Electronic Supplementary Information

Development of an ultra-sensitive electrochemical immunosensor using PPyr-NHS functionalized disposable ITO sheet for detection of interleukin 6 in real human serums

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Characterization of Monomer and Polymers

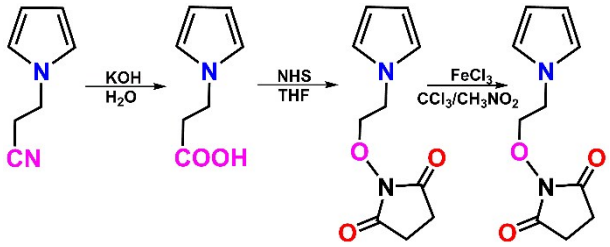

Figure SI-1. Synthesis pathways of succinimide substituted conjugated pyrrole polymer *PPyr-NHS*.

The succinimide functional groupS substituted conjugated *PPyr-NHS* polymer was successfully synthesized a series of reaction steps. These reaction steps were consisted of conversion, esterification and oxidatively polymerization technics. In the first reaction step, acid functional group substituted monomer was prepared by conversion reaction of nitrile group to acid group. In the second reaction step, succinimide functional group substituted monomer (*Pyr-NHS*) was prepared by esterification reaction of N-Pyrrolylpropanoic acid and N-Hydroxysuccinimide. In the third reaction step, succinimide functional group substituted *PPyr-NHS* polymer was synthesized according to chemical oxidative coupling method of pyrrole monomer. The synthesis steps of succinimide-substituted polymer *PPyr-NHS* is shown in Fig. SI-1.

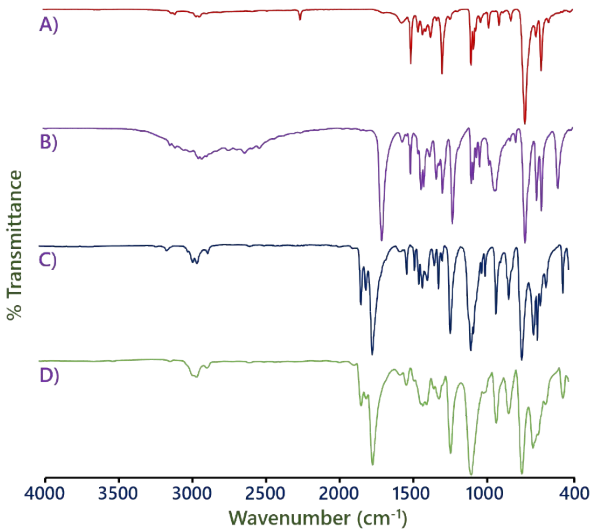

Figure SI-2: FT-IR spectra of 1-Pyrrolepropionitrile (A), N-Pyrrolylpropanoic acid (B), succinimide-substituted monomer *Pyr-NHS* (C), succinimide-substituted polymer *PPyr-NHS* (D).

The FT-IR spectra of 1-Pyrrolepropionitrile, N-Pyrrolylpropanoic acid, succinimide-substituted monomer *Pyr-NHS* and succinimide-substituted polymer *PPyr-NHS* are given in Fig. SI-2, respectively. The 1-Pyrrolepropionitrile showed a sharp peak at 2250 cm^{-1}, originating from the -CN side groups, which disappeared completely after the conversion reaction (Fig. SI-2A)[1]. In addition, the presence of a new carbonyl stretching, and hydroxyl peak proved the successful synthesis of monomer N-pyrrolylpropanoic acid (*Pyr-Pac*) (Fig. SI-2B) [2]. The signal observed around 1695 cm^{-1} and between 3500-2500 cm^{-1} were attributed to C=O stretching vibration of acid groups and -OH peak in pyrrole

monomer *Pyr-Pac*, respectively [3, 4]. The figure SI-2C shows FTIR spectrum of succinimide-substituted monomer (*Pyr-NHS*). The strong signal observed around 1731 cm^{-1} was attributed to C=O stretching vibration of ester carbonyl groups which proved to successful esterification reaction . As shown in Fig. SI-2C, the FT-IR spectra for monomer *Pyr-NHS* shows two strong bands at 1810 cm^{-1} and 1776 cm^{-1} that correspond characteristic carbonyl groups of the NHS ester[5]. After the polymerization of monomer *Pyr-NHS*, the peaks of polymer *PPyr-NHS* were broad and like to monomer spectrum, as shown in Figure SI-2D. The aliphatic C-H peaks of polymer were seen in 2990-2880 cm^{-1} (Fig. SI-2D). The characteristic carbonyl peaks of the NHS ester groups were shown at 1812 cm^{-1} and 1780 cm^{-1} (Fig. SI-2D). The strong signal observed around 1728 cm^{-1} was attributed to C=O stretching vibration of carbonyl groups (Fig. SI-2D).

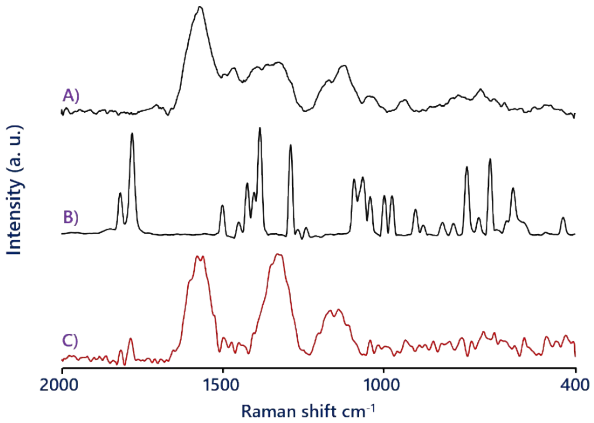

Figure SI-3. Raman spectra of N-Pyrrolylpropanoic acid (A), succinimide-substituted monomer *Pyr-NHS* (B), succinimide-substituted polymer *PPyr-NHS* (C).

The structure of monomer (*Pyr-Pac*), monomer (*Pyr-NHS*), and polymer (*PPyr-NHS*) were also supported by Raman spectroscopy (Figure SI-3). In the related spectrum of Fig SI-3B, the peaks at 1780 cm^{-1} were assigned to carbonyl stretching vibration of succinimide groups of the monomer *Pyr-NHS.* Besides, the Raman spectra for polymer shows two strong bands at 1814 cm^{-1} and 1782 cm^{-1} that correspond characteristic carbonyl groups of the NHS ester[6].

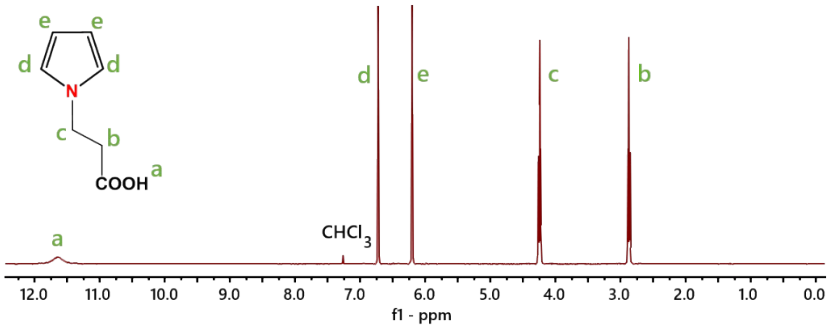

Figure SI-4. ^{1}H-NMR spectrum of acid-substituted pyrrole monomer (Pyr-Pac).

The chemical structure of monomers was also examined via NMR spectral technique. The chemical structure of N-Pyrrolylpropanoic acid monomer *Pyr-Pac* was determined by ^{1}H NMR spectroscopy (Figure SI-4). The ^{1}H-NMR spectrum of acid-substituted pyrrole monomer is given in Fig. SI-4. The two peaks in pyrrole rings appeared in the aromatic region at 6.7 ppm (Hd) and 6.2 ppm (He) were in the integration ratios of 1:1. The peak at 4.2 ppm (Hc) and 2.8 ppm(Hb) were attributable to the methylene group on monomer side group. The protons of hydroxyl group were seen at 11.6 ppm (Ha). The integration ratios of Ha:Hb:Hc:Hd:He was calculated as 1:2:2:2:2.

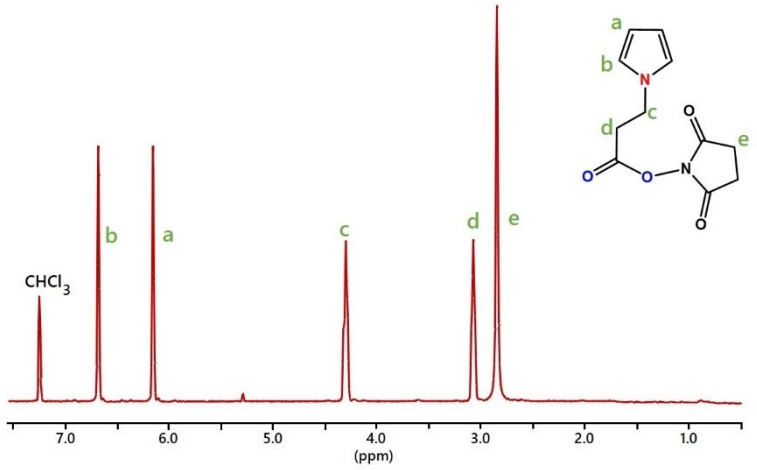

Figure SI-5. ^{1}H-NMR spectrum of monomer *Pyr-NHS*.

The ^{1}H-NMR spectrum of succinimide-substituted monomer is shown in Fig. SI-5. The peaks at 6.17 ppm (Ha, 2H) and 6.7 ppm (Hb, 2H) were attributed aromatic protons of pyrrole rings. The methylene protons peaks of monomer side groups were seen at 4.3 ppm (Hc, 2H) and 3.1 ppm(Hd, 2H). The peak of succinimide groups were seen at 2.85 ppm (He, 4H). The integration ratios of Ha:Hb:Hc:Hd:He was calculated as 1:1:1:1:2.

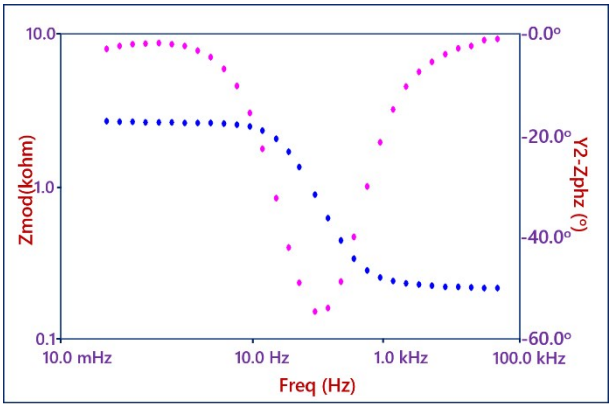

Figure SI- 6. Bode plot .

References

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