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Attenuating the size and molecular carrier capabilities of polyacrylate nanoparticles by a hydrophobic fluorine effect

Raphaël Labruère, Edward Turos *

Center for Molecular Diversity in Drug Design, Discovery, and Delivery, Department of Chemistry, CHE 205, 4202 East Fowler Avenue, University of South Florida, Tampa, FL 33620, USA

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ABSTRACT

This study investigates the effect of introducing alkyl chain fluorination on the properties of polyacrylate nanoparticles prepared in aqueous solution by emulsion polymerization. For this, 2,2,3,3,4,4,4-heptafluorobutyl acrylate (**1**) and methyl trifluoroacrylate (**2**) were tested as monomers as a means to prepare fluorinated polyacrylate nanoparticles to evaluate how side chain fluorination may affect nanoparticle size and drug carrier properties. Our results show that as fluorine content within the polyacrylate matrix increases, the size of the nanoparticle systematically diminishes, from 45 nm (for nanoparticles containing no fluoroacrylate) to ~7 nm (for nanoparticles constructed solely of fluoroacrylate). We also observe that as fluoroacrylate content and hydrophobicity increases, the nanoparticles decrease their ability to incorporate lipophilic molecules during the process of emulsification. These findings have meaningful implications in the implementation of fluorinated nanoparticles in molecular delivery.

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1. Introduction

Recent investigations in our laboratory have centered on the implementation of free radical emulsion polymerization as a route for preparing emulsified polyacrylate nanoparticles as antibacterial drug carriers (Scheme 1).[1,2] For most of our investigations so far, we have relied on the use of either ethyl acrylate as the monomer or butyl acrylate co-mixed with styrene as co-monomers, typically in a 7:3 mass ratio. Emulsion polymerization occurs in water at 60–70 °C upon mixing the neat monomer or mixture of co-monomers with sodium dodecyl sulfate (SDS, 3 wt%) and potassium persulfate (1 wt%). This produces emulsified polyacrylate nanoparticles with a narrow size distribution in the range of 40–50 nm. When done in the presence of certain (primarily lipophilic) antibiotics, the emulsion polymerization process enables the additive to be either covalently or non-covalently incorporated into the nanoparticle matrix. The in vitro and in vivo properties of these nanoparticle emulsions have been examined, and found to be dependent upon not only the antibiotic that is incorporated but also on a variety of other experimental parameters, such as the % drug content in the matrix, the type of covalent linkage between the antibiotic and polyacrylate chain (if covalently attached), the concentration and charge of the surfactant used, and the overall degree of purification of the nanoparticle emulsion.[1–5] Microbiological testing showed that these nanoparticle-bound antibiotics (nanobiotics) have in vitro antimicrobial activity against both methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus anthracis*. Acrylated monosaccharides bearing antibiotic molecules on their pendant side chains can also be used as co-monomers to produce glycosylated nanoparticle antibiotics (glyconanobiotics) as antibacterially-active emulsions.[6,7] These particular nanoparticles turned out to be very interesting to us, for several reasons. First, we found that acrylate monomers containing fairly large (MW > 1000 g mol^{-1}) carbohydrate motifs can be efficiently incorporated into the polyacrylate framework by emulsion polymerization, which was not a priori expected to be the case. Secondly, dynamic light scattering measurements determined that these glycosylated nanoparticles have very similar diameters (~45 nm) and size distributions to those not bearing the carbohydrate, indicating that the sizes of the polyacrylate nanoparticles are inherently independent of the steric demands of acrylate monomer used particularly within a tightly compacted, non-aqueous environment inside the polyacrylate matrix. On the other hand, we also found that the incorporation of polyhydroxylated (monosaccharide) acrylates into the skeletal framework of the nanoparticle matrix by emulsion polymerization, rather than O-protected variants, induces a significant increase in particle size, from 45 nm when no carbohydrate is present to 83 nm when just 10% of the polymer content is comprised of either 3-*O*-acryloyl-D-glucose or 5-*O*-acryloyl-1-methoxy-β-D-ribofuranose. This dramatic size change correlated well with the extent of hydroxylation (amount of unprotected carbohydrate) within the nanoparticle matrix, which we hypothesize helps to draw in water from the aqueous exterior environment to cause nearly a two-fold expansion of the nanoparticle. In an effort to understand and potentially exploit the parameters controlling nanoparticle size and drug

* Corresponding author.
E-mail address: eturos@usf.edu (E. Turos).

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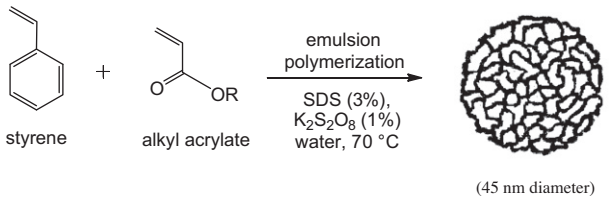


Scheme 1. Emulsion polymerization of styrene with an alkyl acrylate in water to produce emulsified polyacrylate nanoparticles.

carrier properties in these constructs, we postulated that we could also hypothetically induce the inverse effect, the reduction of particle size by repulsion of water from the nanoparticle interior through a hydrophobic effect, while maintaining the structural integrity of the nanoparticle as a stable emulsion in water. To do this, we contemplated the incorporation of fluorination in the acrylate side chain. The introduction of fluorinated residues into the polymeric chain of polymers has been widely investigated as a means to provide additional chemical resistance and stability as well as to enhance water repulsion properties and reducing hydrophilic interactions with the surrounding environment.[8,9] Fluorinated polymers possess unique physico-chemical properties associated with their low surface energy, enhanced chemical and environmental stability, biocompatibility and water repellence. A wide variety of commercial applications of fluoropolymers have also been investigated in automotive and petrochemical industries, surface coatings, microelectronics, aeronautics, textiles and paper manufacturing. Fluorinated biopolymers are highly solvophobic and chemically-resistant, thus making them useful as fouling-resistant coatings for medical implant devices (such as coronary stents)[10] and dental care products that protect the tooth surface from bacterial biofilm formation and acid-promoted demineralization of enamel.[11,12] Fluorine-containing nanoparticles reportedly have enhanced thermal stability and ability to serve as carriers of biomimetic molecules.[13,14] Fluorinated phospholipids, for instance, possess a more stable bilayer, increased drug encapsulation stability, and in vivo serum lifetimes compared to the non-fluorinated variants.[15–19] Likewise, the introduction of fluorine into polymeric nanoparticle matrices could alter morphological structure and physico-chemical properties, such as size, stability, molecular encapsulation and drug delivery. The aim of our present study was to address first whether fluorinated acrylates could be satisfactorily used to prepare stable polyacrylate nanoparticles by emulsion polymerization. We also were attempting to determine if the presence of fluoro atoms in the polyacrylate chain could alter the hydrophobic nature of polyacrylate nanoparticles, and thus influence their size and molecular carrier capabilities.

2. Results and discussion

2.1. Effect of fluorinated acrylates inclusion on the synthesis and physical properties of fluorinated polyacrylate nanoparticle emulsions

Our experiments evaluated two representative fluorinated acrylate monomers, 2,2,3,3,4,4,4-heptafluorobutyl acrylate (**1**) and methyl trifluoroacrylate (**2**), for preparing nanoparticle polymers by emulsion polymerization (Fig. 1). To prepare the nanoparticles, a predetermined amount of the fluoroacrylate monomer (4–66 wt% of the solid content) was added to a stirred, warmed mixture (70 °C) of butyl acrylate–styrene (in a 7:3 w:w ratio) that was then subjected to emulsion polymerization in presence of 3 wt% of SDS and 1 wt% of potassium persulfate (Scheme 2).

2,2,3,3,4,4,4-heptafluorobutyl acrylate (**1**) methyl trifluoroacrylate (**2**)

Figure 1. Fluorinated acrylates used as monomers in this study.

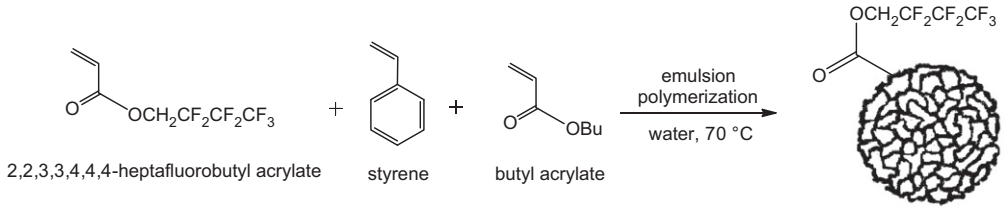
A selection of different fluoroacrylate concentrations was used to establish a correlation between the relative amount of heptafluorobutyl acrylate in the polymeric matrix and the resulting size of the nanoparticles produced (Table 1). The reactions were generally allowed to run for 6–8 h prior to cooling to room temperature and purification of the resulting emulsion as previously reported [4] to remove minor amounts of residual solids and non-emulsified materials. The purified emulsion was then examined by dynamic light scattering to determine particle size distribution and surface charge characteristics. Figure 2 is a plot of the varying weight percent of heptafluorobutyl acrylate **1** incorporated into the nanoparticle matrix versus the resulting nanoparticle size, showing a systematic decrease in nanoparticle size with increasing content of the fluoroacrylate monomer. Figure 3 illustrates the change in turbidity of the emulsions as the concentration of fluoroacrylate is increased from 4% to 96% (in the initial feed for nanoparticles synthesis). Dynamic light scattering analysis confirms an increase in average nanoparticle size as the heptafluoroacrylate content in the matrix is raised from 4% to 96%. The homopolymer derived from the heptafluorobutyl acrylate (labeled as 96% in Fig. 3) formed an almost transparent emulsion of nanoparticles having a mean diameter of only 6.5 nm, as measured by dynamic light scattering. The nanoparticles surface was found to be strongly negative compared to other samples, with a median value of −80 mV, indicative of elevated stability. The zeta potential is indicative of the stability of colloidal dispersions and the estimated absolute value of the zeta potential producing a strong electric repulsion between particles is at least 30 mV.[20] Accordingly, these fluoro-containing nanoparticles were stable for many months without degradation or changes in diameter or zeta potential.

While these emulsions were especially easy to form by emulsion polymerization, our analogous attempts to prepare butyl acrylate–styrene nanoparticles derived from methyl trifluoroacrylate (**2**) failed to afford stable emulsions. We suspect that the additional stability the fluorine provides to the acrylate during radical propagation, by being present directly on the olefin, precludes the successful formation of the polyacrylate nanoparticle. Thus, this suggests that the methodology is somewhat dependent on the type of fluorinated acrylate monomer that is used, and so far, it seems preferable to have the fluorine substituents distal from the olefin via the attached alkyl ester side chain. Upon in vitro microbiological testing of the fluoronanoparticle emulsions, we found moderate antimicrobial activity against MRSA. We attribute this to the presence of SDS in the emulsion, since earlier studies in our laboratory confirmed that weakly associated SDS in nanoparticle emulsions can lead to cytotoxic effects in bacteria.[4]

2.2. Effect of polyacrylate side chain fluorination on molecular entrapment

In order to examine if these fluorinated polyacrylate nanoparticles could potentially serve as molecular delivery vehicles, we studied their capacity to incorporate representative antibiotic molecules into the polymeric matrix during emulsion polymerization. *N*-Thiolated β-lactam **3** and penicillin G (**4**) had previously been used in our earlier investigations and found to be suitable for incorporation into the polyacrylate nanoparticle matrix (Fig. 4).

Acrylated β-lactam **3** had successfully been introduced covalently into poly(butyl acrylate–styrene) nanoparticles, while



Scheme 2. Synthesis of fluorinated polyacrylate nanoparticles by emulsion polymerization.

Table 1
Nanoparticle formulations and physical characteristics of the fluoroacrylate nanoparticles formed in water by emulsion polymerization[a,b]

Components[c]			Particle size (nm)	Zeta potential (mV)
Styrene	Butyl acrylate	2,2,3,3,4,4,4-Heptafluorobutyl acrylate		
28% (320 μL)	64% (753 μL)	4% (28 μL)	24.2 ± 0.3	−83.1 ± 0.4
27% (309 μL)	61% (719 μL)	8% (57 μL)	23.8 ± 0.1	−82.6 ± 0.7
23% (265 μL)	57% (674 μL)	16% (114 μL)	22.5 ± 0.5	−77.6 ± 1.5
20% (232 μL)	43% (517 μL)	33% (236 μL)	19.1 ± 0.4	−79.5 ± 0.8
13% (165 μL)	33% (393 μL)	50% (357 μL)	15.3 ± 0.2	−81.8 ± 0.5
9% (121 μL)	21% (258 μL)	66% (471 μL)	13.9 ± 0.6	−85.6 ± 0.9
—	—	96% (714 μL)	6.4 ± 0.5	−77.9 ± 1.1

[a] The butyl acrylate–styrene ratio (7:3 v:v) is held constant; sodium dodecyl sulfate 3%; potassium persulfate 1%.
[b] The results are expressed as the mean ± S.D of triplicate experiments.
[c] Relative percentages of the solid content and amounts used for polymerization.

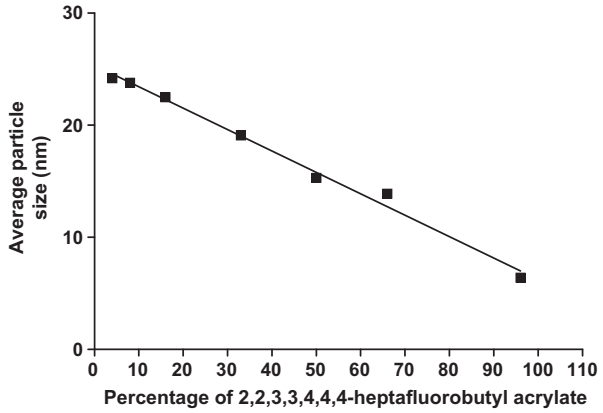


Figure 2. Correlation between weight percentage of heptafluorobutyl acrylate in the poly(butyl acrylate–styrene) nanoparticles versus average nanoparticle size determined by dynamic light scattering analysis.

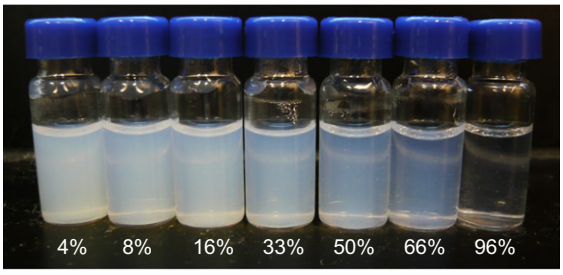
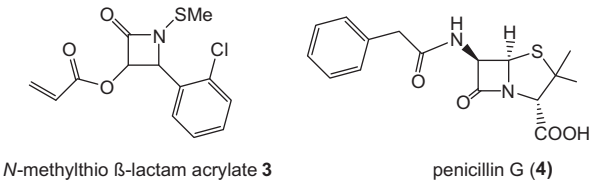


Figure 3. Photographs of seven nanoparticle emulsions made with increasing amounts of 2,2,3,3,4,4,4-heptafluorobutyl acrylate (**1**).

penicillin G (in its carboxylic acid form) was non-covalently incorporated into the nanoparticle by entrapment during the emulsion polymerization process.[1,2] In the case of the fluoroacrylate systems, we were surprised to find that even a small amount (1 wt%) of β-lactam **3** or penicillin G in varying amounts of the fluoroacrylate



N-methylthio ß-lactam acrylate **3** penicillin G (**4**)

Figure 4. Acrylated β-lactam antibiotics.

monomer **1** (4%;16%;50%;96%) and butyl acrylate–styrene (7:3) failed to afford the desired fluoroacrylate nanoparticles as stable emulsions. Thus, while fluoroacrylate **1** can be readily employed in emulsion polymerization to prepare nanoparticles as stable aqueous emulsions, the presence of even small antibiotic molecules attempting to be incorporated into the polymeric matrix completely thwarts the formation of the nanoparticle matrix. This came as a total surprise to us, and in effect, could not be overcome through further experimentation. We attribute this result to the strong hydrophobic interactions of the fluorinated alkyl side chains that cause not only the reduced sizes of the nanoparticles, but also the expulsion of all other small molecules such as water and drug additives during the process of emulsion polymerization. Thus, the use of the fluorinated acrylate in the formation of the nanoparticles effectively reverses the hydrophilic tendencies of polyhydroxylated (saccharide) acrylates previously reported from our laboratory.[7]

3. Conclusions

This study has shown that heptafluorobutyl acrylate **1** can be used as a monomer for preparing polyacrylate nanoparticles by emulsion polymerization. The size of the resulting nanoparticles are dependent on the relative amount of fluoroacrylate that is used in the preparation mixture. We attribute this size versus fluoroacrylate amount dependency on a hydrophobic effect caused by the water repellancy properties of fluorinated alkyl residues being incorporated into the polymeric matrix of the nanoparticle. This phenomenon is complementary to that found previously for hydrophilic acrylate monomers such as carbohydrate acrylates which upon incorporation into polyacrylate nanoparticles cause a

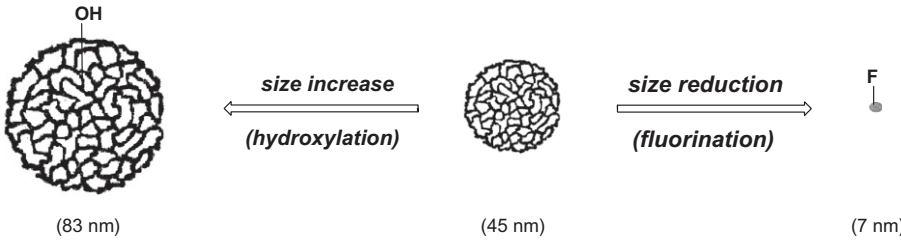


Figure 5. Graphic comparison of the relative size differences of the polyacrylate nanoparticles (center) containing polyhydroxylation (left) versus polyfluorination of the alkyl side chain (right).

corresponding increase in particle size as the amount of hydroxylation increases. An illustration comparing the effect of polyhydroxylation (increasingly hydrophilic) versus polyfluorination (increasing hydrophobic) side chain functionality on nanoparticle sizes is shown in Figure 5. We also found that the fluorinated nanoparticles, although stable in aqueous media, are much less effective at entrapping small drug molecules such as β-lactam antibiotics as drug carriers. This feature may provide utility in applications where molecular absorption may be an undesirable property, such as for biomedical coatings and water-repellent non-absorbing nanomaterials. These are some aspects that our laboratory is interested in further exploring.

4. Experimental

4.1. Materials

The following starting materials were used for this study: styrene, stabilized p.a. (Acros Organics, Morris Plains, NJ), *n*-butyl acrylate, stabilized 99% (Acros Organics); potassium persulfate, electrophoresis grade (Fisher Bioreagents, Fair Lawn, NJ); sodium dodecyl sulfate, 99% (Acros Organics); 2,2,3,3,4,4,4-heptafluorobutyl acrylate, 97% (Acros Organics); methyl trifluoroacrylate (SynQuest, Alachua, FL). No further purification of these materials was necessary.

4.2. Preparation of fluoroacrylate-containing nanoparticles by emulsion polymerization

Fluorinated nanoparticles were prepared by emulsion polymerization.[1] To a 7:3 (w/w) mixture of butyl acrylate and styrene was added 2,2,3,3,4,4,4-heptafluorobutyl acrylate (**1**) in various amounts ranging from 4%; 8%; 16%; 33%; to 66%. The solution was heated at 70 °C for 10 min, followed by pre-emulsification in deionized water (4 mL) with simultaneous addition of the desired amount of SDS surfactant (30 mg, 3 wt%) with rapid stirring under nitrogen. After 30 min the radical initiator, potassium persulfate (10 mg, 1 wt%), was added to the homogeneous emulsion to induce polymerization. The mixture was then stirred for 6 h at 70 °C before being cooled to room temperature for purification and characterization.

Analogously, the poly(2,2,3,3,4,4,4-heptafluorobutyl acrylate) nanoparticles were synthesized accordingly under the same reaction conditions, except without the use of butyl acrylate and styrene co-monomers. Each experiment was done in triplicate.

4.3. Purification of the emulsified polyacrylate nanoparticles

Purification of the nanoparticle emulsions was carried out by centrifugation-dialysis-centrifugation as previously reported.[4] Centrifugation of the initial emulsion was conducted in 2 mL Eppendorf Safe-Lock centrifugation tubes (Eppendorf AG, Hamburg, Germany) using an Eppendorf centrifuge 5415D at 13,000 rpm (16,000 *g*) for 30 min. Then, dialysis was done using a Spectra/Por dialysis tubing of 12 K molecular weight cutoffs (MWCOs). Each 2-mL dialysis bag was placed in a beaker containing 800 mL of deionized water with rapid stirring; the water was changed every 2 h. At the end of the dialysis period (8 h), the material recovered from the dialysis bag was subjected to a second mild centrifugation at 16,000 *g* for 30 min.

4.4. Physical characterization of the emulsified polyacrylate nanoparticles

Particle size and surface charge of the nanoparticles were determined by dynamic laser light scattering at room temperature using a Malvern Nano ZS instrument. Analysis was performed in triplicate and results are expressed as the mean volumetric diameter. Zeta potential measurements were done by micro-electrophoresis on the same instrument and recorded as the average of twelve determinations.

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