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RESEARCH ARTICLE

Aqueous core nanocapsules: a new solution for encapsulating doxorubicin hydrochloride

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Abstract

In this study, we propose a new solution for the nanoencapsulation of hydrophilic anticancer drug, doxorubicin hydrochloride (DOX). The drug molecules are solubilized in the core of aqueous nanoreservoirs, so-called aqueous core nanocapsules (ACN) recently developed by our team, and dispersed in aqueous bulk media. Since it is well acknowledged that the nanoencapsulation of DOX has many advantages, like reducing the sides effects (e.g. cardiac toxicity), we propose through the present study a novel formulation solution for this purpose. After focusing on the formulation process for optimizing the drug encapsulation yield, the DOX-release profiles were followed up and analyzed. Different physicochemical and *in vitro* characterization were performed, and complement activation experiments. ACN were shown efficient to encapsulate DOX reaching yields as high as 80%, followed by a sustained release governed by a diffusion-controlled mechanism. The loaded nanocarriers showed low levels of complement activation, compatible with stealth properties. To summarize, this study brings out a new tool for the nanoencapsulation of hydrophilic anticancers and could open new doors for the administration of this particular class of drugs.

Keywords: Doxorubicin hydrochloride, aqueous core, nanocapsule, nanoemulsion, nanoparticle, release

Introduction

The development of nanodrug-delivery systems irremediably implies an optimized compatibility between the physicochemical properties of the encapsulated drugs and the structure and nature of the carriers. This is particularly true for hydrophilic drugs, since the nanocarriers encapsulating the drug are themselves dispersed in a water-continuous phase. It is precisely the main difficulty related to the nanoencapsulation of hydrophilic compounds, in contrast with lipophilic drugs more stable when encapsulated. During the formulation process, the hydrophilic ones exhibit a strong trend to be fast released toward the external aqueous phase. Aqueous core nanocapsules (ACNs) were emphasized in literature as efficient solution for this purpose[1–6]. ACNs basically consist of a nanoparticle exhibiting a core-shell structure, the core is composed of water solubilizing hydrophilic drugs and the shell is a polymeric capsule. Compared with conventional nanocarriers (e.g. nanospheres), the main advantages of ACNs lies (i) in the high drug loading in the core, (ii) in the low polymer content, and (iii) in the drug confinement and protection within the capsule. However, such a complex structure involves complexes formulation methods[4]. Several strategies of formulations are reported in literature, using polyisobutylcyanoacry late ACNs encapsulating

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oligonucleotides[1,2], azidothymidine-triphosphate or cidofovir[7]. Many molecules, fluorescein, sulforhodamine, DNA, chlorhexidine, magnetite, have been encapsulated in these ACNs[5,8–10]. Herein we focus on the encapsulation of hydrophilic anticancer in ACNs. This technology appears highly beneficial and adapted for the administration of such class of cytotoxic molecules, since ACNs can insure a protection not only for the drug but also for the healthy tissues, reducing the potential toxic side effects.

The present study deals the nanoencapsulation of doxorubicin hydrochloride (DOX), an anthracycline anticancer drug, which has been shown to be efficient in the treatment of a wide range of cancers (leukemia and solid tumors[11]). Due to its irreversible cardiotoxicity[12], the clinical use of DOX remains limited. However, when nanoencapsulated, DOX toxicity can be reduced or prevented. This is actually the main reason why much effort has been dedicated to the development of pharmaceutical and colloidal forms encapsulating this drug. DOX is protected against *in vivo* degradation, the toxic side effects are reduced, the repetitive use of bolus injection or the use of perfusion pumps is avoided, hence increasing patient comfort. Finally, it provides favorable pharmacokinetic results. In addition, encapsulating DOX into nanoparticles has been shown to improve its biodistribution[13] since it limits its accumulation in the heart. Currently, most work involving DOX entrapment into nanocarriers have been performed using polymer nanoparticles. The DOX molecules are linked to the nanoparticles through electrostatic complexation[13–15], or by a chemical conjugation between the drug and the polymer[16]. To date, the nanoencapsulation of hydrophilic anticancers still remains a significant technical challenge, and the research efforts ongoing in this topics, even only related to DOX, are still at present highly sustained. Liposomal forms of doxorubicin hydrochloride (DOXIL® and CAELYX®) are marketed for several years, and have led to reductions of the related drug toxicity[17]. It is actually important to consider the polymeric nanocapsules, such as ACN, as a possible alternative to the existing technologies for DOX nanoencapsulation. Indeed, compared with liposomes, they offer some original aspects, notably regarding their formulation process (i.e. feasibility and repeatability), the fine control of their physicochemical properties and surface functionalization, low cost, and finally the large panel of biodegradable polymers that can be used in their formulation. We can find various recently published formulation strategies for that purpose, for instance, polymeric nanoassemblies[18], silica nanoparticles[19] or even lipid nanoemulsions[20]. However, the DOX is generally entrapped in the polymer matrix or is adsorbed onto the nanoparticle surface, and thus is not protected from the external environment, being released with a burst. However, when the DOX is solubilized in the aqueous core of ACNs, their core-shell structure appears particularly appropriated to the drug protection.

We have recently developed a new solution regarding the formulation of ACNs[5], based on low-energy nanoemulsification methods, organic solvent-free, and avoiding the use of high temperatures or mechanical stresses. This original process appears highly suitable for the nanoencapsulation of fragile or thermosensitive hyrophilic molecules with high encapsulation yields (EY) around 90%. Through the present study, we propose the proof of concept of the adaptability of this new technology on the nanoencapsulation of DOX in ACNs. After focusing on the formulation process for optimizing the drug EY, the DOX-release profiles were followed up and analyzed. Different physicochemical and *in vitro* characterization were performed, and complement activation experiments. This study may bring out a new tool for the nanoencapsulation of hydrophilic anticancer drugs and could open new doors for the administration of this particular class of drugs.

Materials and methods

Materials

Technical grade polyethoxylated surfactant C18E6, was kindly supplied by Stearinerie-Dubois (Boulogne, France). It is a typical commercial product with a Poisson distribution level of about six for ethylene oxide (EO). Light mineral oil, such as, paraffn oil (the standardized denomination to refer to a mixture of saturated hydrocarbons), was purchased from Cooper (Melun, France). Ultrapure water was obtained by the MilliQ filtration system (Millipore, Saint-Quentin-en Yve_{68} lines, France). Sodium chloride was purchased from Prolabo (Fontenay-sous-Bois, France). 2-Methylbutane was provided by Riedel-de-Haën (Seelze, Germany). Finally, tolylene-2,4-diisocyanate (TDI), the monomer used for interfacial polycondensation, and DOX, were obtained from Sigma (Saint-Quentin-Fallavier, France).

Methods

The fabrication of ACNs

The fabrication process of ACNs is a patented process (#WO2009037310 (A2) – 2009-03-26), described elsewhere[5]. Briefly, a (DOX + water)-in-oil nanoemulsion is first formulated by the phase inversion temperature method (DOX-loaded water nanodroplets dispersed in (paraffin oil + 2-methylbutane), stabilized by the non-ionic surfactant). Formulation parameters: water-to-oil weight ratio = 0.4 and surfactant concentration = 10 wt.%. These water nanodroplets sizing around 30 nm are dispersed in the oil phase. Then, we synthesize a polymer (polyurea) shell at the water/oil interface of the droplets, through the polycondensation of TDI, by adding the monomer in the oil-bulk phase. The final step consists in substituting the bulk oily phase for another water phase (dispersing water). To do this, dispersing water is added in the formulation and oil (2-methylbutane) is simultaneously evaporated. The result is a suspension of ACNs dispersed in water encapsulating the DOX molecules. A

schematic illustration based on the previous studies is reported in Figure 1. It is important to note that interfacial membranes of polyurea synthesized from TDI has been used, in literature, in the fabrication of nanocapsules for their nontoxicity, biocompatible and biodegradable properties for biomedical applications[9,10,21,22]. For instance, in Ref. 10, the authors described a TDI-based polyurea nanocapsule fabrication for encapsulating hydrophilic dyes and contrast agents, and show that the obtained results confirmed that the capsules are not toxic and were efficiently internalized into the cell. In Ref. 22, as well, the interfacial polycondensation of TDI is used in the fabrication of ACNs for the encapsulation of silver nanoparticles, considering the polyurea as a biocompatible material, which makes these capsules suitable for biomedical application. Polyurea microcapsules synthesized with diethylene triamine and TDI were found[23] to have excellent oxygen-binding abilities in their use as artificial red blood cells.

DOX encapsulation and quantification

DOX is first solubilized in water, at a concentration of 4.17 mg mL^{-1}. This (DOX + water) was used for the first step of the formulation (nanoemulsion formulation). The drug quantifications were performed following an indirect method: the nanocapsules were first removed by centrifugation (30 min, 20,000 ×g). Due to the paraffin oil in their structure, the centrifugation step induces a creaming of the nanocapsules. Then, the free DOX is collected in the separated water in the bottom of the tubes and quantified using a spectrofluorimetric method, as described elsewhere[24,25] (Fluoroskan Ascent, Saint-Herblain, France). The excitation and emission light levels are 485 and 550 nm, respectively. EY is defined as:

$$EY = \frac{(DOX^{total} - DOX^{free}) \times 100}{DOX^{total}} \quad (1)$$

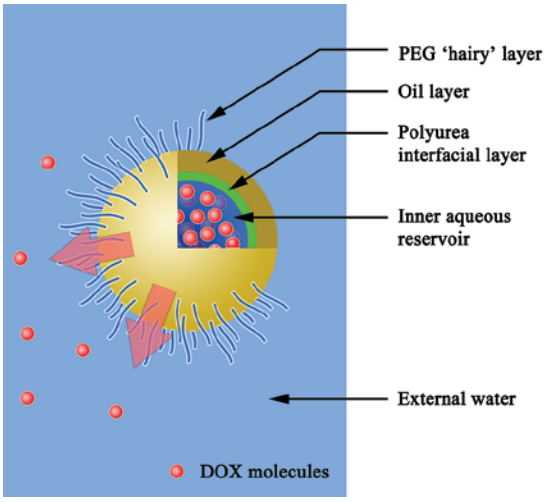

Figure 1. Schematic representation of the aqueous core nanocapsule structure encapsulating hydrophilic molecules.

where DOX_{total} is the total amount of DOX, and DOX_{free} is the amount of DOX in the external water.

DOX release

The DOX-release kinetics were established as follows: 2 mL of freshly prepared suspension of DOX-loaded nanocapsules (monomer concentration = 0.8 mg mL) were enclosed into a 15-kDa dialysis membrane (Spectra/Por Membranes, Fisher Bioblock Scientific, Illkirch, France). They were then added to 40 mL of phosphate-buffered saline solution (PBS; PBS 1X, pH 7.4). The flask was weakly, mechanically stirred (125 rpm) in darkness, and maintained at 37°C. 500 µL of the release medium were collected at specified time points and the DOX concentrations determined by spectrofluorimetry. This collected volume was systematically replaced by the same volume of fresh PBS, in order to ensure the "sink" experimental conditions. Experiments were performed three times. The control with DOX alone shows that the all 100% of the molecules rapidly passes the membrane with negligible time scales compared with the release results. The experimental data of the DOX release profile were fitted with the semiempirical power law presented below in equation 2[26,27]:

$$\frac{M_t}{M_\infty} = \frac{M_b}{M_\infty} + kt^n \quad (2)$$

where M_t, M_1 are the amounts of drug released at time t and infinite time, respectively, and M_b is the burst-release drug quantity. Structural and geometric characteristics of the device are incorporated in the constant k, and the information related to the drug-release mechanism is contained in the value of the constant n, the release exponent. According to such a model proposed by Siepmann and Peppas[26,27], and in the case of spherical geometry (our case), the coefficient values of n range from 0.43 to 0.85, corresponding to Fickian diffusion and Case-II transport, respectively. In addition, since the release profiles at 37°C are assumed to present a burst effect[26,27], the value of the M_b/M_∞ is fixed equal to 0.03.

Surface characterization, complement activation

The ζ-potential was assessed using a Nano ZS (Malvern Instruments). We used the Smoluchowski model, linking electrophoretic mobility and zeta potential. The Helium-Neon laser, 4 mW, was operated at 633 nm, with the scatter angle fixed at 12.8°, at a constant temperature of 25°C. Suspending medium was MilliQ water.

Complement consumption was assessed in normal human serum (NHS) by measuring the residual hemolytic capacity of the complement system after contact with blank ACN, DOX-loaded ACNs or nonpolycondensed nano-objects. The technique consisted of determining the amount of serum required to hemolyze 50% of a fixed number of sensitized sheep erythrocytes (CH50 units). A veronal, saline buffer containing 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+} was prepared as previously described

(VBS^{++}). Sheep erythrocytes were sensitized by rabbit, antisheep erythrocyte antibodies, and suspended at a final concentration of 1.10^8 cells mL^{-1} in VBS^{++}. To assess the consumption of CH50 units in the presence of the particles during a constant incubation time, increasing amounts of particle suspensions were added to NHS diluted in VBS^{++} so that the final dilution of NHS in the reaction mixture was 1/4 (v./v.) in a final volume of 1 mL. After 60 min. of incubation at 37°C with gentle agitation, the suspension was diluted at 1/25 (v./v.) in VBS^{++}; then, aliquots at different dilutions were added to a given volume of sensitized sheep erythrocytes.

After 45 min of incubation at 37°C, the reaction mixture was centrifuged at 2,000 rpm for 10 min. The absorption of the supernatant was determined at 415 nm with a microplate reader (Multiskan Ascent, Labsystems SA, Cergy-Pontoise, France) and compared with the results obtained with the control serum. After determining the CH50 units remaining in the serum, the results were ex pressed as the consumption of CH50 units as a function of the nanoparticle surface area calculated as described elsewhere[28] in order to compare nanoparticles of different average diameters. High activating control experiments were performed with a suspension of poly(methyl methacrylate) (PMMA) nanoparticles.

Results and discussion

The objectives of this proof of concept study is to show that these new nanocapsules are an interesting solution for nanoencapsulating DOX. To do this, we first focus the presentation on the process, showing the strategies undertaken for optimizing the EY, and then we present the full physicochemical characterization, DOX release, and complement activation. The first critical point of the encapsulation of DOX relies in optimizing the formulation to reach the higher EY with the lower amount of monomer. This is solved by studying the impact of the TDI concentration on the nanoemulsion oily phase (step 2 of the formulation), on the resulting EY value. These results are reported in Figure 2. The polycondensation has been shown[5] to be completed around 1-1.5 h, thus it was fixed at 2 h in the present study. The EY appear higher than 80%. This is a significant result, confirming the compatibility of this technology for the encapsulation of DOX. In addition, in this formulation the weight of DOX encapsulated as a fraction of the total weight of the formulation is calculated, giving 83 μg/g. The high values of EY are mainly attributed to the use of two distinct phases during the formulation process. The size of these DOX-ACNs were determined around 50 nm by CryoTEM (data not shown in the present paper, but already described in Ref. 5). The relationship disclosed between the EY and the monomer amount confirms that the polycondensation occurs at the water/oil interface. Moreover, the gradual increase of EY signifies that the number of "closed" nanocapsules (i.e. with any holes allowing the DOX to escape) gradually increases as well with the quantity of polymer.

The *in vitro* DOX-release profiles (see Figure 3) disclosed that the drug progressively escapes from the nanocapsule reservoirs. The cumulative release finally reaches 100% within about 15 h. Let us consider equation 2 for extrapolating the experimental values. It finally follows the values of the parameters k = (5.2 ± 0.8) and n = (0.44 ± 0.03), with R = 0.988. This result clearly reveals that Fickian diffusion governs the DOX-release mechanism.

Besides the physicochemical characterization, another important results are brought out by the complement

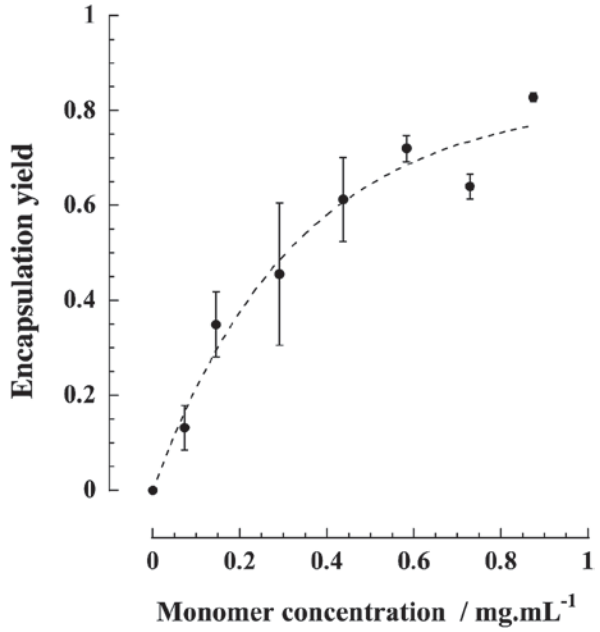

Figure 2. Influence of the monomer amount on the DOX encapsulation yield into aqueous core nanocapsules. Polycondensation time is fixed at 2 h. The dashed line is only for visual aid.

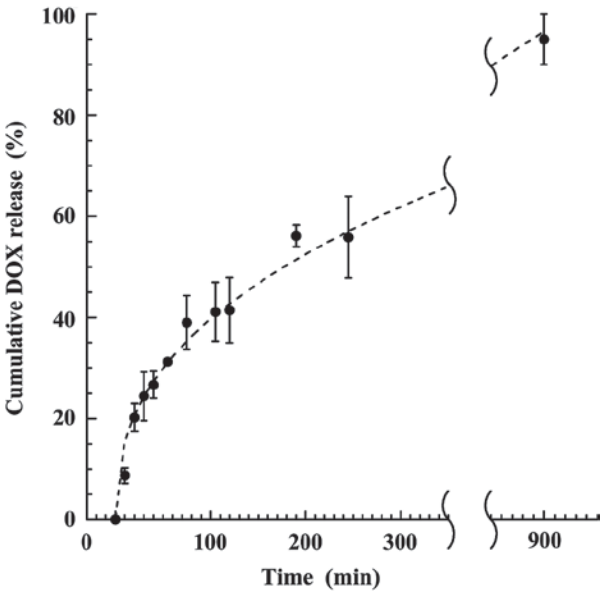

Figure 3. Cumulative DOX release from aqueous core nanocapsules at 37°C in PBS. The dashed line presents the extrapolation from the semiempirical power law of equation 2.

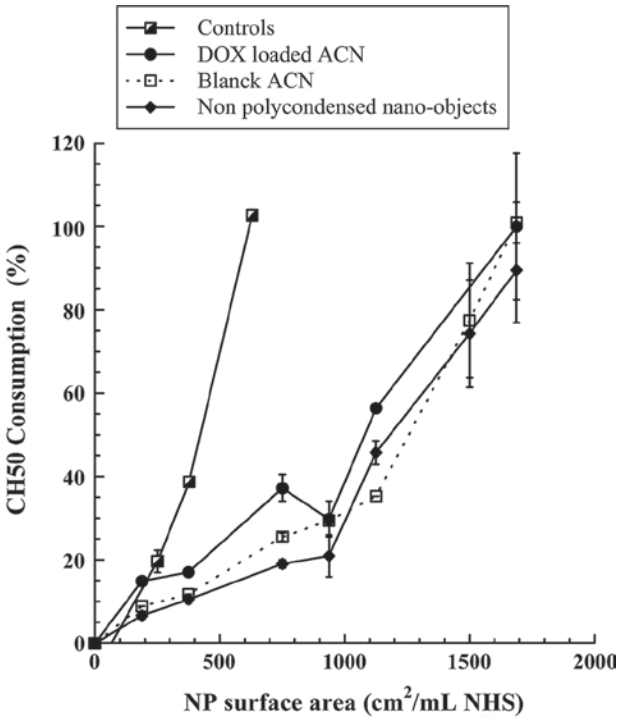

Figure 4. Consumption of CH50 units as a function of nanoparticle surface area, in the presence of DOX-loaded ACNs, blank ACNs, and polymer-free nanoparticles.

activation and the cell viability experiments. Complement activation experiments, reported in Figure 4, were performed as a complementary study to assess the ACNs surface behavior. Figure 4 shows CH50 consumption as a function of the nanoparticle surface area for blank ACNs, DOX-loaded ACNs and nonpolycondensed nano-objects (blanks ACNs are nonloaded ACNs, and nonpolycondensed nano-objects are blanks ACNs without polymer). As expected, a dose-dependant consumption of CH50 units was obtained in all cases. The three activation profiles are quite similar, revealing comparable surface behavior for the blank or DOX-ACNs and the nonpolycondensed nano-objects versus complement proteins. This indicates similar surface properties of the different particles and thus that the polymer layer is efficiency covered by the surfactant. These results may also indicate that the DOX is not entrapped into the shell, but rather encapsulated in the aqueous core. This is confirmed by ζ-potential measurements giving (–28.0 ± 0.7) mV, (–20 ± 1) mV and (–27.6 ± 0.9) mV, respectively for blank ACNs, nonpolycondensed nano-objects, and DOX-loaded ACNs.

Even if the activation profiles given in Figure 4 appear between the ones of weak activators[28,29] and the ones of highly activating nanoparticles, they remain closer that the ones of the weak activators. For instance, when compared with PMMA nanoparticles, for which CH50 consumption is around 90%, for a nanoparticles surface area around 300 cm^2/mL NHS, ACN nanoparticles appear weakly activating, with a maximum of 15% of CH50 consumption. This is compatible with stealth properties of ACNs. It is likely due to their mixed polymer/lipid/PEG-6 surfactant interfacial structure. These profiles comparing DOX-ACNs, blank ACNs, and polymer-free blank ACNs, indicate that not only the polymer but also the DOX, are effectively entrapped within the layer and into the aqueous core, respectively. Their surface properties are therefore not influenced. This result is corroborated by zeta potential measurements. ACNs appears very stable in solution and showing stealth properties. This is likely due to the polyethylene glycol (PEG) chains surrounding the particles. Actually, this assumption corroborates the schematic representation presented in Figure 1 giving a structure decorated by the PEG chains.

Conclusion

This study proves that the DOX is efficiently encapsulated (with yields up to 90%), and fully released in these poorly toxic ACNs. The aqueous suspensions of DOX generated allows to stabilize and protect this anticancer in water, exactly meeting the fixed specifications. The DOX encapsulation and the PEG decoration of such ACNs were confirmed by the surface characterization and activation complement experiments.

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Declaration of interest

The authors report no declaration of interest.

References

1. Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. (2002). Nanocapsule technology: a review. Crit Rev Ther Drug Carrier Syst, 19:99–134.
2. Lambert G, Fattal E, Pinto-Alphandary H, Gulik A, Couvreur P. (2000). Polyisobutylcyanoacrylate nanocapsules containing an aqueous core as a novel colloidal carrier for the delivery of oligonucleotides. Pharm Res, 17:707–714.
3. Hillaireau H, Le Doan T, Chacun H, Janin J, Couvreur P. (2007). Encapsulation of mono- and oligo-nucleotides into aqueous-core nanocapsules in presence of various water-soluble polymers. Int J Pharm, 331:148–152.
4. Anton N, Benoit JP, Saulnier P. (2008). Design and production of nanoparticles formulated from nano-emulsion templates-a review. J Control Release, 128:185–199.
5. Anton N, Saulnier P, Gaillard C, Porcher E, Vrignaud S, Benoit JP. (2009). Aqueous-core lipid nanocapsules for encapsulating fragile hydrophilic and/or lipophilic molecules. Langmuir, 25:11413–11419.
6. Vrignaud S, Benoit JP, Saulnier P. (2011). Strategies for the nanoencapsulation of hydrophilic molecules in polymer-based nanoparticles. Biomaterials, 32:8593–8604.
7. Hillaireau H, Le Doan T, Besnard M, Chacun H, Janin J, Couvreur P. (2006). Encapsulation of antiviral nucleotide analogues azidothymidine-triphosphate and cidofovir in poly(isobutylcyanoacrylate) nanocapsules. Int J Pharm, 324:37–42.
8. Paiphansiri U, Dausend J, Musyanovych A, Mailänder V, Landfester K. (2009). Fluorescent polyurethane nanocapsules prepared via inverse miniemulsion: surface functionalization for use as biocarriers. Macromol Biosci, 9:575–584.

9. Baier G, Musyanovych A, Dass M, Theisinger S, Landfester K. (2010). Cross-linked starch capsules containing dsDNA prepared in inverse miniemulsion as "nanoreactors" for polymerase chain reaction. Biomacromolecules, 11:960–968.
10. Rosenbauer EM, Landfester K, Musyanovych A. (2009). Surface-active monomer as a stabilizer for polyurea nanocapsules synthesized via interfacial polyaddition in inverse miniemulsion. Langmuir, 25:12084–12091.
11. Mdicaments antitumoraux et perspectives dans le traitement des cancers; vol. 6; chap. AFECT: Anthracyclines utilises en thrapeutique 2003, 364–411.
12. Allen A. (1992). The cardiotoxicity of chemotherapeutic drugs. Semin Oncol, 19:529–542.
13. Fundarò A, Cavalli R, Bargoni A, Vighetto D, Zara GP, Gasco MR. (2000). Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: pharmacokinetics and tissue distribution after i.v. administration to rats. Pharmacol Res, 42:337–343.
14. Janes KA, Fresneau MP, Marazuela A, Fabra A, Alonso MJ. (2001). Chitosan nanoparticles as delivery systems for doxorubicin. J Control Release, 73:255–267.
15. Wong HL, Rauth AM, Bendayan R, Manias JL, Ramaswamy M, Liu Z et al. (2006). A new polymer-lipid hybrid nanoparticle system increases cytotoxicity of doxorubicin against multidrug-resistant human breast cancer cells. Pharm Res, 23:1574–1585.
16. Park JH, Cho YW, Son YJ, Kim K, Chung H, Jeong SY et al. (2006). Preparation and characterization of self-assembled nanoparticles based on glycol chitosan-bearing adriamycin. Colloid Polym Sci, 284:763–770.
17. Rahman AM, Yusuf SW, Ewer MS. (2007). Anthracycline-induced cardiotoxicity and the cardiac-sparing effect of liposomal formulation. Int J Nanomedicine, 2:567–583.
18. Cho HJ, Yoon IS, Yoon HY, Koo H, Jin YJ, Ko SH et al. (2012). Polyethylene glycol-conjugated hyaluronic acid-ceramide self-assembled nanoparticles for targeted delivery of doxorubicin. Biomaterials, 33:1190–1200.
19. Liu Y, Mi Y, Zhao J, Feng SS. (2011). Multifunctional silica nanoparticles for targeted delivery of hydrophobic imaging and therapeutic agents. Int J Pharm, 421:370–378.
20. Vrignaud S, Anton N, Gayet P, Benoit JP, Saulnier P. (2011). Reverse micelle-loaded lipid nanocarriers: a novel drug delivery system for the sustained release of doxorubicin hydrochloride. Eur J Pharm Biopharm, 79:197–204.
21. Jagielski N, Sharma S, Hombach V, Mailander V, Rasche V, Landfester K. (2007). Nanocapsules synthesized by miniemulsion technique for application as new contrast agent materials. Macromol Chem Phys, 208:2229–2241.
22. Crespy D, Stark M, Hoffmann-Richter C, Ziener U, Landfester K. (2007). Polymeric nanoreactors for hydrophilic reagents synthesized by interfacial polycondensation on miniemulsion droplets. Macromolecules, 40:3122–3135.
23. El-Gibaly I, Anwar M. (2004). Hemolysate-filled polyethyleneimine and polyurea microcapsules as potential red blood cell substitutes: effect of aqueous monomer type on properties of the prepared microcapsules. Int J Pharm, 278:25–40.
24. Soma CE, Dubernet C, Bentolila D, Benita S, Couvreur P. (2000). Reversion of multidrug resistance by co-encapsulation of doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles. Biomaterials, 21:1–7.
25. Rahimi M, Wadajkar A, Subramanian K, Yousef M, Cui W, Hsieh JT et al. (2010). *In vitro* evaluation of novel polymer-coated magnetic nanoparticles for controlled drug delivery. Nanomedicine, 6:672–680.
26. Siepmann J, Peppas NA. (2001). Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev, 48:139–157.
27. Missirlis D, Kawamura R, Tirelli N, Hubbell JA. (2006). Doxorubicin encapsulation and diffusional release from stable, polymeric, hydrogel nanoparticles. Eur J Pharm Sci, 29:120–129.
28. Vonarbourg A, Passirani C, Saulnier P, Simard P, Leroux JC, Benoit JP. (2006). Evaluation of pegylated lipid nanocapsules versus complement system activation and macrophage uptake. J Biomed Mater Res A, 78:620–628.
29. Béduneau A, Saulnier P, Anton N, Hindré F, Passirani C, Rajerison H et al. (2006). Pegylated nanocapsules produced by an organic solvent-free method: Evaluation of their stealth properties. Pharm Res, 23:2190–2199.