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A Mini-Review on Conjugated Polymer-Based Fluorescence Probes for Bilirubin Detection

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ABSTRACT

This review report provides information on the specific conjugated polymer (CP)-based fluorescence probes for bilirubin detection. Few CPs are available, used to detect bilirubin at the nano level. These polymers are soluble in the aqueous phase, appropriate for detecting bilirubin. The sensing efficiency of the CP is demonstrated by the fluorescence resonance energy transfer (FRET) emission and quenching of the polymer fluorescence. DLS provides the particle size of polymer and interaction between the polymer and bilirubin shown by ITC. The different developed CP by the researcher can detect the bilirubin in an aqueous medium and human serum, despite other biomolecules and metal ions with the limit of detection (LOD) of up to 6.9 pM.

Abbreviations: CP: Conjugated Polymer; LOD: Limit of Detection; MOF: Metal Organic Framework; FRET: Fluorescence Resonance Energy Transfer

Introduction

Bilirubin is a bile pigment originating in two ways in the human body. The first one is hemoglobin disintegration (75%-85%), and the second one is myoglobin and cytochromes degradation (15-25%).[1,2] Generally, normal level of bilirubin concentration in the blood range from 0.3 to 0.19 miligrams per 100 mL [3,4]. If the concentration of bilirubin exceeds the mentioned appropriate concentration, it is toxic for the human being; it may lead to jaundice and brain hemorrhage due to its accumulation on body organs. A low concentration of bilirubin is also harmful and causes coronary heart diseases [5-8]. For the detection of bilirubin, several techniques were developed by the researchers, including the Chemiluminescence based techniques [9], spectroscopic techniques, some diazo reactions, [10,11], and polarographic techniques [12]. Due to a lack of adequate selectivity and affectability, the researcher adopted different fluorescence techniques for bilirubin detection. Fluorescence Techniques are unique because of their simplicity, quick response, and economic nature. Various fluorescence probes like metal-organic framework (MoF) [13], quantum dots, and conjugated polymer (CPs) [14-17] have been reported to detect bilirubin. CPs have arisen as a flexible category of nanomaterials with incredible capability in biosensing and bioimaging. These CPs are soluble in the aqueous phase, appropriate for bio application. The solubility in water of rigid CPs is achieved due to functionalized with either nonionic neutral side chains or charged side chains. The polymer's backbone exhibits a hydrophobic nature, compensated by the hydrophilic pendant groups, enhancing the solubility and interacting with the specifically targeted analytes. Due to the nonspecific interaction of nonbiological and multiple biological substances like DNA, RNA, and protein, selectivity is compromised. At the same time, conjugated polymer carried charged pendants, which can bind with the serum protein differentially. This report aims to provide information regarding conjugated polymer-based fluorescence Techniques for bilirubin detection.

Bilirubin Visual Sensing Via Self-Assembled Polyfluorenes [14]

This research report designed and synthesized two bulky appended containing polymers via Suzuki coupling polymerization, one homopolymer (PDP-PF) and the second copolymer (PDPPF-co-Ph), respectively. The sensing of both polymers toward bilirubin was shown by the FRET-based bilirubin green emission and polymer

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fluorescence quenching as presented in Figure 1. With the help of a UV lamp, it can be easily observed that the change in color from blue to green is due to bilirubin addition. The PDPPF-co-Ph polymer's porous spherical assembly is able to adsorb analytes better than the simple micellar assembly, which produces an excellent spectral overlap and greater efficiency for FRET -energy transfer. An unbound bilirubin sensing was performed in Water/THF mixture to maintain the equilibrium between bound (water-soluble) and unbound (THF- soluble). The coupling between bilirubin emission increment and polyfluorene quenching emission creates this approach, appropriate and adaptable for the fluorimetric emission color change-based sensor. Fluorescence quenching efficiency of structural analogues of bilirubin like porphyrin and biliverdin showed poor; due to this reason, these new polymers can highlight the sensitivity and selectivity of the FRET-based sensing in the context of bilirubin.

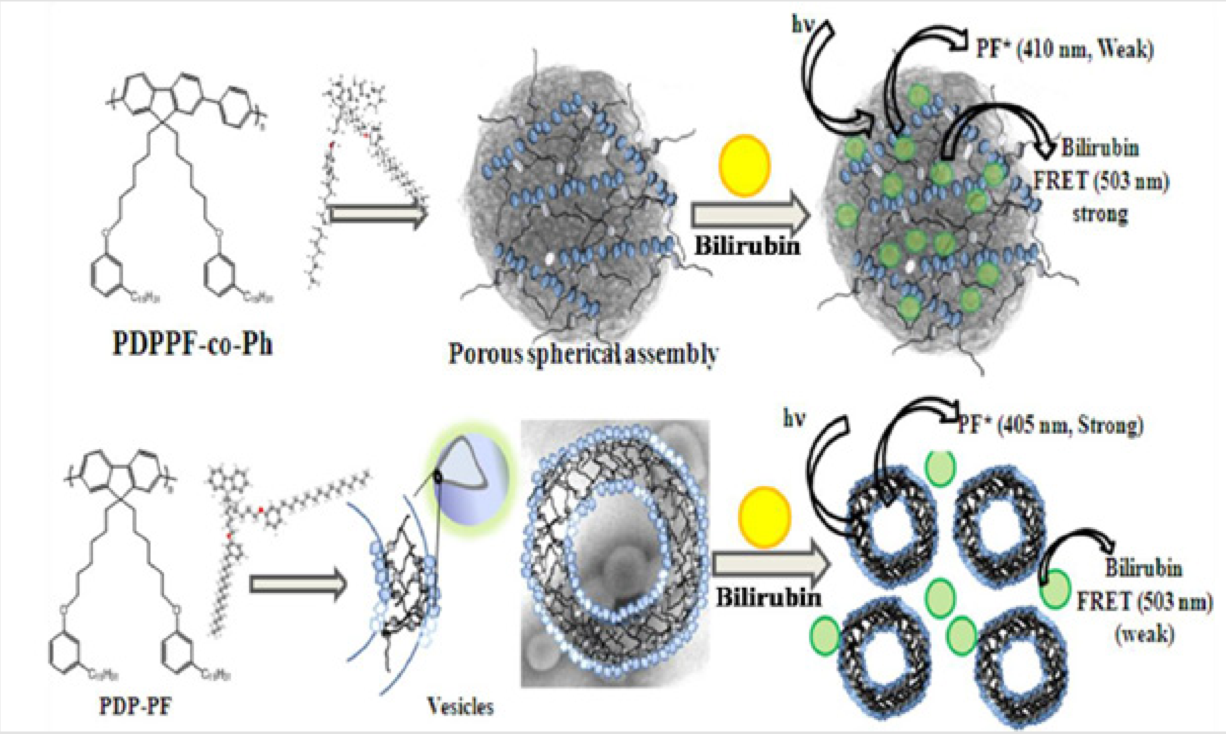

Figure 1: FRET induced mechanism of bilirubin sensing in PDP-PF and PDPPF-co-Ph. Reproduced with permission from Ref. [14] Copyright (2013) American Chemical Society.

Bilirubin Sensing in Human Serum Via Fluorescent Probe Based on Polyfluorene: [15]

Senthilkumar et al.; designed a new conjugated polymer-based fluorescence sensing technique to detect bilirubin in water and human blood serum. This process acts on the FRET and plays a vital role in obtaining the emission of FRET mediated bilirubin which can change the blue color into green. This new polymer was synthesized in a two-step. In the first step, polyfluorenes are functionalized with glucuronic acid side chains; this is a click chemistry-based cross-coupling reaction followed by a palladium catalyst. The new polymer polyfluorenes attached with the appendage of D-glucuronic acid provide the selective sensing of bilirubin in the human blood serum without sticking with any serum protein because of its nonreceptor nature towards to protein as given in Figure 2. The tendency to form large aggregates in the serum by the homopolymer was supported by Dynamic light scattering (DLS). DLS is conducted in an aqueous medium as well as in human serum. In the aqueous medium, PF-GlcA showed a 330 nm particle size while PF-Ph-GlcA exhibited a small particle size of 110nm. With the help of the DLS result, it can be understood that a high volume of glucuronic acid with homopolymer PF-GlcA shows the tendency to stick to the protein, which is hydrophilic; this leads to the aggregation of polymer in the serum. So that in the design of the polymer, the glucuronic acid appendage should be optimum to avoid the proteins adherence. Interaction between the bilirubin and appendage of D-glucuronic acid of the polymer was identified with the help of an isothermal titration calorimeter. During the interaction, bilirubin act as a ligand, and polymer acts as a macromolecule; the result of this interaction produces heat which the ITC measured. At the end of the analysis, results reveal that the interaction between bilirubin and polymer is exothermic in nature. The stability of the polymer in human serum was found excellent under extreme basic conditions. Despite the various interferences like biliverdin, cholesterol, glucose, metal ions, and proteins in the human serum, the polymer exhibits high selectivity and sensitivity towards free bilirubin. This novel polymer could sense the bilirubin at the nano-level (~150 nm).

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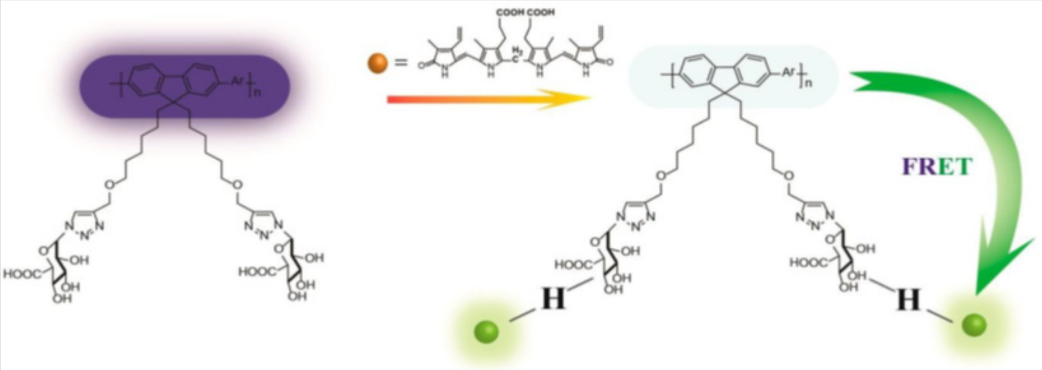

Figure 2: FRET-induced Mechanisms of bilirubin sensing in human serum using polymer PF-Ph-GlcA. Reproduced with permission from Ref.xx Copyright (2015) American Chemical Society.

Bilirubin Detection Via "Turn-On" Fluorescence Using Water-Soluble Conjugated Polymer [16]

In this report, a conjugated polymer was designed and synthesized, which is water-soluble named PF-BT-GlcA poly(fluorenes-alt-benzothiadiazole) used for turn sensing of bilirubin as indicated in Figure 3. The synthesis of the polymer was carried out in two-step, in the first step was performed via Suzuki cross-coupling polymerization reaction between glucuronic acid-functionalized fluorene monomer and benzothiadiazole bisboronic ester and produced the polymer PF-bT-GlcP. In the 2nd step, deprotection of PF-BT-GlcP takes place, and the final product PF-BT-GlcA was obtained as a polymer. The GPC data and NMR data indicate the deprotonation. After the preparation of the polymer, the bilirubin detection is performed in water. The polymer's solubility in an aqueous medium plays a crucial role, essential for sensing studies. As per the emission and absorption spectra of polymer, with increasement in bilirubin concentration, increasement in the absorption and emission was observed at 520nm and 462nm, respectively. After successful detection in water, further studies were done with the human serum. During this experiment, many obstructions were included in the human serum, such as cholesterol, hemoglobin, protein, triglyceride, and metal ions. The bilirubin detection in human serum was led by the polymer PF-BT-GlcA blended in human serum with free bilirubin; the emission spectra illustrate a similarly sharp peak at 520nm. The report concludes the work by demonstrating the turn-on fluorescence sensing of bilirubin using conjugated polymer.

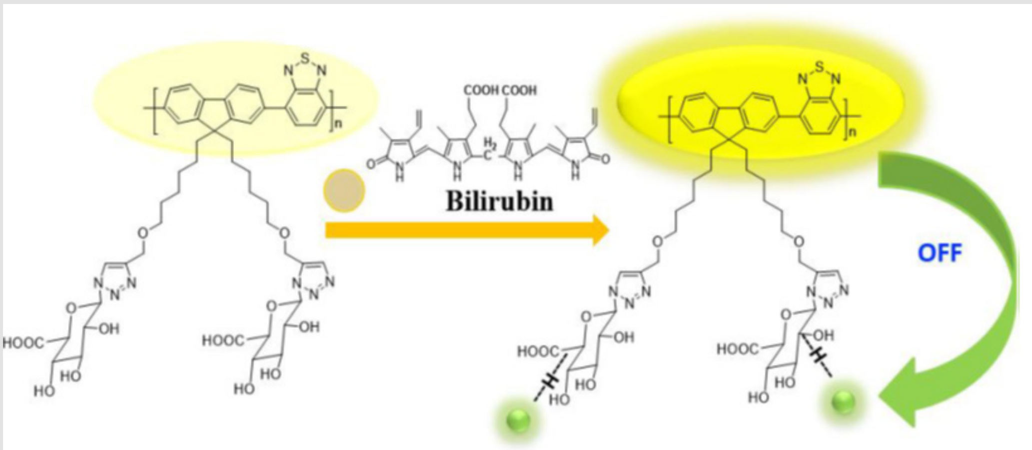

Figure 3: FRET-induced mechanism of bilirubin sensing using polymer PF-BT-GlcA in human serum. Reproduced with permission from Ref. [16] Copyright (2022) American Chemical Society.

Bilirubin Detection in Human Serum Via Fluorescence Probe Based on Conjugated Polymer Nanoparticle [17]

In this research work Iyer et.al., designed and synthesized a polyfluorene derivative conjugated polymer poly1,1′-((2,7-dimethyl-9H fluorene-9,9-diyl) bis(hexane-6,1-diyl) bis(1H-benzo[d]imidazole) (PFBZ) with special receptor via oxidative coupling polymerization reaction. Further, this polymer utilizes for the detection of bilirubin. The complexation between bilirubin and PFBZ polymer and FRET is initially responsible for the sensing mechanism. PFBZ polymer can be utilized in human blood serum for the bilirubin; results were appropriate with a very low standard deviation. In the aqueous medium, PFBZ polymer forms nanoparticles spontaneously and can exhibit the limit of detection of 6.9 pM. After obtaining good results, the researcher developed a fabricated paper-based fluorescence test kit that seems very simple and gives quick response times as shown in Figure 4.

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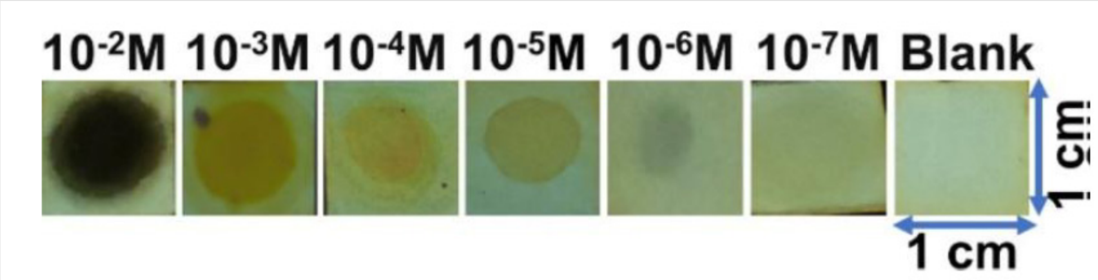

Figure 4: Fluorescence kit based on the color of paper under a UV lamp (365nm) after adding different concentrations of bilirubin. Reproduced with permission from Ref [17] Copyright (2022) American Chemical Society.

Conclusion

Different researchers successfully designed and synthesized the conjugated polymers via click chemistry and Suzuki polymerization. The further analysis provides information regarding the particle size and interaction between bilirubin and polymer. The analysis results reveal bilirubin acts as a ligand and polymer acts as macromolecules. The FRET emission and polymer fluorescence quenching demonstrate the sensing efficiency of the polymer towards the bilirubin. Table 1 summarizes the various conjugated polymer based fluorescent probe for the detection of bilirubin with different LOD values. Conjugated polymers are excellent materials for the detection of bilirubin in water as well as human serum as low as 6.9 pM.

Table 1: Comparison of some previously reported conjugated polymer-based fluorometric sensors for bilirubin detection.

Sensing Polymer	LOD	Ref.
PDP-PF, (PDPPF-co-Ph)	1 nM	14
PF-GlcA, PF-Ph-GlcA	~150 nm	15
PF-BT-GlcA	~180 nm	16
PFBZ	6.9 pM	17

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