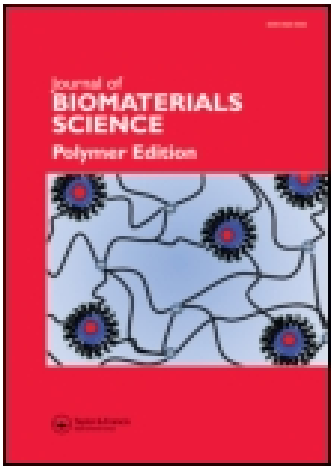


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# Detection of DNA Sequence with Enhanced Sensitivity and Higher FRET Efficiency Using a Light-Emitting Polymer, Peptide Nucleic Acid Probe and Anionic Surfactant System

Nidhi Mathur [a] , Anamika Aneja [b] , P. K. Bhatnagar [c] & P. C. Mathur [d]

[a] Materials Laboratory, Department of Electronic Science, University of Delhi South Campus, Benito Juarez Road, New Delhi-110021, India

[b] Materials Laboratory, Department of Electronic Science, University of Delhi South Campus, Benito Juarez Road, New Delhi-110021, India

[c] Materials Laboratory, Department of Electronic Science, University of Delhi South Campus, Benito Juarez Road, New Delhi-110021, India

[d] Materials Laboratory, Department of Electronic Science, University of Delhi South Campus, Benito Juarez Road, New Delhi-110021, India

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# Detection of DNA Sequence with Enhanced Sensitivity and Higher FRET Efficiency Using a Light-Emitting Polymer, Peptide Nucleic Acid Probe and Anionic Surfactant System

**Nidhi Mathur** *, **Anamika Aneja**, **P. K. Bhatnagar and P. C. Mathur**

Materials Laboratory, Department of Electronic Science, University of Delhi South Campus, Benito Juarez Road, New Delhi-110021, India

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**Abstract**

An improved strategy has been developed for detection of DNA sequence by using water-soluble cationic conjugated polymer (PFP)/single-strand (ss) DNA and peptide nucleic acid labeled with fluorescent dye (PNAC*), where an anionic surfactant (sodium dodecyl sulphate, SDS) system has been used to improve the sensitivity of the sensor. The method of detection is simple to use, fast and cost-effective. This method uses the phenomenon of Forester Resonance Energy Transfer (FRET). The detection sensitivity of the biosensor has been improved by about ten times by using the anionic surfactant. It is observed that the effect of surfactant is to increase the photoluminescence (PL) intensity of the PNAC* when the sequence of the DNA is complementary (to that of PNA probe). On the other hand when the two sequences are non-complementary, the PL intensity of the PNAC* is further reduced as compared to the case when surfactant was absent.


**Keywords**

DNA sequence detection, light-emitting polymer, anionic surfactant, FRET, photoluminescence

## 1. Introduction

Techniques for detecting DNA sequences are important for medical diagnostics and biomedical research applications. There is need for techniques, which are low cost, fast, sensitive and reliable. Methods of identifying biological molecules such as enzyme-linked immunosorbent assay (ELISA) use some specific antibody/antigen interactions with further addition of secondary reagents for the identification of specific molecules [1]. These techniques are time-consuming and require multistep procedures such as polymerase chain reaction (PCR) [2–5] to get the results at the detectable levels. DNA sequence detection technique based on the Forester Reso-

* To whom correspondence should be addressed. Tel.: (91-11) 2411-6718; Fax: (91-11) 2411-5270; e-mail: nidhim_nidhi@rediffmail.com

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nance Energy Transfer (FRET) technique is attractive because of its simplicity of operation and use of standard optical equipment [6]. There is also a growing interest in using conjugated polymers for chemical and biological detection schemes because they are water-soluble and have optoelectronic properties, which support FRET [7, 8]. In this process, the excitonic energy is transferred non-radiatively from an excited donor molecule to an acceptor in the ground state *via* induced dipole–dipole interaction. For FRET to be efficient, it is necessary that there should be maximum overlap between the PL spectrum of the donor and absorption spectrum of the acceptor. Also, the distance between the donor and acceptor must be within the Foresters radius ($R_0$), which is defined as the critical distance between the donor and the acceptor molecules at which efficiency of energy transfer becomes 50%. It can be calculated using the following relation:

$$R_0\ (\text{nm}) = 0.0211(K^2 \cdot J(\lambda) \cdot \eta^{-4} \cdot Q_\text{D})^{1/6}, \tag{1}$$

where $K^2$ is a factor describing the relative orientation in space between the transition dipoles of the donor and acceptor, $J(\lambda)$ is the spectral overlap integral of donor emission and acceptor absorbance (with wavelength in nanometers), $\eta$ is the refractive index of the medium and $Q_\text{D}$ is the quantum yield of the donor.

The donor–acceptor separation $R$ is related to $R_0$ by the equation:

$$R = R_0\left[\left(\frac{1}{E_\text{T}}\right) - 1\right]^{1/6}, \tag{2}$$

where $E_\text{T}$ is the efficiency of energy transfer through FRET [9].

An assay of cationic conjugated polymer (CCP) and PNA labeled with a fluorescent dye (PNAC*) has been successfully used by many workers [10–15], including ourselves [14, 15] for detecting the sequence of ssDNA for medical diagnostics. Peptide nucleic acids (PNAs) are DNA analogs where the four nucleotides, adenine (A), thymine (T), guanine (G) and cytosine (C), are attached to a N-(2-aminoethyl) glycine backbone instead of the negatively-charged deoxyribose phosphate backbone in DNA. PNAs were first described in 1991 and have since then attracted broad attention within the fields of bioorganic chemistry, physical chemistry and medicinal chemistry due to its chemical and physical properties, in particular with regard to efficient and sequence specific binding to DNA. If DNA and PNA have complementary sequences, the PNAC*/DNA will form a negatively-charged hybridized complex and molecules of this complex will be attracted towards the positively-charged CCP dissolved in water. If the distance between the two is less than the Forster critical radius, the excitonic energy may be transferred from CCP (donor) to the PNAC*/DNA complex (acceptor) and the PL emission from the donor may be quenched while the PL emission from the acceptor is enhanced.

In the present paper, we report an improved strategy for the detection of *Escherichia coli* sequences in known ssDNA sequences by adding an anionic surfactant (sodium dodecyl sulphate, SDS) to the above-mentioned assay. The four

components of the assay are (i) blue light-emitting cationic conjugated polymer, polyfluorenephynelene (PFP), (ii) a PNA probe labeled with a fluorescent dye, Fluorescein (PNAC*), (iii) ssDNA which may be complementary or non-complementary to the PNA sequence and (iv) anionic surfactant (SDS). FRET between the donor polymer and the acceptor PNAC* probe is used to detect the presence of specific ssDNA sequence. It is cost-effective, as it requires a very small amount of ssDNA and PNAC*, i.e., 25 nM.

The effect of surfactant is to increase the PL intensity of the PNAC* when the sequence of the DNA is complementary to that of PNA. On the other hand, when the two sequences are non-complementary, the PL intensity of the PNAC* is reduced significantly as compared to the case when surfactant was absent. The presence of surfactant is, therefore, a big help for improving the efficiency of the biosensor.

The reason for the observed increase of fluorescence quantum yield (FQY) of PNAC* for complementary ssDNA, in the presence of the surfactant is that the surfactant breaks the dimers and trimers of the fluorescent dye into monomers [16–18]. In the case of non-hybridized, i.e., non-complementary DNA, since there is no negative charge in the solution, the surfactant molecules are able to surround the neutral PNAC*, thereby reducing its solubility in water and, hence, lowering the FQY of PNAC*. However, when PNAC* is complementary due to negative charge of the hybridized complex the surfactant molecules are not able to surround the PNAC* densely, increasing the FQY of PNAC*. The effect of surfactant is found to be very sensitive to its concentration. We have also determined the sensitivity of our biosensor for complementary and non-complementary assays, the results of which are also presented.



## 2. Materials and Methods

The blue-light-emitting polymer, poly[9,9-di(6,6′-N,N′-trimethylammonium) hexylfluorenyl-2,7-diyl)-alt-co-(1,4-phenylene)]dibromide salt (PFP) was purchased from American Dye Source. Sodium dodecyl sulphate (SDS) was purchased from Sigma-Aldrich. A 15-mer PNA probe corresponding to a segment of *E. coli* labeled with Fluorescein dye was purchased from Panagene. PNA is known to display hydrophobic interactions leading to aggregation at high concentrations, suggesting that the experiments must be carried out at very low concentrations ($<10$ μM).

A 15-mer strand labeled with Fluorescein dye (PNAC*) corresponding to the following sequence of *E. coli* was used (direction from N-terminus to C-terminus):

PNA 15-mer: Flu-TCAATGAGCAAAGGT.

The oligonucleotide sequences used in the experiment (listed in Table 1) were procured from Sigma-Aldrich, USA. Deionized water (DI) used for preparing the solutions was obtained from a TKA water filtration system.

The solution of polymer was prepared in ethanol and DI water in the ratio of 1:1 (v/v). The PNAC* was dissolved in a solution of pH 5.5 consisting of HCL and DI

while solutions of DNA primers were prepared in 1 × TE buffer. Absorption spectra of PFP and PNA probe were observed using Shimadzu UV Visible Spectrophotometer model UV-2450. Fluorescence studies of PFP and PNAC* were carried out using Shimadzu Spectrofluorophotometer model RF-5301-PC equipped with a xenon lamp excitation source in the range 350 nm to 700 nm. The PNA probe for *E. coli* was annealed at approx. 55–60°C, both with its complementary ssDNA sequence and with the non-complementary ssDNA sequence separately in equimolar concentrations for about 30–40 min, in order to improve hybridization between the ssDNA and PNAC*. The volume of ssDNA and PNAC* used in the present experiment is 150 µl, which is equivalent to a weight of approx. 25 ng each. The solution was allowed to cool down slowly to room temperature and, subsequently, PFP was added in small steps of 20 µl to the above solution, i.e., PNAC*/ssDNA complex. To mix the components well, the PFP/PNAC*/ssDNA samples were placed in a vortex mixer after each addition of the polymer before taking the fluorescence measurement. To observe the change in emission intensity of PFP and PNAC*, the PL measurements of the samples were recorded (at an excitation wavelength 376 nm) after each successive addition of PFP.

In the second stage of optimization (reduction of the unwanted leakage signal) due to non-complementary ssDNA, two different co-solvents (ethanol and SDS) were used. To see the effect of the co-solvent, initially 2 µl ethanol was added to the solutions (for both complementary and non-complementary DNA sequences). The solutions were placed in the vortex mixer to mix the components well. The PL measurements were taken again to see the effect of the ethanol. Similarly, 2 µl anionic surfactant (SDS) was added to the solutions (for both complementary and non-complementary DNA sequences). The solutions were placed in the vortex mixer to mix the components well. The PL measurements were taken again to see the effect of the surfactant.

## 3. Results and Discussion

The spectral response of PFP in different solvents (DI water, DI water/ethanol (1:1, v/v), ethanol and propanol) was studied at different concentrations (2.3 µM and 230 nM) to obtain maximum overlap between the PL of the PFP and the absorption of the PNAC* in order to obtain a efficient FRET. For PNAC*, a solution of pH 5.5 consisting of HCL and DI water was used at 25 nM concentration. It was found [14] that there is a large spectral overlap between the PL spectra of the PFP and the absorption spectra of the PNAC*, indicating that efficient FRET between the polymer and the dye can be obtained for DI water/ethanol in the ratio 1:1 (v/v) as the solvent for PFP at 230 nM concentration.

In the next step, PL studies were performed for the composite solutions (CCP/ssDNA/PNA) with and without surfactant for complementary and non-complementary DNA. The FRET spectra of the composite solution (CCP/ssDNA/PNA) for complementary DNA with and without the anionic surfactant excited at 376 nm are

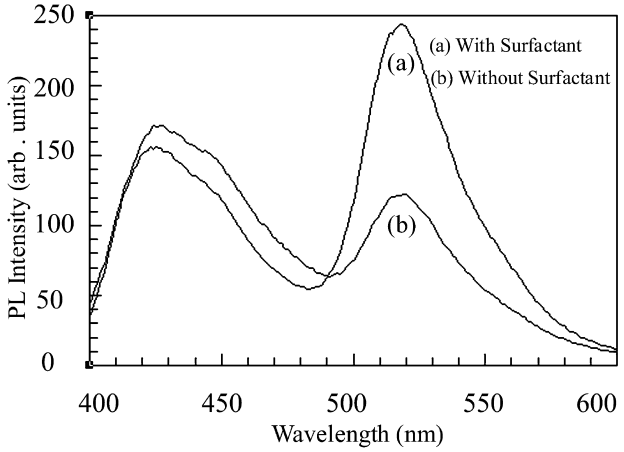


**Figure 1.** FRET spectrum of the composite solution (230 nM PFP/25 nM PNAC*/25 nM ssDNA) (a) with anionic surfactant (1 mM) and (b) without anionic surfactant for complementary DNA (25 nM).

shown in Fig. 1. It was observed that in the presence of the surfactant the PL intensity for the complementary ssDNA was significantly enhanced (about two times) as compared to the PL intensity obtained in the absence of the surfactant.

We have determined the sensitivity of the biosensor for complementary and non-complementary DNA and the effect of the anionic surfactant on the sensitivity of the biosensor has been studied with and without surfactant. The results are shown in Figs 2 and 3. In order to determine the sensitivity of the biosensor, we first determined the noise level of the device. For this purpose, the PL spectrum of the solution containing PFP/PNAC* was determined in absence of DNA (with surfactant). For determining the sensitivity we added the DNA to the solution and kept on decreasing the concentration of the DNA until the signal to noise ratio reached 4. The detection limit, thus, determined for our biosensor is 2.5 nM. The results are shown in Fig. 2. We also determined the detection limit of the biosensor without surfactant and the detection limit, thus, determined was 25 nM. Hence, we can say that the anionic surfactant improves the detection sensitivity 10-fold. Similar studies were performed for a non-complementary sequence and the results are shown in Fig. 3. Curve (c) of Fig. 3 represents the intensity of PL emission as a function of wavelength in presence of PFP/PNAC* and surfactant in the absence of target DNA. This curve represents the noise level of the biosensor. At a wavelength of 520 nm, which corresponds to PL emission from PNAC*, the PL intensity is 6 (arbitrary units, au). On adding 25 nM non-complementary DNA (without surfactant) the intensity of the acceptor for PNAC* increased to 30 au, which is well above the noise level and the signal to noise ratio is 5. On the other hand, in the presence of the surfactant the PL intensity increased only to 12 au, which is quite close to the noise level. Therefore, presence of surfactant helps in increasing the efficiency of the biosensor in case of non-complementary DNA.

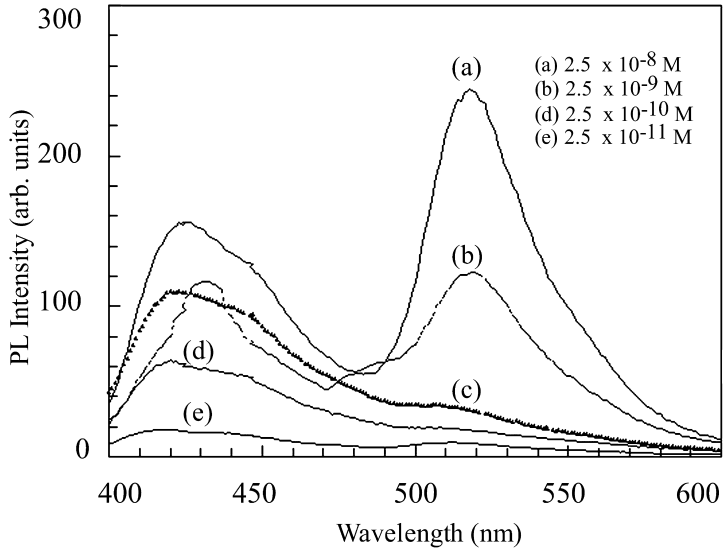


**Figure 2.** FRET spectrum of the composite solution (230 nM PFP/25 nM PNAC*/25 nM ssDNA) for different molar concentrations (curves a, b, d and e) of complementary DNA (with 1 mM surfactant). Curve (c) depicts emission from the composite solution in the absence of the target DNA with surfactant (1 mM).

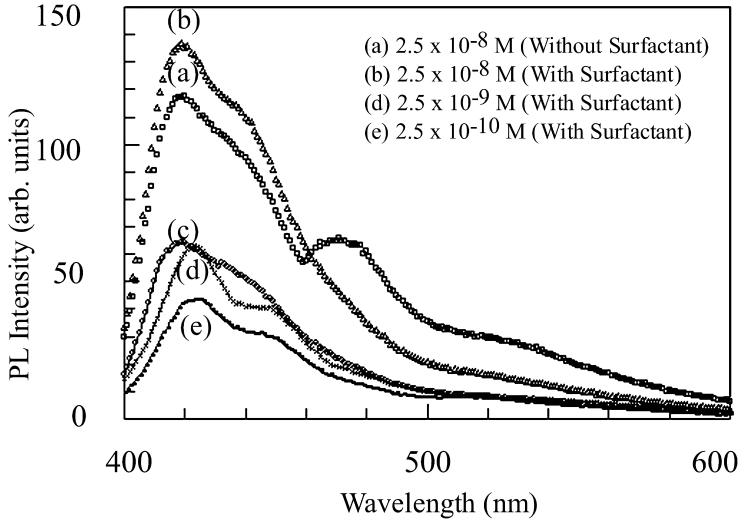


**Figure 3.** FRET spectrum of the composite solution (230 nM PFP/25 nM PNAC*/25 nM ssDNA) for different molar concentrations (curves a, b, d and e) for non-complementary DNA (with 1 mM surfactant). Curve (c) depicts emission from the composite solution in the absence of the target DNA with surfactant (1 mM).

The sensitivity of the biosensor is enhanced by about ten times in the presence of the surfactant as discussed above (Figs 2 and 3). The results indicate that the use

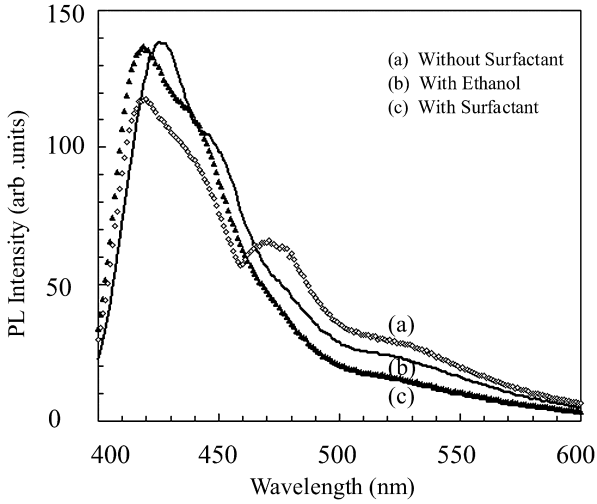


**Figure 4.** FRET spectrum of the composite solution (230 nM PFP/25 nM PNAC*/25 nM ssDNA) (a) without anionic surfactant, (b) with anionic surfactant (1 mM) and (c) with ethanol for non-complementary DNA (25 nM).



of anionic surfactant provides a strategy to improve the detection sensitivity and selectivity significantly for the biosensor. Al Attar *et al*. [18] reported an increase in PL intensity of only 5% when a non-ionic surfactant was used, whereas in our case the increase is 100% by using the anionic surfactant SDS.

To further improve the efficiency of the sensor, reduction of the leakage signal (due to non-complementary DNA) was done by adding ethanol and SDS to the composite solution. It was observed that with ethanol the unwanted signal due to non-complementary DNA signal was reduced partially. However, when the surfactant was used the leakage signal reduced almost completely as shown in Fig. 4. In the case of non-complementary DNA, since there is no negative charge in the solution, the surfactant molecules are able to surround the neutral PNAC*, thereby reducing its solubility in water and, hence, lowering the FQY of PNAC*. The FRET efficiency as a function of surfactant concentration was also studied for three different molar concentrations of the surfactant; 10 μM, 100 μM and 1 mM. It was found that the FRET intensity is maximum for the 1 mM concentration, as shown in Fig. 5. We have repeated the experiment 4 times to get the consistent results within 5%. We have also performed the ageing studies for our samples. For this purpose, solutions were prepared at the molar concentration of 25 nM, containing the anionic surfactant at a concentration of 1 mM. Observations were taken at an interval of 20 h. Between observations the samples were stored at −20°C. Before taking the observations the solutions were placed in a vortex mixer to mix the components well. Ageing studies (Fig. 6) reveal that the sensor is more efficient for freshly made solutions and samples only. A major reduction in the FRET signal (PL intensity from the acceptor) was observed in case of older samples.

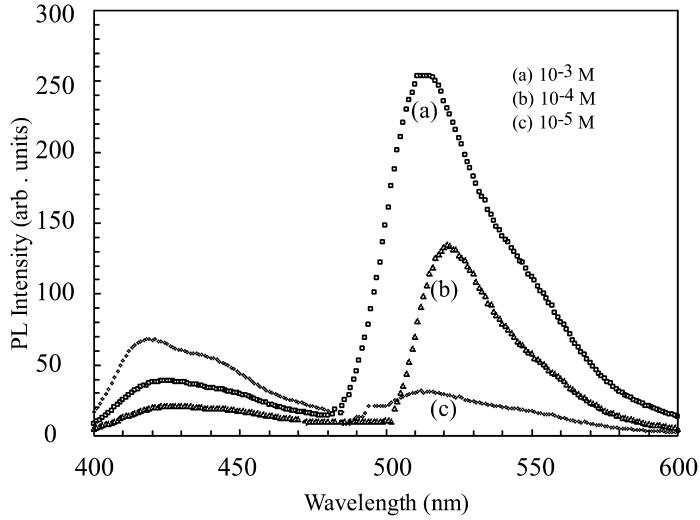


**Figure 5.** FRET intensity as a function of surfactant concentration for complementary DNA (25 nM).

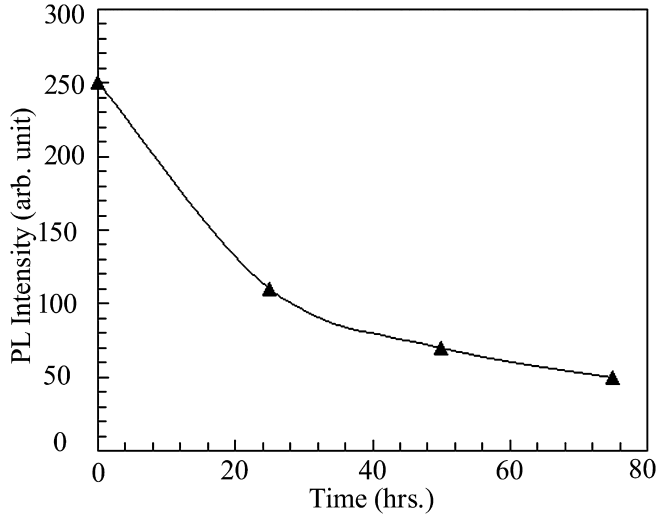


**Figure 6.** Ageing effect in the sensor for the composite solution (230 nM PFP/25 nM PNAC*/25 nM ssDNA) with surfactant (1 mM).

**Table 1.**
Oligonucleotides used in the study

| Primer name | Primer sequence (5′ to 3′) |
|---|---|
| Complementary DNA | ACCTTTGCTCATTGA |
| Non-complementary DNA | GTTGGGTGGATAGTG |
| PNA probe | TCAATGAGCAAAGGT |

## 4. Conclusions

In summary, we have demonstrated that an anionic surfactant can be used in combination with a cationic conjugated polymer and peptide nucleic acid probe to develop a sensor for DNA sequence detection with enhanced detection efficiency. By using the anionic surfactant, the PL intensity of the PNAC* for complementary ssDNA is enhanced significantly and that for the non-complementary ssDNA is significantly reduced. The detection sensitivity is also improved by about 10-fold using this assay. Thus, it can be concluded that this method improves both the sensitivity and selectivity of our sensor. Work with some other anionic surfactants such as sodium cholate and sodium laureth sulphate (SLES) is under progress and we are also studying the mechanism of ageing in samples and effect of ageing on the sensitivity of the senor.

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