

## pH-Responsive Nanocarriers for Delivery of HIV Antiviral Drugs

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**Statement of Purpose:** Sexually transmitted infections including HIV are a major health burden worldwide, where in sub-Saharan Africa it is the leading cause of death among women of reproductive age<sup>1</sup>. Topical delivery strategies that deliver ARV drugs locally to mucosal tissue are important prevention strategies against sexual HIV transmission. Here, we describe pH-sensitive nanocarriers that can undergo triggered drug release upon exposure to alkaline semen. A healthy vaginal flora, which has nearly no buffering capacity, has a pH < 5<sup>2</sup>. In contrast, seminal plasma is alkaline, leading to neutral pH of the vagina during and after intercourse<sup>3</sup>. As such, pH-sensitive drug delivery systems are designed to release their drug payload upon exposure to the virus or infected cells in semen. We developed of pH-sensitive nanofibers and nanoparticles for delivery of dapivirine (DPV) and etravirine (ETR), which are non-nucleoside reverse transcriptase inhibitors (NNRTI) used in the treatment or prevention of HIV/AIDS. DPV and ETR share similar physicochemical properties because they are analogs of the same class of diarylpyrimidine compounds and differ only in substitution of two of the three core isomeric pyrimidine heterocycles.<sup>4,5</sup> We employed a pH-sensitive poly(methacrylic acid-co-methyl methacrylate) (PMAA-co-PMMA) to modulate drug release kinetics of DPV or ETR from the different carriers. Both nanocarriers facilitated rapid and sustained release of DPV and ETR as a function of pH. We expect these delivery systems to expand the methods for HIV prevention.

**Methods:** DPV loaded pH-sensitive poly(methacrylic acid-co-methyl methacrylate) (PMAA-co-PMMA) nanofibers were prepared by electrospinning. ETR nanoparticles were prepared by an electrostatic co-precipitation technique by combining ETR and pMAA-co-MMA in acetone with an aqueous chitosan solution containing PVA as a stabilizer. The mixture was stirred at room temperature for 3 h, and diluted upon addition of water to result in a nanoprecipitation. We used scanning electron microscopy (SEM) to characterize nanocarrier size and morphology, and differential scanning calorimetry (DSC) to characterize the amount of crystalline drug particulate in the different carriers. Release assays were performed under sink conditions using a phosphate buffer/methanol mixture (3:1).

**Results:** Electrospinning PMAA-co-PMAA with DPV resulted in uniform fibers of 400 nm with a smooth surface morphology (Fig. 1a). DPV-NFs show a high drug content of 20 wt% and is an amorphous dispersion within the fiber. A decrease in the T<sub>g</sub> of PMAA-co-PMMA by 20 °C suggests that DPV interacts with the copolymer and acts as a plasticizer.

Electrostatic co-precipitation of the polyanionic PMAA-co-PMMA and polycationic chitosan was used to

form ETR-NP of 150 nm diameter (Fig. 1a). By altering the pH and copolymer concentration, we tailored the ETR-NP size from 500 nm to 100 nm. The size of ETR-NP decreased with pH, but increased with copolymer concentration. ETR-NP had high encapsulation efficiency (~70 %) and drug loading of 14 wt%. ETR was also observed by DSC to be an amorphous dispersion in the nanoparticles.

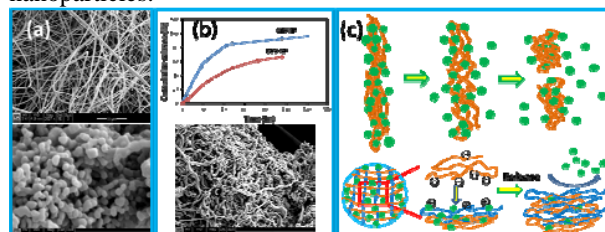


Figure 1. (a) SEM images of DPV loaded nanofibers and ETR loaded nanoparticles (from top to bottom). (b) DPV and ETR *in vitro* release in pH 4 buffer solution from nanofibers and nanoparticles. SEM images of DPV nanofibers after incubation in pH 4 media for 7 days. (c) Schematic of drugs release mechanism from nanofibers and nanoparticles.

Both carriers resulted in sustained drug release under acidic conditions (pH ~4) and burst drug release in alkaline media (pH ~7). At pH 4, SEM micrographs show that the fibers appear to contract and break along the long axis (Fig. 1b), whereas at pH 7, the fibers appeared to decrease in diameter and dissolve. ETR-NP exhibited significantly faster drug release than DPV-NF at pH 4 and 7. The faster drug release rate from copolymer nanoparticles compared to nanofibers may be due to enhanced dissolution of the particles caused by copolymer and chitosan dissociation (Fig. 1c).

**Conclusions:** We successfully fabricated DPV-loaded nanofibers (DPV-NF) and ETR-loaded nanoparticles (ETR-NP) using a pH-sensitive PMAA-co-PMMA copolymer that undergoes a gel-to-solution phase transition in response to alkaline pH. Under acidic conditions, nanocarriers remained intact and result in sustained release of DPV and ETR. However, under alkaline conditions, the nanocarriers rapidly dissolve and burst release DPV or ETR. Although pH modulated the same burst or sustained release profile from either carrier, nanoparticle carriers exhibited much faster release kinetics compared to nanofibers. These materials may be useful for delivery of other ARV drugs or drug combinations with programmable release kinetics.

**References:** (1) Mallipeddi, R. Rohan, C.L. 2010 *Intern J Nanomedicine*, 5: 533–547. (2) Huang, C. et al., 2012 *Biomaterials*, 33: 962-969. (3) Bouvet, J.P. et al., 1997 *Clinic Microbio Infect*, 3(1):19-23. (4) Bousquet L. et al., 2009, *Antimicrob. Agents Ch.* 53: 896-902. (5) Lee C.G.L. et al., 1998, *Biochem.*, 37: 3594-3601.