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In situ encapsulation of Nile red or Doxorubicin during RAFT-mediated emulsion polymerization via polymerization-induced self-assembly for biomedical applications

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Hydrophobic agents, a fluorescent dye (Nile red, NR) or an anti-cancer drug (doxorubicin, DOX), were encapsulated into poly(3-(dimethylamino) propyl) methacrylamide)-b- poly (methyl methacrylate) (PDMAPMA-b-PMMA) nanoparticles (NPs) via one-pot RAFT-mediated emulsion polymerization in water. The macroRAFT, PDMAPMA, was chain-extended with the methyl methacrylate (MMA), with the hydrophobic agents soluble in MMA, resulting in loaded NPs, with either NR or DOX via polymerization-induced self-assembly (PISA). The NR-loaded NPs were visualized by structured illumination microscopy (SIM), thus indicating the successful loading of the fluorescent dye into the PMMA-core. The DOX-loaded NPs exhibited a sustained release profile over five days, showing a small burst effect during the first two hours, as compared with the free DOX. The DOX-loaded NPs show higher cell toxicity than the free DOX in RAW 264.7 cell line. The results demonstrate the potential of using emulsion polymerization for synthesis of tailored and reproducible NPs encapsulating hydrophobic agents.
ToC Abstract Figure represents the *in situ* hydrophobic agents encapsulated NPs *via* PISA

**Introduction**

In most developed countries, cancer is the second leading cause of death after cardiovascular diseases. Cancer is characterized by the uncontrolled cell growth in certain tissues invading other organs (metastasis) in the body.[1] The prevalence of cancer is increasing based on GLOBALCAN’s estimation, in which about 14.1 million new cancer cases and 8.2 million deaths occurred worldwide in 2012. Lung, prostate and colorectal cancer are the most frequently diagnosed cancer in males and breast, lung and colorectal cancer are diagnosed in females.[2] Approximately, 40% of all deaths caused by cancer can be prevented by appropriate treatment. The challenge remains to discover effective treatments at acceptable costs. Traditional approaches of cancer treatment include chemotherapy, radiotherapy and/or surgery. Most of the chemotherapeutic agents are highly toxic and poorly soluble in water and biological media leading to low bioavailability. The low molecular weight chemotherapeutics’ suffer from short circulation time, low therapeutic efficacy and adverse side effects. Hence, the use of a nano-carrier system as reservoir, that retain and locally deliver an anti-cancer drug allows for the preparation of effective formulations for cancer treatments.[3] Promising nano-carriers includes both inorganic[4, 5] and organic[6] nanoparticles (NPs). Examples of organic NPs include micelles of block copolymers[7] and dendrimers/dendritic polymers.[8, 9] Recently, nanocarriers based on block copolymers have received significant attention due to the use of controlled radical polymerizations (CRP) and such as the reversible addition-fragmentation
chain-transfer (RAFT) polymerization.\textsuperscript{10, 11} The field of polymerization-induced self-assembly (PISA) of block copolymers in water- or organic solvents controlled by RAFT is rapidly expanding rapidly.\textsuperscript{11-15} One significant advantage with PISA is that NPs are formed in a reproducible manner, which can be a severe challenge for many other NPs platforms obtained by chemical- and/or assembling procedures.\textsuperscript{16} Further advantages are that various chemical functionalities\textsuperscript{11} and NP morphologies\textsuperscript{12, 17, 18} can be obtained by adjusting the solids content\textsuperscript{18, 19} with no need for stabilizers or surfactants, as the NPs can be efficiently stabilized by the hydrophilic shell.\textsuperscript{20} RAFT combined with PISA can be utilized to produce stable NPs as nano-carriers for biomedical applications, such as drug delivery and bio-imaging.\textsuperscript{17, 21-26} A pioneering example was presented by Davis and co-workers\textsuperscript{24} demonstrating one-pot \textit{in situ} encapsulation of NR using PISA, in a dispersion polymerization using methanol as solvent. The aim of the present study is to demonstrate the encapsulation of hydrophobic agents concurrently with the formation of NPs, using RAFT-mediated emulsion polymerization and PISA in water. PDMAPMA, a hydrophilic macroRAFT agent, was chain-extended with methyl methacrylate (MMA) to accomplish, colloidal-stable loaded NPs. The targeted hydrophobic agents were Nile red (NR) and Doxorubicin (DOX) which are both soluble in MMA, and could therefore be encapsulated in the hydrophobic core during PISA. To the best of the author’s knowledge, this is the first demonstration of the \textit{in situ} physical encapsulation of a hydrophobic compound (NR or DOX) during particle formation via RAFT-mediated PISA in water. The DOX-loaded NPs showed a controlled \textit{in vitro} drug release profile at physiological pH (7.4). The unloaded NPs showed no cell toxicity towards macrophages cell line (RAW 264.7) and breast cancer cell line (MCF-7) at concentrations below 1 mg mL\textsuperscript{-1}. The DOX-loaded NPs show higher cell toxicity than the free DOX in RAW 264.7 cell line.

\textbf{Experimental}

\textbf{Synthesis of PDMAPMA-\textit{b}-PMMA\textsubscript{NR}}
Following previously published protocols\textsuperscript{[27]}, the NPs were synthesized targeting DP 400 of the hydrophobic core and Nile red was left allowed to dissolve in the monomer (MMA) prior to addition. In a typical experiment macroRAFT agent PDMAPMA (100 mg) is dissolved in deionized water for 10 minutes under magnetic stirring. For a PMMA-based NPs, hydrophobic monomer MMA (0.9 g) with Nile Red (catalytic amount) is added to the flask dropwise, targeting a DP of 400 and final dry content 10-15 wt%. Round bottom flask is kept in ice bath and radical initiator AIBA (from 3.4 g L\textsuperscript{-1} (aq) 0.739 mg) is added during stirring. Flask is then degassed for 30 minutes under argon flow and subsequently immersed in an oil bath pre-heated to 70 °C. Reaction was left for 120 minutes to reach a final conversion at 100%. The resulting NPs are kept in the fridge and analysed by SEM and DLS together with dried samples analysed by SEC in DMF, Table 1.

**Synthesis of PDMAPMA\textsubscript{b}-PMMA\textsubscript{DOX}**

In this study DOX as a model drug for cancer therapy. A stock solution of DOX was prepared by dissolving DOX, HCl and TEA (1:3 or 1:6 mol eq) in DCM (1 mg mL\textsuperscript{-1}). The DCM was then partially evaporated and the DOX was re-dissolved in the MMA monomer under mild magnetic stirring. The DOX-MMA mixture was added dropwise to a round bottom flask, kept in ice, containing PDMAPMA-macroRAFT solution under magnetic stirring (targeting a DP of 400). This followed by addition of AIBA solution during stirring. Flask is then degassed for 30 minutes under argon flow and subsequently immersed in an oil bath pre-heated to 70 °C for 2 h. After the predetermined period, a sample was taken carefully by syringe and dried at room temperature overnight to calculate MMA conversion gravimetrically. The resultant drug-loaded NPs was recovered by dialysis (MWCO 3.5 kDa) with distilled water for 2 h to remove the free drug. The concentration of DOX in the NPs was determined by comparing the fluorescence intensity at the wavelength 485/595 nm (excitation/emission) of a sample diluted with DMSO: H2O (4:1) compared to a standard curve (three replicates). The drug entrapment
efficiency percentage was calculated as \[\frac{\text{amount of drug loaded into the NPs}}{\text{total amount of drug added initially}} \times 100\%\].

**In vitro drug release**

The drug release experiments were performed of free DOX or DOX-loaded NPs in PBS (pH 7.4) at 37 °C. Briefly, free DOX or DOX-loaded NP solutions (2.7 mL) were transferred into 3 mL dialysis cassettes (MWCO 3.5 kDa, Slide-A-Lyzer, Thermo) suspended in 4 L of PBS. Samples (10 μL) from inside cassettes were collected (in triplicates of each) at the following time intervals: 0, 2, 4, 6, 8, 10, 24, 48, 96 and 120 h, and transferred into 96-well plates, containing 100 μL DMSO: H2O (4:1) in each well. The fluorescence intensity of each well was determined with a BioTek Synergy MX plate reader at the wavelength 485/595 (excitation/emission) nm. A calibration curve of DOX in DMSO: H2O (4:1) was performed in triplicates to calculate the EE % and assess the drug release.

**Cell viability study**

Cytotoxicity of PDMAPMA-\(b\)-PMMA and PDMAPMA-\(b\)-PMMA\textsubscript{DOX} NPs were evaluated on macrophages cell line (RAW 264.7) and breast cancer cell line (MCF-7) using the AlamarBlue assay. RAW 264.7 or MCF-7 cells were seeded on 96-well plates at the concentration of 1×10\(^4\) cells/well in 100 μL DMEM medium, and then incubated for 24 hours prior to analysis. The old medium was replaced by fresh medium containing samples at designed concentrations: 0.1–100 μg mL\(^{-1}\) for neat NPs or 0.1–10 μg mL\(^{-1}\) for DOX/DOX-NPs (equivalent DOX concentration). Six parallel wells were set for each concentration. After 72 hours of incubation, 10 μL of AlamarBlue reagent was added into each well. After an additional 4 hours, the fluorescent intensity was measured at ex/em 560/590 nm.

**Results and Discussion**

**Design of PDMAPMA-\(b\)-PMMA NPs**
Following a previously reported protocol\cite{27}, NPs were synthesized with RAFT-mediated emulsion polymerization using PISA. The hydrophilic macroRAFT, PDMAPMA, of a low molecular weight was chain extended with MMA, Figure 1. The characteristics of the synthesized NPs can be found in Table 1. The resulting PDMAPMA-\textit{b}-PMMA NPs exhibited narrow PdI values (< 0.02), hydrodynamic diameter (\(D_H\)) of 200 nm from DLS measurements and were found to be colloidally stable as an effect of the charged cationic corona (\(\xi \geq +40\) mV). Given by the theory of PISA using chain-extension of a hydrophilic polymer with a hydrophobic polymer (insoluble in water above a critical molecular weight resulting in a critical molecular weight resulting in self-assembly). The molecular weight assessed by SEC was higher than targeted (~40 000 g mol\(^{-1}\), DP400 for the PMMA) and the polydispersity (\(D\)) was higher (~1.5) than expected for a well-controlled polymerization. However, the results are in agreement with previously published findings for PDMAPMA-stabilized NPs and partly due to the fact that the SEC is a relative method to assess the molecular weights of polymers.\cite{27}

\textit{Table 1.} Results from surfactant-free emulsion polymerization of MMA, with and without the hydrophobic agents (Nile Red or DOX) using macroRAFT PDMAPMA. Reactions were performed at 70 °C using AIBA as the thermal initiator.

| Sample               | DC\(^a\) | \(M_n\)\(^b\) | \(M_n\)\(^c\) | \(D\)\(^d\) | Pdl \(^d\) | \(D_H\) | Zeta potential \(\xi,\ \text{mV}\)\(^d\) |
|----------------------|----------|----------------|----------------|-------------|------------|--------|-----------------------------------|
| PDMAPMA-\textit{b}-PMMA | 15       | 39 000         | 260 000        | 1.5         | 0.01       | 200    | +47                               |
| PDMAPMA-\textit{b}-PMMA\textsubscript{NR} | 15       | 39 000         | 260 000        | 1.5         | 0.01       | 170    | +40                               |
| PDMAPMA-\textit{b}-PMMA\textsubscript{DOX} | 15       | 40 000         | -              | 1.5         | 0.02       | 240    | +40                               |

\(^a\) Targeted dry content (DC) assuming 100% monomer conversion. \(^b\) Theoretical molecular weight using conversion calculated from gravimetrical analysis. \(^c\) Experimental molecular weight and dispersity from DMF-SEC using PMMA standards. \(^d\) Polydispersity index (Pdl) and hydrodynamic diameter (\(D_H\)) from DLS measurements in Milli-Q of crude NPs.
Figure 1. Schematic representation of the synthesis of PDMAPMA macroRAFT and its chain extension with MMA via PISA to produce PDMAPMA-b-PMMA NPs. The hydrophobic agent (NR or DOX respectively) was simultaneously encapsulated during PISA.

In situ encapsulation of Nile red into PDMAPMA-b-PMMA NPs

The fluorescent dye Nile red (NR) was conveniently encapsulated in the hydrophobic core of the PDMAPMA-b-PMMA NPs, referred to as PDMAPMA-b-PMMA\textsubscript{NR}. NR is hydrophobic and dissolves readily in MMA and thus, resides in the monomer droplets during the emulsion polymerization until nucleation of particles occurs. Upon the subsequent polymerization-induced self-assembly (PISA) the dye becomes encapsulated in the hydrophobic core of the NPs. The subsequent swelling of the NPs by the NR-containing monomer results in fluorescent, stable, NPs (Figure 2). It is possible to observe the progression of the encapsulation process by the eye; at the start of the reaction the mixture contains strongly coloured, dispersed, monomer droplets which is transformed into a homogeneous dispersion upon polymerization of the hydrophobic MMA (Figure 2b), a video can be found in SI. PDMAPMA-b-PMMA\textsubscript{NR} NPs have very similar characteristics as the reference NPs, PDMAPMA-b-PMMA, $D_H \sim 170$ nm and a narrow Pdl (Table 1). We hypothesize that the solubility of NR in MMA entails that NR is encapsulated in the hydrophobic core upon polymerization. This hypothesis can be further corroborated by a solubility study, showing that NR is completely soluble in MMA but insoluble in water at the utilized concentration, Figure S1.

Characterization of PDMAPMA-b-PMMA\textsubscript{NR}

The successful loading of the NR into the hydrophobic core of the PDMAPMA-b-PMMA\textsubscript{NR} NPs was further verified using structured illumination microscopy (SIM). Figure 2a.\cite{28} Grey
circles were observed in the corresponding micrograph, indicating that the hydrophobic interior of the NPs is loaded with NR. The sizes of the NPs were found to be ~ 200 nm. Figure 2a.

Figure 2. a) SIM’s image of PDMAPMA-\textit{b}-PMMA\textsubscript{NR} NPs on a glass slide, scale bar in red is 0.2 \(\mu\)m and white scale bar is 2 \(\mu\)m. Visual appearance of b) the PDMAPMA-\textit{b}-PMMA\textsubscript{NR}, and c) PDMAPMA-\textit{b}-PMMA\textsubscript{DOX} NPs, both after full conversion (2 hours).

To assess the shape of NPs and their internal structure as well as compare their sizes, PDMAPMA-\textit{b}-PMMA and PDMAPMA-\textit{b}-PMMA\textsubscript{NR} were also analysed with TEM or SEM as seen in Figure 3a and 3b, respectively. The TEM micrograph (Figure 3a) of PDMAPMA-\textit{b}-PMMA NPs showed very monodisperse and highly regular spheres. More interestingly, a core-shell nanostructure was clearly seen at higher magnification (x 880K inset Figure 3a) with an average shell-thickness of ca. 20 nm and a core of ca.110 nm (see SI). As PMMA (\(T_g \geq 100^\circ\)C) was used as the hydrophobic core polymer, SEM successfully visualized the PDMAPMA-\textit{b}-PMMA\textsubscript{NR} NPs in the dry state, showing spherical and uniform NPs (neither the NPs morphology nor size distribution were disturbed by encapsulation of NR). The NPs size observed by TEM or SEM is comparable with the result from DLS measurement, considering the loss of the hydrated layer of the PDMAPMA-corona (as measured by DLS) when dried for SEM analysis, which resulted in decreasing the NP diameter. SEM reveals the robust nature of the NPs; there seems to be only very subtle differences between PDMAPMA-\textit{b}-PMMA (Figure 3a) and PDMAPMA-\textit{b}-PMMA\textsubscript{NR} (Figure 3b) suggesting that the PISA is not detrimentally hampered by the concurrent encapsulation process.
Figure 3. a) TEM micrograph of PDMAPMA-b-PMMA NPs with an inset of a single NP (particle size histogram, upper right) and b) SEM micrograph of PDMAPMA-b-PMMA_NRs NPs (particle size histogram, lower right), both after drying at room temperature.

Evaluation of cell viability of PDMAPMA-b-PMMA NPs

Given the excellent reproducibility offered by RAFT-mediated PISA and the facile encapsulation of a hydrophobic compound, it is of interest to explore the use of the NPs as nano-carriers for drug-delivery applications. However, the use of the cationically charged NPs (5.6 meq g⁻¹)²⁷ may be problematic as cationic NPs have been reported to exert a higher cytotoxic effect than neutral, or negative, particles.²⁹ To assess this, the cell toxicity was investigated by evaluating the cell viability of macrophages (RAW 266.7) and a breast cancer cell line (MCF-7) after incubating the PDMAPMA-b-PMMA NPs with cells for 72 hours (Figure 4). Our findings suggest that the crude and the purified NPs showed no toxicity at concentrations < 1 mg mL⁻¹, but increasing with increasing NPs concentration. The increase of the cytotoxicity of the crude NPs could be due to the presence of free PDMAPMA homopolymer (~ 20 %) in the water.²⁵ To remove any free PDMAPMA, the crude NPs were purified by successive centrifugation-redispersing cycles as described elsewhere.²⁵ The work-
up increased the cell viability further and it was found that the NPs have no toxicity at concentrations 1 mg mL\(^{-1}\) in the MCF-7 cell line, **Figure 4**. The fact that the NPs could be successively centrifuged and redispersed with no noticeable traces of aggregates further confirm their excellent stability.

**Figure 4.** Cell viability of crude and purified NPs (PDMAPMA-\(b\)-PMMA) after incubation with RAW 264.7 and MCF-7 cell lines for 72 h.

**In situ encapsulation of DOX to form PDMAPMA-\(b\)-PMMA\(_{\text{DOX}}\) NPs**

Inspired by the successful encapsulation of the hydrophobic NR, attempts were made to encapsulate the anti-cancer drug DOX to form drug loaded NPs (PDMAPMA-\(b\)-PMMA\(_{\text{DOX}}\)) using the same approach. No previous study could be found on the *in situ* encapsulation of DOX in NPs synthesized by RAFT-mediated emulsion polymerization using PISA. Karagoz *et al.*\(^{[23]}\) studied the non-aqueous post-PISA chemical conjugation of DOX to poly[oligo(ethylene glycol) methacrylate]-\(b\)-[poly(styrene)-co-poly(vinyl benzaldehyde)] NPs. The conjugation was accomplished by a pH-sensitive linkage and the drug loading efficiency was determined to be 67 %. Another study by Liu *et al.* investigated the loading of
DOX by covalent attachment to aldehyde groups in the core of a star-polymer based system, resulting in drug loading efficiency of ~28 wt %, seemingly determined by the amount of reactive monomer incorporated in the core of the NPs.\cite{30} The concurrent self-assembly and encapsulation would be a versatile alternative to drug-conjugation. In the current study, it was found that the hydrophilic DOX (DOX.HCl) had to be converted to the hydrophobic form by treating it with triethylamine to neutralize the HCl. By this treatment, DOX became sufficiently hydrophobic to completely dissolve in MMA where after the mixture could be subjected in the emulsion polymerization using PISA. Full monomer conversion was reached after 2 hr and resulted in a ‘reddish’ dispersion of PDMAPMA-\textit{b}-PMMA\textsubscript{DOX}, Figure 2c, indicating successful encapsulation in the hydrophobic core. It was found that the loading efficiency of DOX during PISA is significantly dependent on the hydrophobicity of the DOX and increased when a larger excess of TEA was used (10 % to 30 %), reflecting the crucial neutralization step. The $D_H$ of PDMAPMA-\textit{b}-PMMA\textsubscript{DOX} as measured by DLS, Figure 5, was found to be 240 nm with a PdI of 0.02, which is in the same range as PDMAPMA-\textit{b}-PMMA and PDMAPMA-\textit{b}-PMMA\textsubscript{NR} NPs, Table 1. The crude NPs were then dialyzed for 2 h to remove any residual macroRAFT (PDMAPMA chains) and free DOX, where after DLS measurements confirmed an essentially unaffected size and size distribution as well as the absence of aggregates (Figure 5). These results again demonstrate the potential of using PDMAPMA-\textit{b}-PMMA NPs for encapsulation of hydrophobic moieties due to the stability during purification processes and loading capacity.
Figure 5. DLS measurements of PDMAPMA-\(b\)-PMMA\(_{\text{DOX}}\) NPs before and after the dialysis to remove any macroRAFT and free DOX. Samples were prepared in de-ionized water with a concentration 0.1 g L\(^{-1}\).

**In vitro drug release kinetics of PDMAPMA-\(b\)-PMMA\(_{\text{DOX}}\) NPs**

The drug release kinetics of free DOX and PDMAPMA-\(b\)-PMMA\(_{\text{DOX}}\) were studied in PBS buffer to mimic physiological conditions (pH 7.4 and 37 °C) using a dialysis cassette and UV spectroscopy. The cumulative release of PDMAPMA-\(b\)-PMMA\(_{\text{DOX}}\) and free DOX as a control was evaluated as a function of time, **Figure 6.** The free DOX showed a fast release and 97 % of DOX was released from the dialysis cassette within 24 hours. In contrast, PDMAPMA-\(b\)-PMMA\(_{\text{DOX}}\) NPs exhibited a small burst effect during the first two hours (~20 % released) and there after a sustained release over five days (~ 50% released).
Figure 6. Cumulative DOX release profiles of free DOX and PDMAPMA-b-PMMA_{DOX} NPs in PBS buffer at pH 7.4 and 37 °C.

**Investigating cell toxicity of PDMAPMA-b-PMMA_{DOX}**

The cell toxicity of DOX-loaded PDMAPMA-b-PMMA_{DOX} NPs was evaluated using RAW 264.7 and MCF-7 cell lines, and compared to free DOX, Figure 7. For the RAW 264.7 cell line at DOX-concentrations above 0.5 µg/mL, the NPs resulted in enhanced toxicity, compared to free DOX. This could be due to a specific interaction between PDMAPMA-b-PMMA_{DOX} and the cells, thus facilitating the internalization of DOX. One plausible explanation for this enhanced effect can be the cationic corona (PDMAPMA-chains) of the NPs, as previously shown when comparing the effect of anionic and cationic NPs on RAW 264.7 cell line.[31] For the breast cancer cell line (MCF-7) the toxicity is only slightly increased for the PDMAPMA-b-PMMA_{DOX} NPs compared to free DOX.
Figure 7. Cell viability of PDMAPMA-b-PMMA_{DOX} NPs and free DOX in RAW 264.7 and MCF-7 cell lines. Concentrations are given in amount of DOX.

**Doxorubicin stability**

DOX, an anthracycline antibiotic, is commonly used for the treatment of a wide range of cancers.\(^{32,33}\) DOX is a cytostatic and cytotoxic drug which interchelate with the DNA of the host cell and inhibits its replication.\(^{34-36}\) DOX consists of a tetrahydroxy-anthraquinone chromophore with a pendant six-membered daunosamine sugar. The DOX, besides its therapeutic function, shows an intrinsic fluorescence at excitation/emission wavelengths of 485/595 nm which is useful for cancer imaging.\(^{37-40}\) Preserving the stability of DOX is crucial to maintain its biological activity. It has been reported that the stability of DOX is influenced by the pH and the temperature of the medium.\(^{41}\) The degradation can be monitored by fluorescence spectroscopy where the original peak pattern is affected and a new blueshifted peak appears, below 550 nm, referring to the degradation products of DOX.\(^{30}\) Herein, we investigated the effect of the TEA, used to neutralize the HCl in the DOX, and the polymerization temperature (70 °C) on the DOX stability, by observing the unperturbed peak at 595 nm in the emission spectra. Figure 8 shows the fluorescence spectra of the TEA-treated
DOX (adjusted pH to 7) at both RT and after heating at 70 °C for 2 h. The untreated DOX (DOX.HCl), before encapsulation, showed a characteristic peak at 595 nm referring to the DOX fluorescence. The TEA-treated DOX at RT or after heating at 70 °C revealed an emission signal at the same wavelength as the untreated DOX (595 nm). The fluorescence spectrum of the DOX released from the NPs, at the physiological conditions (buffer pH 7.4 at 37 °C), also exhibited a maximum emission at 595 nm. No traces of degradation of DOX could be observed in the fluorescence spectra (Figure 8). These results confirm the stability of the DOX under the applied conditions (TEA/RT or 70 °C) at pH 7. Furthermore, the cell viability of the free DOX and DOX-loaded NPs (PDMAPMA-b-PMMA_{DOX}) towards RAW 264.7 (Figure 7) showed toxicity at DOX-concentrations above 0.5 µg mL^{-1}. The cytotoxicity caused by the DOX corroborates its preserved biological activity as a cytotoxic drug after encapsulation using the investigated method.

![Fluorescence spectra](image)

*Figure 8.* Fluorescence spectra of untreated DOX.HCl, treated DOX/TEA/RT, treated DOX/TEA/70 °C and DOX released from NPs.

**Conclusions**

We have demonstrated a successful *in situ* encapsulation approach for the formation of functional NPs, using two hydrophobic agents, NR or DOX, in water. The NPs were formed
with RAFT-mediated emulsion polymerization using PISA in a one-pot system. The NPs are composed of a cationic hydrophilic PDMAPMA-corona and a hydrophobic PMMA-core, resulting in monodisperse NPs, ca 200 nm in diameter. The NR and DOX were both miscible with MMA allowing for the in situ encapsulation during PISA. The NR-loaded NPs were fluorescent and therefore show promising imaging probe properties. The purified NPs exhibited no detectable cell toxicity in RAW 264.7 and MCF-7 cell lines. DOX was successfully loaded into the NPs with an encapsulation efficacy of 30 %. The DOX-loaded NPs exhibit a sustained release profile over 5 days, which hold promise for controlled drug delivery applications. The NPs enhanced the cell internalization of DOX as compared to free DOX. The DOX was stable under the PISA reaction condition preserving its biological activity in vitro. We envision that these nanocarriers could be further explored for theranostic applications.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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