Controlled synthesis of two fluorescent derivatives of poly(2-dimethyl-aminoethyl-methacrylate) adopting Atom Transfer Radical Polymerization (ATRP)

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INTRODUCTION

Natural or synthetic polycations are daily used in clinic for human and veterinary purposes (Protamine, Eudragit®, Chitosan,..). In view to establish other medical applications for new polycations extensive in vitro and in vivo toxicological and pharmacological investigations are requested to analyze their safety, biodistribution, possible metabolism and clearance rate. To follow easily the behavior of these polymers in vitro and in vivo, the cell biologists have today an arsenal of wonderful cell / molecular imaging tools. Therefore when a drug becomes available under a fluorescent form, these methodologies allow to the biologists to follow crucial biointeraction steps measurable at different scale and time ranges such as : membranes adsorption, cell internalization and intracellular trafficking. However when grafting a fluorescent probe several questions should be addressed from the beginning such as :

- precaution should be taken in order to assure that this chemical modification of the polymers does not alter significantly their biological/biochemical behavior;
- define the most optimal density and distribution of the fluorochrome molecules within the macromolecules;
- selection of the most appropriate fluorescent marker based on its imaging characteristics (excitation and emission wavelength, quenching characteristics, quantum yield, extinction coefficient, fluorescent life time, complementary labeling for the purpose of colocalization tool.

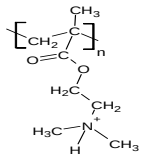


Figure 1 : Molecular structure of PDMAEMA

AIM

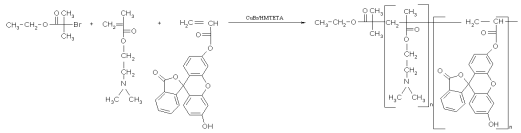
In view to get more in-depth knowledge on the parenteral behavior of poly(2-(dimethylamino)ethyl methacrylate polymer (PDMAEMA), we have labeled this synthetic polycations adopting ATRP as polymerization mechanism and considering two different synthesis routes relying upon :

- a copolymerization of a functional co-monomer giving readily access to the covalent grafting of fluorescent probes;
- the use of a labeled initiator which label the polycation either at its a-extremity or either to its center.

First approach : Polycation labeling

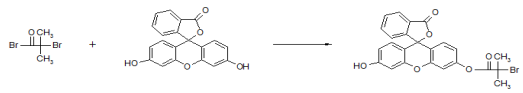
First step : Synthesis of fluorescent comonomer, fluorescein acrylate

Second step : Copolymerization of DMAEMA with fluorescein acrylate

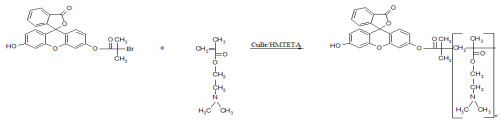


Second approach : Mono labelling

First step : Preparation of fluorescein-BiB



Second step : Copolymerization of DMAEMA with fluorescein-BiB



ATRP was conducted using copper bromide as the catalyst complexed by HMTETA ligand and 2-ethylbromoisobutyrate (EBiB) as initiator. For the sake to simplify the polymerization and to avoid any solvent, we have compared the polymerization realized either in bulk, either in solution (DMF).

RESULTS AND DISCUSSION

First approach : Multiple labelling

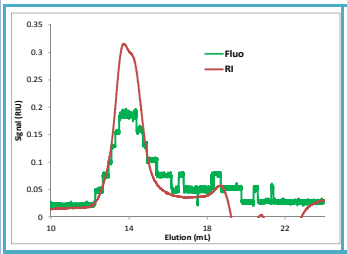


Figure 2 : Chromatogramme SEC

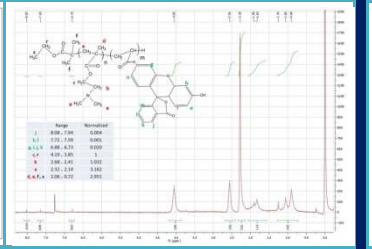


Figure 3: Proton NMR spectrum

Second approach : Mono labelling

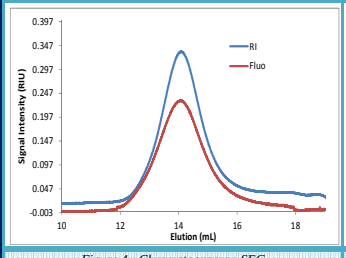


Figure 4 : Chromatogramme SEC

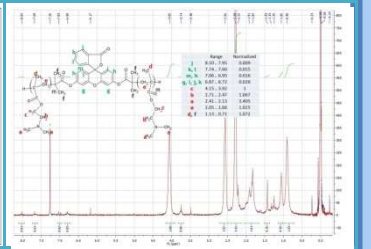
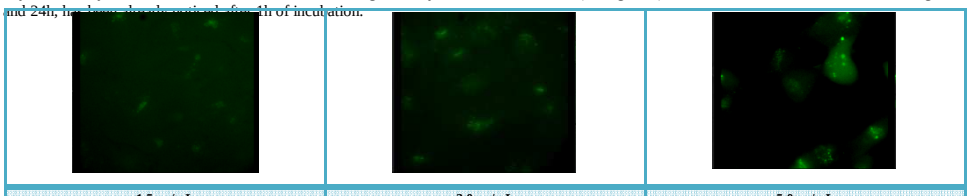


Figure 5: Proton NMR spectrum

Once purified these fluorescent polymers have been characterized by 1H.NMR and SEC chromatography adopting refractive index and fluorescent detections. The double detection (RI and fluorescence) adopted for size exclusion chromatography has allowed us to verify the labeling homogeneity of the polycations. As highlighted on figure the SEC analysis clearly highlights the presence of one single polymer population which contains all the fluorescence detected during this chromatography separation.
It should be stressed that the very low signal/noise ratio of the fluorescent trace of these SEC analysis is the result of a quenching of the fluorescence by the mobile phase adopted for this analysis, i.e. : THF (TEA : 2%). Indeed by comparison with batch-mode measurements performed with a spectrofluorimeter, we have observed up to a 2000 times quenching of the fluorescence intensity of free and bounded fluorescein when dissolved in this solvent by comparison to an aqueous medium. Future analyses are actually running in order to explain this fluorescent inhibition which does not fit to the expected evolution with solvent polarity.

A first prescreening test to verify the stability of our fluorescent marker and the labeling intensity on some animal cells (see figure 6). Cells are visible in all concentration ranges assessed. A cell internalization just screened at 2 time points, 1h and 24h, has been detected after 4h of incubation.



1,5 µg/mL	3,0 µg/mL	5,0 µg/mL

Figure 6 : Fluorescent labelling of endothelial cells after their incubation for 24h with fluorescent PDMAEMA (JV024) at 3 concentrations (images acquired with the collaboration of the Centre for Pharmaceutical Nanotechnology and Nanotoxicology, Pharm Nanotech (Copenhagen University).