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# Modulation of electrostatic interactions to improve controlled drug delivery from nanogels

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ABSTRACT

The synthesis of nanogels as devices capable to maintain the drug level within a desired range for a long and sustained period of time is a leading strategy in controlled drug delivery. However, with respect to the good results obtained with antibodies and peptides there are a lot of problems related to the quick and uncontrolled diffusion of small hydrophilic molecules through polymeric network pores. For these reasons research community is pointing toward the use of click strategies to reduce release rates of the linked drugs to the polymer chains. Here we propose an alternative method that considers the electrostatic interactions between polymeric chains and drugs to tune the release kinetics from nanogel network. The main advantage of these systems lies in the fact that the carried drugs are not modified and no chemical reactions take place during their loading and release. In this work we synthesized PEG-PEI based nanogels with different protonation degrees and the release kinetics with charged and uncharged drug mimetics (sodium fluorescein, SF, and rhodamine B, RhB) were studied. Moreover, also the effect of counterion used to induce protonation was taken into account in order to build a tunable drug delivery system able to provide multiple release rates with the same device.



## 1. Introduction

In the last years a lot of efforts and studies were devoted to the synthesis of novel drug delivery systems consisting of polymeric nanocarriers [1–4]. The need to improve classic drug administration routes like oral, intravenous and intra-arterial was and still is one of the main goals of these researches [5,6]: indeed, while pills and injections have enabled significant medical advances, these methods are inadequate for the delivery of drugs with short half-lives, poor permeability in membranes, and serious toxicity when delivered systemically in large doses. In particular, the main improvement would be the possibility to maintain drug level in plasma at an effective level for a sustained period of time, avoiding under- and over-dosing [7,8]. Moreover, novel drug delivery systems should guarantee: (*i*) drug protection from hostile environment; (*ii*) controlled release in response to environment stimuli like pH or temperature; (*iii*) drug targeting and selectivity to specific organs, tissues or cells thus improving the pharmacodynamic characteristics of the drug [9–11]. Following these objectives and criteria, researchers and companies focused their attention on the design of new smart drug vehicles made of polymeric colloidal dispersions [2,12]. In this framework nanogels (NGs) appear as a promising tool: they are nanosized networks composed by physically or chemically cross-linked polymers and characterized by high biocompatibility and biodegradability. They provide large specific surface, which guarantees the interaction with physiological compartments and enhances stability and bioavailability of the loaded drugs and proteins [13,14]. Despite the good results obtained in many applications, several critical aspects are related to the fact that drug release is mostly driven by pure diffusion mechanism that is very quick due to the high clearance observed *in vivo* [15,16]. The hydrophilic nature of encapsulated drugs and biomolecules is not enough to control the release mechanisms, therefore, the necessity to develop NGs able to delay release rates or allow multiple release kinetics, different from pure-Fickian ones, is very demanding [17,18].

In order to overcome these drawbacks and so attenuate diffusional release of biomolecules Vulic et al. [19] introduced biorthogonal strategies to create an affinity bond between peptide and polymeric network, covalently linking a small protein receptor module to her gel systems. Similar procedures were also followed by several other groups [16,20,21] that used different functionalization strategies to link drugs to polymers. In these cases, drug release is not driven by Fickian diffusion but delayed by the stability or affinity of the bond between drug and polymer: higher the stability and slower the release kinetics. This approach guarantees the controlled and sustained release of drugs/biomolecules on one hand, but on the other hand, the chemical modification of active principles may change their efficacy [19,21,22]. With the aim of

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avoiding the formation of covalent bonds between therapeutic compounds and polymers, but taking advantages from the interactions that could take place among solutes and polymers [15,17,23], we decided to consider electrostatic interactions.

In particular in this work we synthesized NGs based on polyethylene glycol and polyethylenimine PEG-PEI, suitable for biomedical applications [24], with different protonation degrees. The colloidal dispersions were characterized in terms of dimensions, polydispersity and ζ-potential and then loaded with two different drug mimetics. The first one was sodium fluorescein (SF), a commonly used drug-mimetic molecule [25–27], chosen for its steric hindrance and its resemblance to many corticosteroids and anti-inflammatory drugs (for example, methylprednisolone, ibuprofen, and estradiol) used in pharmacotherapy. Moreover, SF is a sodium salt like several drugs already listed and in water it is dissociated into fluorescein anion and sodium cation [26]. The second one was Rhodamine B (RhB), drug mimetic with the same dimension of SF, in order to neglect the steric hindrance contribution in the experiments [28,29]. RhB is a neutral molecule at pH = 7.4 and slightly positive at acid pH: this behavior can be found in several amine-based drugs like lidocaine or trandolapril [30]. To validate and confirm the results collected with the charged drug we compared them with release studies performed with rhodamine.

As a significant consequence of this study, it could be possible to tune the delivery of charged therapeutic compounds from NGs, according to specific pharmacological and medical needs, tuning polymer protonation degrees. This strategy is able to guarantee suitable delivery kinetics avoiding chemical modification of loaded drugs/biomolecules.

## 2. Materials and methods

### 2.1. Materials

All experiments were performed using the following polymers: polyethyleneimine linear (MW = 2500 Da, by Polysciences Inc., Warrington, USA) and polyethylene glycol 8000 (MW = 8000 Da, by Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany); all other used chemicals were purchased from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Deisenhofen, Germany). The materials were used as received, without further purification. Solvents were of analytical grade. Synthetized products containing fluorescein sodium salt or rhodamine B were stored at 4 °C in dark. The NMR experiments were carried out on a Bruker AC (400 MHz) spectrometer using chloroform ($CDCl_3$) as solvent, and chemical shifts were reported as δ values in parts per million with respect to TMS as internal standard.

### 2.2. Synthesis of NG-$c^+$

Nanogels constituted by PEG and PEI with high cationic charge density, labelled as NG-$c^+$, were synthetized referring to the modified emulsification-evaporation method [31]. Briefly, PEG was activated using 1,1′-carbonyldiimidazole (CDI) as discussed in our previous work [24] and the modified polymer (200 mg, 0.025 mmol) was dissolved in $CH_2Cl_2$ (3 ml). PEI (52 mg, 0.021 mmol) was added to distilled water (5 ml), under stirring at 30 °C until complete dissolution. Then, HCl 1 M was added to adjust pH at 4.5. Successively, the organic solution was poured dropwise to PEI solution under vigorous stirring and the resulting mixture was sonicated for 30 min and $CH_2Cl_2$ was evaporated. The obtained solution was left stirring for 17 h at 25 °C and purified performing a dialysis against aqueous solution at pH 4.5 (using a membrane of $M_w$ cutoff = 3500 Da), prepared from distilled water (2000 ml) and HCl 37% w/w (0.72 ml). Finally, the resulting mixture was lyophilized.

The product was then characterized by $^1$H NMR analysis and nanogel size and charge were investigated using DLS and AFM techniques.

### 2.3. Synthesis of NG-u

Nanogels synthetized by PEG and uncharged PEI, named NG-u, were prepared following the procedure illustrated in the previous section, using alkaline conditions both in nanostructure formation and dialysis, instead of acidic ones. In summary, PEI (52 mg, 0.021 mmol) was dissolved in distilled water (5 ml) and the solution was carried to pH 10.5 with NaOH 1 M. The organic solution of PEG bis-activated CDI was added dropwise to PEI alkaline system and sonicated for 30 min. Then the organic solvent was removed under reduced pressure and the system left stirring for 17 h at 25 °C. The resulting solution was dialysed against aqueous solution at pH 10.5, consisting of distilled water (2000 ml) and NaOH 1 M (2 ml) and lyophilized. The system was analyzed through $^1$H NMR spectroscopy, while DLS and AFM gave information about size and ζ-potential of the nanogels NG-u.

### 2.4. Nanogel characterization

The hydrodynamic diameters as well as ζ-potentials and the polydispersity index of nanogels NG-$c^+$ and NG-u were determined by dynamic light scattering (DLS) measurements using a Zetasizer Nano ZS from Malvern Instruments. The reported data are an average value of three measurements of the same sample: for NG-$c^+$, the sample was dissolved in acidic solution (pH = 4.5); whereas NG-u one was dissolved in alkaline solution (pH = 10.5). Atomic force microscopy (AFM) analysis was performed using a NT-MDT Solver Pro instrument operating in non-contact mode with silicon tips.

Samples were prepared by dropping nanogel latexes onto silicon substrate and drying. AFM images on 1 × 1 μm areas were recorded for the preliminary morphologic evaluation; 500 × 500 nm image were then cropped and height line profile performed for single nanostructure. The evaluation of the surface morphology and nanogel size were obtained by flattening of the images (first order) using NTMDT software.

### 2.5. Loading of nanogels with fluorescein and rhodamine B

Two drug mimetic solutions were prepared dissolving, separately, SF and RhB in distilled water (both solutions at concentration of 1 mg/ml). Lyophilized nanogels NG-$c^+$ and NG-u were independently suspended in aqueous solution (20 mg/ml). Then, 1 ml of drug mimetic solution was added dropwise (1 ml/min) to 1 ml of nanogel solution under stirring and the system was left to stir for 17 h at 25 °C, in dark. Following this procedure, we performed the loading of SF and RhB within the network of each type of synthetized nanogel: fluorescein was entrapped within NG-$c^+$ and NG-u and, in the same manner, other samples of NG-$c^+$ and NG-u carried rhodamine. Successively, loaded nanogels were dialyzed (membrane $M_w$ cutoff = 3500Da) against aqueous solution in order to remove free molecules. Loading efficiency (% loading) was calculated referring to the equation:

$$\%\ \text{loading} = \frac{\text{drug entrapped within NGs}}{\text{initial amount loaded}} \cdot 100 \quad (1)$$

### 2.6. In vitro drug delivery

Drug release mechanism was investigated in two different release environments: one at pH 7.4 using a phosphate buffered saline solution (PBS), and the other at pH 4.5. In details, each nanogel sample was placed in excess of PBS and in excess of acidic solution (2.5 ml) and aliquots (3 × 100 μl) were collected at defined time points, while the sample volume was replaced by fresh solution, in order to avoid mass-transfer equilibrium with the surrounding release environment. The experiments were performed at 37 °C. Percentages of released SF and RhB