

Polydopamine Nanoparticle as a Stable and Capacious Nano-Reservoir of Rifampicin

Tasnuva Tamanna, Aimin Yu

Abstract—Application of nanoscience in biomedical field has come across as a new era. This study involves the synthesis of nano drug carrier with antibiotic loading. Based on the founding that polydopamine (PDA) nanoparticles could be formed via self-polymerization of dopamine at alkaline pH, one-step synthesis of rifampicin coupled polydopamine (PDA-R) nanoparticles was achieved by adding rifampicin into the dopamine solution. The successful yield of PDA nanoparticles with or without the presence of rifampicin during the polymerization process was characterized by scanning electron microscopy, Fourier transform infrared spectroscopy, and Raman spectroscopy. Drug loading was monitored by UV-vis spectroscopy and the loading efficiency of rifampicin was calculated to be 76%. Such highly capacious nano-reservoir was found very stable with little drug leakage at pH 3.

Keywords—Drug loading, nanoparticles, polydopamine, rifampicin.

I. INTRODUCTION

IN the last decade, nanoscale drug delivery has emerged as an interdisciplinary, independent and fascinating field of research. A wide range of nanoparticles have been developed as carrier of drugs, vaccine, enzyme, recombinant proteins, and nucleotides etc. Biocompatibility and negligible toxicity of polydopamine (PDA) nanoparticles allow their implementation in drug/gene delivery and other biomedical applications [1], [2].

The polymerization of dopamine at alkaline pH actually generates PDA [3]. Dopamine is one of the significant neurotransmitters that contains catechol and amine functional groups in its structure. The oxidation of catechol functional group is considered as the possible strategy behind the self-polymerization of dopamine to PDA at alkaline pH [4]. Based on this mechanism, researches are synthesizing PDA as thin film, nanocapsule, nanoparticles as well as exploring their beneficial aspect in biomedical science. For example, PDA nanoparticles with particle size ranging from 75-400 nm were synthesized via changing the pH values from 7.5 to 9. These PDA nanoparticles were then implemented as nanocarriers of amphotocin and maximum 34% drug entrapment was accomplished [5]. In addition, those PDA nanoparticles showed no apparent hemolysis, and no acute toxicity to the A549 and HeLa cells. Not only the PDA nanoparticles but also

the PDA capsules were reported for the controlled drug delivery system [6], [7]. Now-a-days, PDA has been applied as a film over other nanoparticles to develop stimuli responsive drug release system. Recently, PDA coated mesoporous silica nanoparticles (MSNs) were synthesized to obtain a pH-responsive release of doxorubicin [8]. PDA coating was provided over the MSNs as gate-keepers and ensured “zero premature release” in neutral media. The release was triggered by exposing the drug loaded nanocarriers to acidic pH. R. Liu and co-workers reported Fe₃O₄ polydopamine core-shell nanoparticles for pH controlled delivery of bortezomib wherein pH-sensitive release was achieved via reversible bonding between catechol and boronic acid groups of PDA and bortezomib [9].

Based on the discussion above, PDA is worth enough to study in the field of drug delivery system. In this study, we aim to synthesize PDA nanoparticles as well as co-loading of antibiotic (rifampicin) into PDA nanoparticles by a single step. The synthesis of PDA is monitored in both the presence and absence of rifampicin. Characterization of both PDA and PDA-R are carried out via scanning electron microscopy, Fourier transform infrared spectroscopy, Raman spectroscopy. Drug loading efficiency is analyzed via UV-vis spectroscopic assay.

II. MATERIALS AND METHODS

A. Materials

Rifampicin and 3-hydroxytyramine hydrochloride (DA) were purchased from Sigma-Aldrich. All other chemicals used during the following experiments were of analytical grade reagents. Tris buffer (50 mM, pH 8.5) was prepared by dissolving Tris base and pH was adjusted by using 1 M hydrochloric acid (HCl). Ultrapure water was obtained from a Millipore water purification system (Milli-Q) and used throughout all experiments.

B. Synthesis of PDA and Rifampicin in Loaded PDA

PDA was synthesized by adding 12 mg dopamine into 15 mL Tris buffer at pH 8.5. This mixture was kept under stirring for 5 days. Encapsulation of rifampicin in PDA was performed similarly i.e. by adding 12 mg dopamine into 15 mL of Tris buffer containing rifampicin (0.6 mg/mL conc.). This mixture was allowed to react for 5 days with constant stirring. Finally, both PDA and rifampicin loaded PDA (PDA-R) products were collected via centrifugation of precipitate followed by three times washing with Milli Q water. Drug loading was measured via UV-vis assay. Samples were diluted 5 times with Tris

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buffer to carry out the UV-vis assay before and after the loading.

C. Stability Test of PDA-R

In order to carry out the stability study, 1 mg of PDA-R and PDA were dispersed in 3 mL pH 3.0 buffer. Temperature was maintained at 37°C throughout the study period. Aliquots were collected by centrifugation and the fraction of drug leakage was monitored via UV-vis spectroscopic assay. The volume of the medium was kept constant via returning the sampling aliquots to the release medium.

D. Material Characterization

Scanning electronic microscope (SEM) images were obtained on a field emission scanning electron microscope (FeSEM, ZEISS SUPRA 40VP, Germany) at an acceleration voltage of 3 kV. The UV-Vis absorbance was carried out with a Halo RB-10 UV-Vis spectrophotometer (Australia). Raman spectra were obtained at room temperature using a Raman microscope (Renishaw, inVia) with 514 nm laser light. FTIR analysis was done with the help of Thermo Scientific Nicolet iD5 spectrometer (USA).

III. RESULTS AND DISCUSSION

A. Characterization

The SEM images of PDA and PDA-R (Figs. 1 (a) and 1 (b)) illustrate that both PDA and PDA-R were roughly spherical in shape. PDA particles were larger (~ 65 nm) in diameter when compared to PDA-R (~ 35 nm) as identified from SEM images (Figs. 1 (a) and 1 (b)). This kind of result suggests that presence of rifampicin controls the polymerization of dopamine (DA) to PDA, thereby, controls the size of rifampicin coupled PDA.

However, FTIR spectra provided additional information regarding the chemical interaction between rifampicin and PDA. Rifampicin could be encapsulated into the PDA via hydrogen bonding as both PDA and drug molecule contains hydroxyl groups. In addition, FTIR spectra of PDA and PDA-R also supported the successful polymerization of dopamine to PDA. The chemical structure of PDA and rifampicin are shown in Fig. 3.

Briefly, characteristic peaks at 1585 and 1497 cm^{-1} were observed for both PDA and PDA-R due to the stretching vibration of aromatic C=C and C-N-C residing in the indole ring [10] of PDA (Fig. 2). In addition, other featured peaks within a range of 3160-3300 cm^{-1} and at 1040 cm^{-1} caused by the stretching vibration of catechol O-H, and hydroxyl C-O and/or C-N respectively [11]. However, a new peak was noticed at 3200 cm^{-1} in the spectrum of PDA-R because of H-bonding between the catechol O-H group and free O-H groups in drug molecule, which was unobserved in PDA spectrum. These data backed up the successful preparation of PDA in presence and/or absences of rifampicin and proved the incorporation of rifampicin in PDA-R.

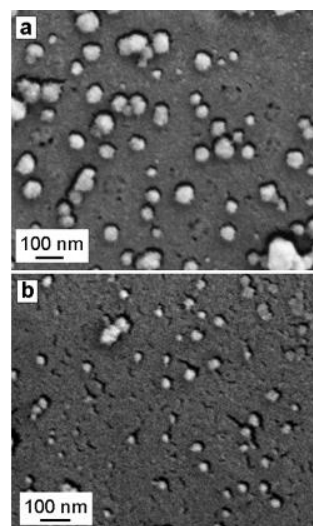


Fig. 1 SEM image of (a) PDA and (B) PDA-R

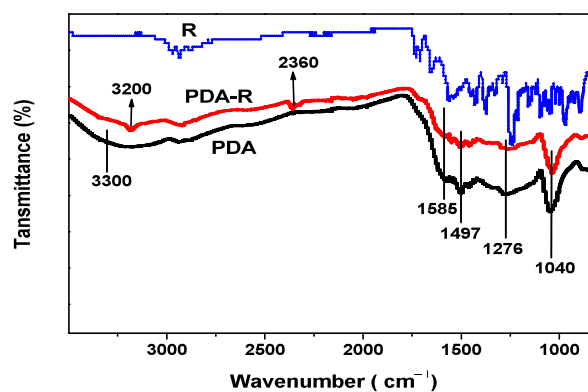


Fig. 2 FTIR spectra of rifampicin (R), PDA and PDA-R

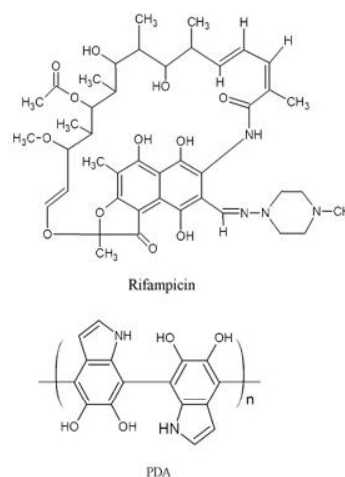


Fig. 3 Structure of rifampicin and PDA

Furthermore, Raman spectrum of PDA showed the featured broad peaks at 1580, 1390 and 1350 cm^{-1} (Fig. 4) due to the stretching and deformation of aromatic ring (catechol) confirming successful synthesis of PDA [12], [13]. Conversely, these intensity peaks were unnoticed in PDA-R due to the luminescence of rifampicin in PDA-R. Such

noticeable changes in Raman spectra of PDA and PDA-R also reinforced existence of pure PDA and rifampicin in PDA-R.

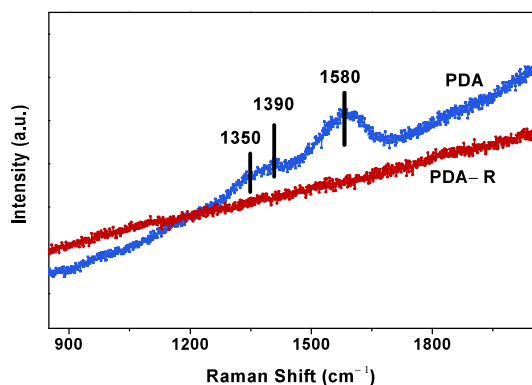


Fig. 4 Raman shift for PDA and PRA-R

B. PDA as High Capacious Reservoir for Rifampicin

High loading of drug is vastly desirable for any effective nano drug reservoir. PDA served as a good nanocarrier to accommodate high bulk of rifampicin. Rifampicin loading was quantified via UV-vis spectrophotometry as rifampicin showed typical absorbance at 249, 339 and 473 nm (Fig. 5 (a)). Absorbance at 473 nm has been used to calculate the drug quantity [14]. In our study, 473 nm was also preferable to compute the drug loading efficiency as it minimizes the absorption interference from PDA. Moreover, the absorbance of rifampicin solutions with varying concentrations showed good linearity at 473 nm with R square value of 0.9921 (Fig. 5 (b)).

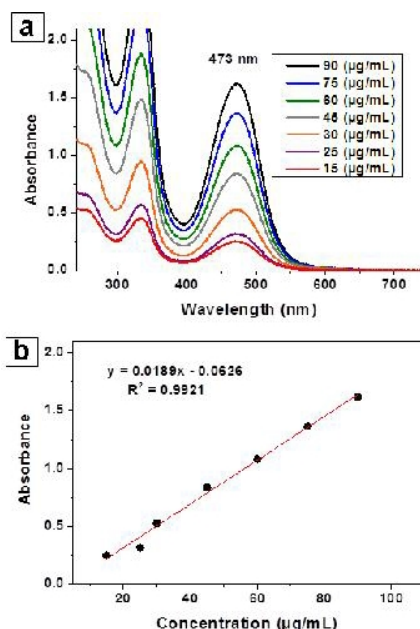


Fig. 5 (a) UV-vis spectra of rifampicin and (b) linearity of concentration vs. absorbance

The loading capacity of rifampicin in PDA was measured to be 76 %. Such high loading capacity of PDA proved itself as a

highly capacious reservoir of rifampicin. The following equation was used to calculate the loading efficiency.

$$\text{Loading capacity (\%)} = \frac{\text{Amount of drug encapsulated}}{\text{Total amount PDA-R synthesized}} \times 100$$

Although very negligible but PDA showed absorbance at 473 nm (Fig. 6 (A)). Thereby, absorbance of PDA was taken into account while analyzing drug entrapment efficiency.

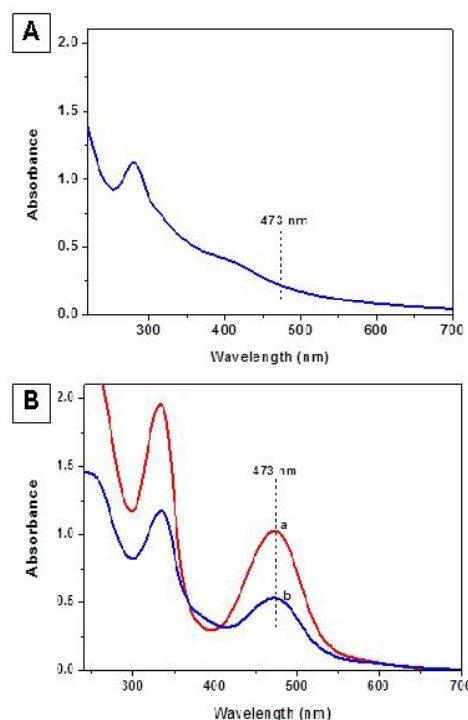


Fig. 6 (A) UV-vis spectra of PDA, (B) rifampicin before (a) and after (b) the drug loading

C. PDA as a Stable Nano Reservoir for Rifampicin

Indeed, the stability is an important factor for any nanocarrier. A very good stability profile of rifampicin coupled PDA (PDA-R) was observed at acidic pH 3.0. PDA-R particles were stable for 6 days without any burst release of loaded rifampicin. A very negligible leakage of rifampicin i.e. 22.3 ng and 23.0 ng at 2nd and 4th day respectively, was noticed which eventually reached to plateau phase on 6th day of immersion.

This data suggested that PDA is a highly stable reservoir for rifampicin to retain the antibiotic for prolong period with very little leakage. However, there is limitation of this highly stable particle. It may cause a large fraction of drug trapped inside. Such confinement could even be toxic. Thereby, there is still enough scope to work on the modulation of rifampicin release from PDA. In this study, we confirmed the high loading capacity PDA for rifampicin. Further work on release behavior can suggest these PDA as nano drug carrier for controlling the release profile of loaded rifampicin.

IV. CONCLUSION

To conclude with, a simple and one-step process was established for the synthesis of PDA along with co-drug loading. Notably, the presence of rifampicin interfered in the polymerization process of dopamine. Thereby, PDA-R particles were observed smaller than the PDA particles though prepared in the same experimental condition. However, the FTIR and Raman spectra strongly supported the presence of PDA both in bare and drug loaded particles. Moreover, PDA appeared as highly capacious nano vehicle of rifampicin. Loading efficiency of 76% was achieved by 5 day of loading. These rifampicin loaded PDA (PDA-R) showed excellent stability over 6 days at acidic pH 3.0. Significant drug (rifampicin) loading efficiency of PDA drew the attention for further study. A release study in presence of different stimuli such as different pHs, chemical agent needs to carry out as future work to develop this PDA as controlled drug delivery system.

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