesion of nanoparticles to bacteria can be expected due to electrostatic attraction forces. Similarly, adsorption of cationic liposomes with a relatively large positive zeta potential (+60 mV) to *S. aureus* with a negative zeta potential of −30 mV has already been described (Kim et al., 1999). Because of electrostatic repulsion, PLGA nanoparticles will probably not adsorb onto negatively charged microorganisms.

The size of the round-shaped *S. aureus* in sterile PBS is comparable to that of the rod-shaped *P. aeruginosa*. The size increase of *P. aeruginosa* after treatment with f–PLGA-E-RL nanoparticles points at adhesion of the nanoparticles to this bacterium. Since *P. aeruginosa* is negatively charged and Eudragit® containing particles positively charged, adhesion probably occurs through electrostatic attraction. Interestingly, size of *S. aureus* did not increase after treatment with f–PLGA-E-RL nanoparticles. Moreover, a size decrease was observed for both types of bacteria after incubation with f–PLGA nanoparticles. In this case, both bacteria and particles are negatively charged and repel each other. When there is no or slight adhesion of the nanoparticles to the bacteria, two populations co-exist during size measurements. It is reported that in a mixture of bacteria and nanoparticles the light scattering intensity from the much larger bacteria (diameter 800–1000 nm) predominates that from the nanoparticles (diameter 150–250 nm) (Kim et al., 1999). The software of the computer averages the sizes of both populations (bacteria and nanoparticles), therefore a net size decrease is observed.

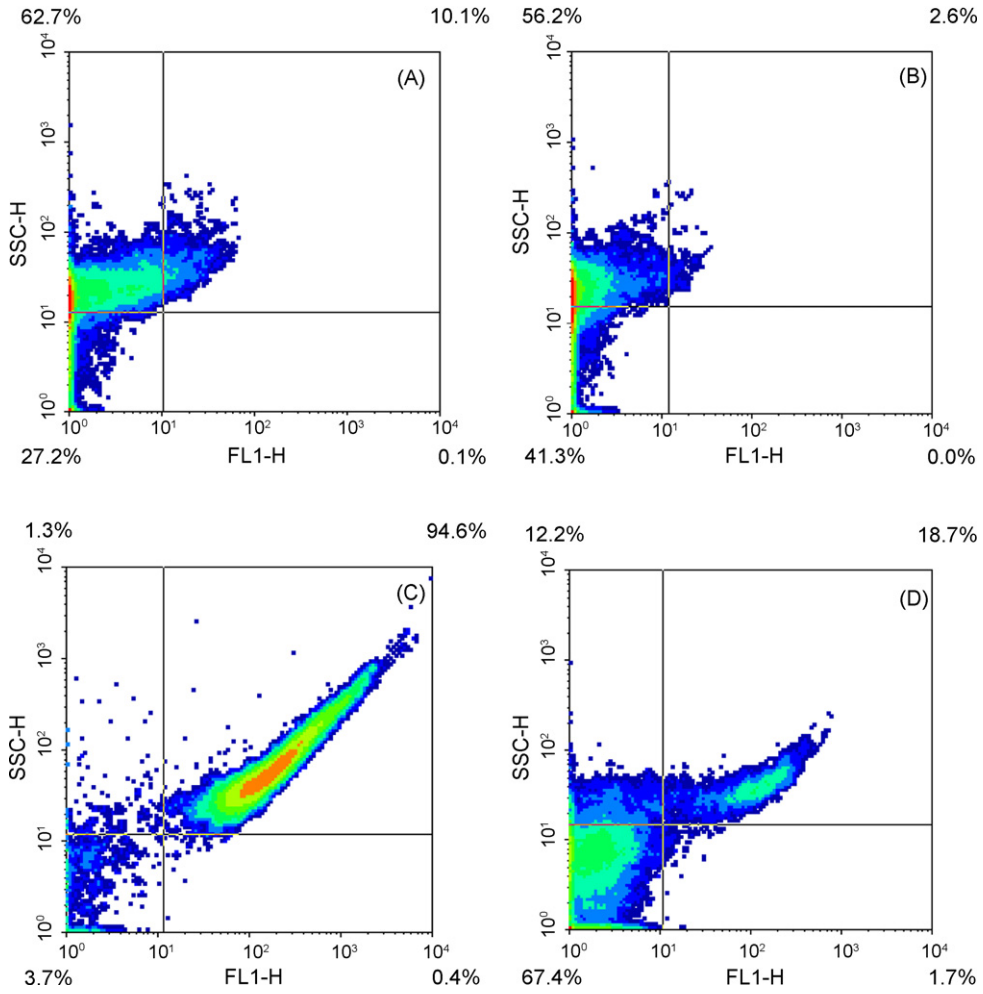


Fig. 3. Densitograms of Sa + f–PLGA-E-RL (A), Sa + f–PLGA (B), Pa + f–PLGA-E-RL (C), and Pa + f–PLGA (D). Sa stands for *S. aureus* and Pa for *P. aeruginosa*.

Flow cytometric measurements were used to confirm the adhesion of nanoparticles to bacteria. Flow density plots of non-fluorescent and fluorescent nanoparticles are shown in Fig. 2A and B. All particles containing f–PLGA were fluorescent. Fluorescent particles can clearly be differentiated from microorganisms as the latter possess no or minimal background fluorescence (Fig. 2C and D).

The density plot of bacteria (Fig. 3) incubated with nanoparticles showed a higher fluorescence intensity for cells treated with f–PLGA-E-RL nanoparticles in comparison to f–PLGA nanoparticles. For *S. aureus* incubated with f–PLGA-E-RL nanoparticles, a maximum of 10% of the microorganisms (high SSC) showed a higher fluorescence (Fig. 3A). When the experiment is repeated with f–PLGA nanoparticles, only 2.6% of the cells have a higher fluorescence intensity (Fig. 3B). This higher intensity could at least partly be explained due to co-incidence during measurement of the mixtures. For *P. aeruginosa* treated with f–PLGA-E-RL nanoparticles, more than 94% of the bacteria analysed have fluorescent particles attached (Fig. 3C). For f–PLGA nanoparticles, this percentage is about 20% (Fig. 3D). Nanoparticles were thus observed to adsorb onto the surface of *P. aeruginosa*, as evidenced by flow cytometry and confirmed by the change in the diameter of the microorganisms. Positively charged colloidal drug carriers showed interesting properties with respect to negatively charged bacteria. Adhesion is thus favoured by electrostatic attraction. Although *P.aeruginosa* and *S. aureus* both have a negative zeta potential value, the positively charged nanoparticles only seemed to interact with *P.aeruginosa* and not with *S. aureus.* This could probably be attributed to a different structure of the cell wall of both bacteria. *P.aeruginosa*, being gram negative, has an outer membrane containing lipopolysaccharides (LPS). Of these LPS, most phospholipids as well as lipid A provide a net negative charge to the bacteria (Caroff and Karibian, 2003). *S. aureus*, being gram positive, posesses a cell wall consisting mostly of teichoic acid polymers. The great number of phosphate groups confers teichoic acid polyanionic properties.

D-alanine incorporation, however, introduces positively charged amino groups leading to a partial neutralisation of these polymers. Most of the bacterial phospholipids such as phosphatidylglycerol, cardiolipin, and others are characterised by a negative charge. However, *S. aureus* modifies a great amount of phosphatidylglycerol with L-lysine, which leads again to neutralisation of the cell surface net charge and to reducing binding of cationic peptides or proteins (Fedtke et al., 2004). Probably, the structure of the LPS of *P.aeruginosa* offers

a better interaction with PLGA nanoparticles than the cell wall of *S. aureus*, where part of the negative charges are compensted by positively charged groups.

The results of both size measurements and flow cytometric experiments were not influenced by the presence or absence of the drug.

Previous research showed that the release of the antibiotic from the nanoparticle matrix was prolonged and diffusion controlled. Moreover, the antimicrobial activity of both PLGA as PLGA-E-RL nanoparticles was comparable with that of a ciprofloxacin solution containing the same amount of drug (Dillen et al., 2006). Since positively charged nanoparticles interact with bacteria components due to electrostatic interactions, the continuous diffusion of the antibiotic from the nanospheres matrix through the bacterial membrane can provide a sustained antimicrobial activity (Lboutounne et al., 2002).

5. Conclusion

This study has shown that treatment of *P. aeruginosa* with positively charged Eudragit® containing PLGA nanoparticles can cause nanoparticle adhesion to their surface. This adhesion was shown by an increase in size, after incubation with nanoparticles, and confirmed by flow cytometric measurements. An electrostatic interaction between the cationic nanoparticles and negatively charged molecules in the bacterial cell wall is most likely responsible for the adhesion. An adhesion of ciprofloxacin-loaded nanoparticles could locate the drug carriers directly onto bacteria during an eye infection. This could improve the therapeutic effect of the drug, by releasing it nearby its target.

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