Journal of Macromolecular Science, Part A

Photo-Physical Properties of a New Norfloxacin-Containing Polymer for Protons and Fe³⁺ Sensing

Yulin Hu[a]; Baoyan Wang[a]; Zhixing Su[a]

[a] Institute of Polymer and Engineering, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, People's Republic of China

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis
Taylor & Francis Group

Photo-Physical Properties of a New Norfloxacin-Containing Polymer for Protons and Fe³⁺ Sensing

YULIN HU, BAOYAN WANG and ZHIXING SU*

Institute of Polymer and Engineering, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, People's Republic of China

A novel norlfoxacin-containing fluorescent polymer was synthesized via copolymerization of two derivatives of norfloxacin and methylmetacrylate (MMA). It could emit blue fluorescence in both the solution and film states. Fluorescence characteristics of the polymer as a function of pH were investigated in aqueous solution. The polymer solution showed weaker fluorescence between pH 5-9. When the pH of the solution was higher than 9 or lower than 5, stronger fluorescence could be seen. Responses of metal cations (Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}) to the fluorescence intensity of this polymer were obtained that only Fe^{3+} could quench efficiently the fluorescence intensity of the polymer in solution and film state. The results suggested the possibility that this newly synthesized compound might work as a polymeric sensor responding to water polluted by protons and Fe^{3+}.

Keywords: photo-physical properties, fluorescence intensity, intramolecular charge transfer (ICT), protons, Fe^{3+}

1 Introduction

In recent years, the environmental pollution has been a major concern of the present industrial societies. The design of highly selective and sensitive sensor materials has become a very important scientific goal. With this regard, fluorescence-based sensors have been of particular interest because of their high sensitivity, versatility and fairly good selectivity. Most of the research in this scope was focused on polymers with pendant chromophore or polymers with chromophore in their backbone to prevent the pervasion of fluorescent compounds in use. T.S. Lee et al. (1) have synthesized an acridine-containing polymer with properties of an optical sensor for Fe^{3+}. Grabchev et al. (2–5) had evaluated a series of naphthalimide labeled polymeric materials to detect protons and metal cations by monitoring the change of the fluorescence intensity.

Norfloxacin (NF) [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)quinoline-3-carboxylic acid] is a synthetic, broad-spectrum fluoroquinolone antibacterial agent for oral administration, which has *in vitro* activity against Gram-positive and Gram-negative aerobic bacteria. With its intrinsic fluorescence, norfloxacin (NF) has been widely studied on its photophysical and photochemical properties because of the intramolecular charge transfer (ICT) process taking place from the nitrogen on piperazinyl ring to the aromatic fluoroqulinone system (6–10). Bernard Valeur (11) considered that when a cation receptor was linked to an intramolecular charge transfer fluorophore so that the bound cation can interact with either the donor group or the acceptor group, the ICT was perturbed; the consequent changes in photophysical properties of the fluorophore can be used for sensing cations. We report here, for the first time, the usage of the poly(methyl methacrylate) grafted by two kinds of norfloxacin derivatives for spectrofluorimetric determination of protons and Fe^{3+} cations. In the text, we described the influence of protons and metal ions on the photophysical properties of the compounds and explained the mechanism of the action in detail.

The chemical structures of NF derivatives were shown as Scheme 1.

In the compound ACNF (named as 1-ethyl-6-fluoro-7-[(4-acryloyl) piperazin-1-yl]-4-oxo-1,4-dihyroquinolin-3-carboxylic acid) was synthesized as described in our previous work (12) and compound GMANF (named as 1-ethyl-6-fluoro-7-{4-[2-hydroxy-3-(2-methylacryloyloxy) propyl] piperazin-1-yl}-4-oxo-1,4-dihyroquinolin-3-carboxylic acid) was synthesized as described in the lierature (13).

---

*Address correspondence to: Zhixing Su, Institute of Polymer and Engineering, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, People's Republic of China. Tel: +86-0931-8912391; Fax: +86-0931-8912582; E-mail address: suzx@lzu.edu.cn
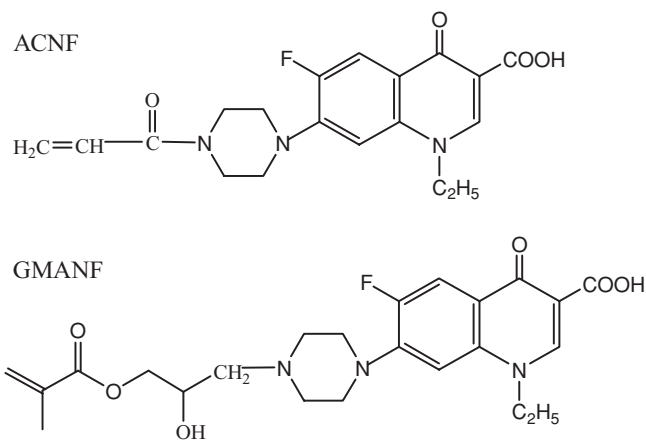
ACNF

GMANF

Sch. 1. Structures of compound ACNF and GMANF.

2 Experimental

2.1 Materials

Norfloxacin (NF), analytical grade, was purchased from Shenda Industry & Trade Company, Guangxi, China. The monomer MMA was distilled from copper powder under reduced pressure before use. Acrylic acid and benzoyl chloride, which were used to prepare acryloyl chloride, were of analytical grade. Triethylamine was refluxed with acetic anhydride and then distilled, refluxed with KOH, and again distilled. Glycidyl methacrylate (GMA) was of reagent grade. Azo-bis-isobutylonitrile (AIBN) was recrystallized from ethanol. Reagents such as N,N-dimethylformamide (DMF) and methanol were of guaranteed reagent grade and used without further purification. The water used in this work was double distilled.

ACNF was synthesized by the reaction of NF with acryloyl chloride in the presence of triethylamine as describe in detail in our previous work (12). GMANF was synthesized by the reaction of NF with GMA in DMF at 40°C for 24 h as described in the literature (13).

The copolymer named poly(MMA-co-ACNF-GMANF) was prepared by the copolymerization of ACNF, GMANF and MMA with AIBN as an initiator. A solution of 0.075 g (2.0×10^{-4} mol) ACNF, 0.092 g (2.0×10^{-4} mol) GMANF, 2.0 g (2.0×10^{-2} mol) MMA, and 0.032 g (2×10^{-4} mol) AIBN in 5ml DMF was introduced into a dry polymerization tube. The solution was deoxygenated by purging with N$_2$ gas for 5 min. The tube was sealed and placed in a regulated thermostat bath at 70°C for 24 h. The solution was allowed to precipitate in excessive methanol. The modified copolymer was dissolved in DMF and precipitated three times with excessive methanol in order to remove the unreacted monomers and low-molecular weight oligomers. FT-IR (KBr, ṽ/cm^{-1}): 2997 (νCH$_3$), 2952 (νCH$_2$), 2844, 1731 (ν C=O), 1677 (ν O=CN-), 1626 (ν COO$^-$), 1482 (δCH$_2$),

1450 (δ_{as} CH$_3$), 1387 (δ_s CH$_3$). Mn = 22,000 gmol^{-1}, Mw = 30,500 gmol^{-1}; the polydispersity was 1.39.

2.2 Preparation of the Copolymer Films

The film were obtained by casting 0.08 ml of the polymer solution, with mass concentration of 1.0%, over glass plates that had the same dimension of 1.0 cm × 2.5 cm which could be inserted in the quartz cell, and kept at room temperature for 30 min to move out the solvent. The film samples were stored in a desiccator before fluorescent measurements.

2.3 Apparatus and Methods

FI-IR spectra were recorded on a Nicolet Neus 670 FI-IR spectrophotometer. The molecular weights of the copolymers were determined using a GPCaV Water 2000 apparatus. Fluorescent excitation and emission spectra were measured with an LS 55 Luminescence Spectrometer (Perkin-Elmer). UV-visible spectra were recorded using a Lambda 35 UV/Vis Spectrometer (Perkin-Elmer). The pH of the solutions was adjusted with pHs-25 digital meter (made in Shanghai, China).

For fluorescence emission measurements, a 10 × 10 mm quartz cell was used for detection. Fluorescence intensities against pH were obtained by recording the emission in solutions with different pHs at a fixed wavelength of 325 nm. During the measurement, concentrated HCl and NaOH were used to adjust the pH to the desired value and samples were carried out at room temperature. The pH range of the sample was changed from 0.5 to 13.5 by a digital pH controller. The effect of the metal ions on fluorescence intensity was examined by adding a few microlitres of stock solution of the metal cations (by using a microlitre injector) to a known volume of the polymer solution (3 ml). The addition was limited to 0.10 ml so that the dilution of the polymer solution remained insignificant. The effect of the Fe^{3+} cations on fluorescence intensity of the polymer film was also investigated under injection conditions; the polymer film was placed into the sample quartz cell containing 2.0 ml distilled water. CuCl$_2$·2H$_2$O, NiCl$_2$·6H$_2$O, FeCl$_3$·6H$_2$O, CoCl$_2$·6H$_2$O, ZnCl$_2$ and MnSO$_4$·4H$_2$O were used as sources of metal cations.

3 Results and Discussion

3.1 Spectral Characteristics of the Dyes and the Polymer

Excitation and emission spectra of ACNF and GMANF were recorded in aqueous solution. As shown in Figure 1, compound ACNF and GMANF displayed the maxima excitation at 283 nm and 325 nm. Their emission spectra were strong, broad structureless bands with a large Stokes' shift and with the maximum at about 435 nm. It indicated that substitution in the amino group produced no significant influence on the fluorescence spectra.
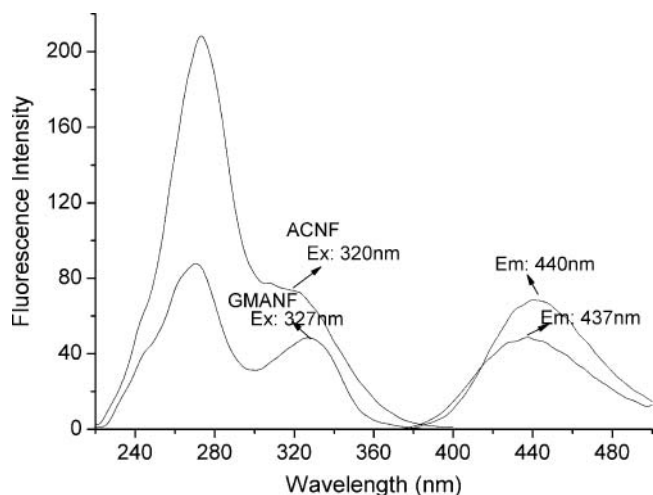
Fig. 1. Excitation and emission spectra of ACNF (pH 11.5) and GMANF (pH 3.0) in aqueous solution.

Fig. 2. Excitation and emission spectra of the fluorescent polymer in DMF/water solution and in film state.

The ability of the compound to emit the absorption energy was characterized quantitatively by the quantum fluorescence yield (Φ_F). The quantum fluorescence yield of the compound in the pH with the strongest fluorescence intensity was calculated using a 0.1 N NaOH solution of fluorescein ($\Phi_{st} = 0.86$) as a standard according to Equation 1, where the Φ_F is the emission quantum yield of the sample, Φ_{st} is the emission quantum yield of the standard, A_{st} and A_u represent the absorbance of the standard and sample at the excited wavelength, respectively, while S_{st} and S_u are the integrated emission band areas of the standard and sample, respectively, and n_{Dst} and n_{Du} is the solvent refractive index of the standard and sample, subscripts u and st refer to the unknown and standard, respectively.

$$\Phi_F = \Phi_{st} \frac{S_u A_{st} n_{Du}^2}{S_{st} A_u n_{Dst}^2} \tag{1}$$

Compound ACNF had quantum yield values $\Phi_F = 0.12$ at pH 11.5 and compound GMANF had quantum yield values $\Phi_F = 0.21$ at pH 3.0.

Figure 2 showed the excitation and emission spectra of the compound poly(MMA-co-ACNF-GMANF) in a DMF/water(1:4) solution and in film state. They had the emission wavelength at about 435 nm as same as the dyes.

3.2 Influence of Protons on the Fluorescence Intensity of the Two Fluorescent Compounds and the Polymer

The influence of protons on the photophysical properties of the fluorescent compound ACNF and GMANF in aqueous solution were investigated in the pH range from 0.5 to 13.5. The fluorescence intensity of them against pH was plotted in Figure 3. ACNF had stronger fluorescence intensity in a more basic medium. When the pH increased from 1.5 to 11.0, the fluorescence intensity of ACNF increased steadily, then gradually decreased from pH 11.0
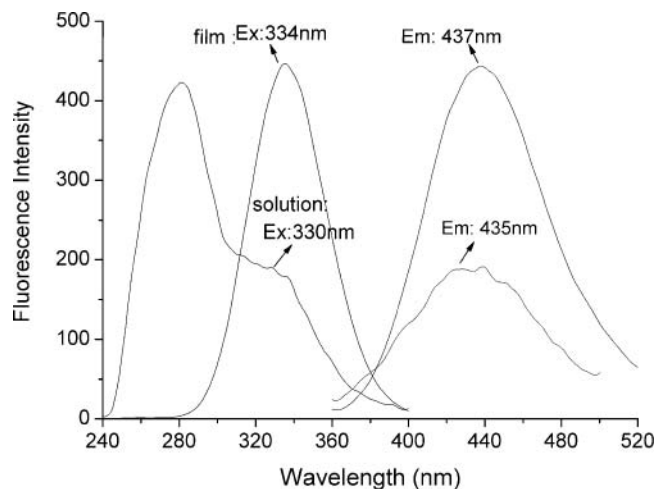
to 13.0, leading to a peak of fluorescence intensity at pH 11.0. However, GMANF had stronger fluorescence intensity in more acidic medium and the strongest fluorescence intensity was at pH 3.0.

Having the same chromophore moiety, the characteristic excitation and emission properties of AcNF and GMANF had few differences. We will give an explanation of why they behave as they do. It suggested that it was the different substitute not the main chromophore unit determined the pH dependence. ACNF had a carbonyl group at position N(4') and GMANF had an equivalent alkyl substitute at N(4'). It would be explained by the mechanism described in Schemes 2 and 3, respectively. The ICT taking place from the N(1') of the piperazinyl ring to the fluoroquinolone system was an efficient energy-wasting pathway which could deactivate the excited-state of the molecule and quench the fluorescence intensity of the compound. As shown in
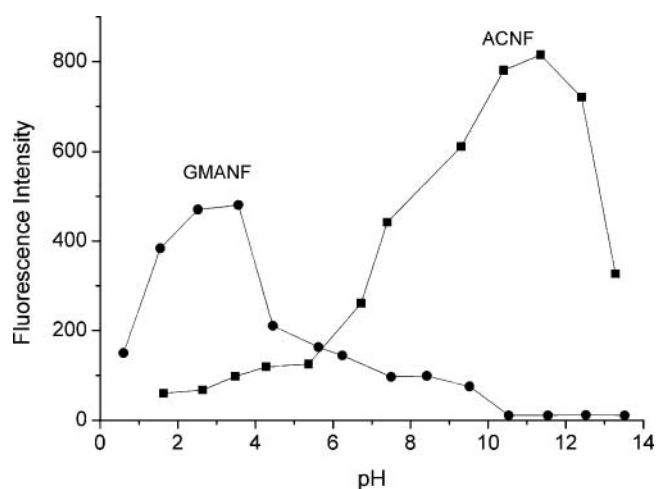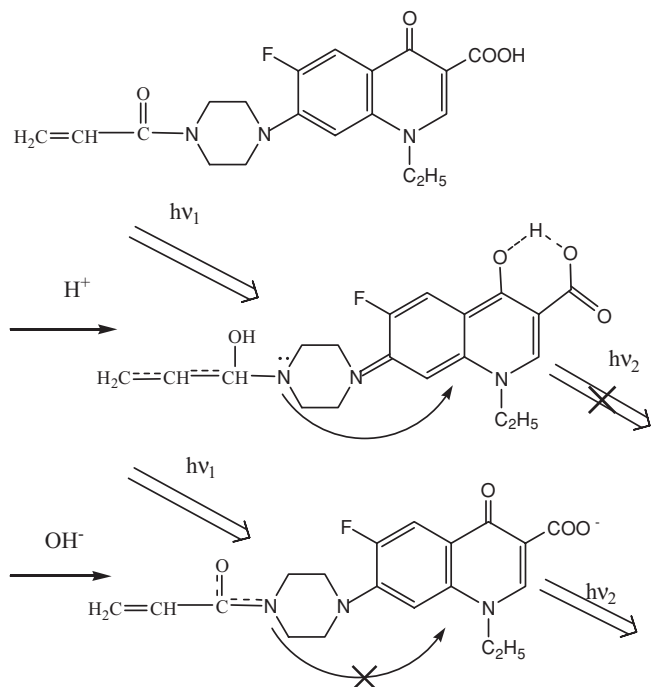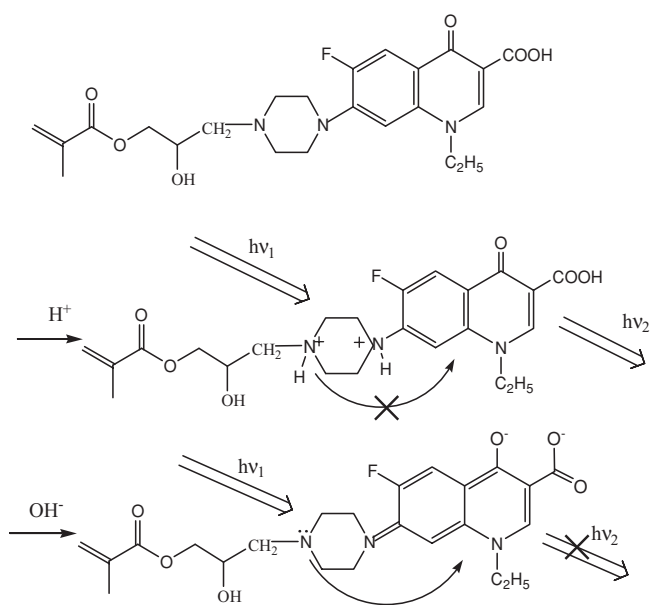

Fig. 3. Effect of pH on fluorescence intensity of the compound ACNF and GMANF in aqueous solution.

Sch. 2. Fluorescence mechanism of compound ACNF in different pH medium.

Scheme 2, for compound ACNF in acidic medium, the protonation of the carbonyl conjoined with $N(4')$ decreased its conjugation with the lone pair of $N(4')$ and increased the occurrence possibility of ICT from the lone pair of N to the fluoroquinolone system. This deactivation pathway was not possible for the ACNF form in basic medium, whose $N(4')$ lone pair was blocked as a result of conjugation with

Sch. 3. Fluorescence mechanism of compound GMANF in different pH medium.
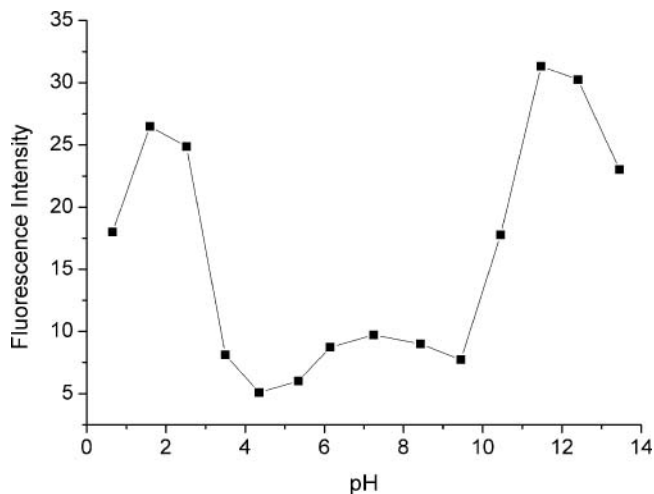

Fig. 4. Effect of pH on fluorescence intensity of the DMF/water solution of the designed polymer.

carbonyl group. So it showed stronger fluorescence in basic medium. The reverse phenomenon ocurred on compound GMANF as shown in Scheme 3. The protonation of the $N(4')$ and $N(1')$ in acidic medium decreased the possibility of ICT process which would take place with no hindrance in basic medium. As a result, compound GMANF showed stronger fluorescence in acidic medium, while there was nearly no fluorescence when the pH was higher than 10.0.

This finding discredited the earlier conclusions by P.Bilski et al. (14) who considered that it was the main chromophore, not the different substitute that determined the pH dependence.

Grafting the two molecules in the polymer chain, the synthesis polymer have stronger fluorescence intensity both in acidic and basic medium compared to the neutral aqueous solution as shown in Figure 4.

3.3 Influence of Metal Cations on the Fluorescence Intensity of the Two Fluorescent Compounds and the Polymer

When mixing the metal cations to the fluorophore solutions, the most likely binding sites were indicated to be the 3-carboxy moiety and the piperazine ring. Two hypotheses for the effect of metal ions on the fluorophore were possible:

1. If metal ions bind the dyes mainly at the 3-carboxy group, intramolecular charge transfer (ICT) from the N(1') to the aromatic system will be accelerated. This energy-wasting pathway may quench the fluorescence of the fluorophore.
2. If the main binding site was the the piperazine ring, the lone pairs of N will be blocked. ICT from N(1') to the main rings would not take place efficiently. Thus, the energy will be emitted as fluorescence and the fluorescence of the compounds will be enhanced with the metal ions' presence.

Table 1. I/I_0 values of the two fluorescent molecules and polymer solutions in the presence of different metal cations of the same concentration 6.67×10^{-4} M.

I/I_0 values of products	No ions	Mn^{2+}	Fe^{3+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
ACNF	1	0.28	0.00	0.15	0.16	0.09	0.96
GMANF	1	0.61	0.07	0.21	0.11	0.08	1.43
Polymer	1	0.87	0.14	0.83	0.81	0.73	1.03

The response of compound ACNF, GMANF and the fluorescent polymer to some transition metal cations(Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}) have been tested in aqueous solution by monitoring the change in their fluorescence spectra in the presence of various cations. The relative fluorescence intensity (I/I_0) were used to measure the effect of the metal cations on the fluorescence intensity of compounds. I_0 and I were the fluorescence intensity of the compound free and have metal cations, respectively. As shown in Table 1, nearly all cations exhibited quenching effects on the fluorescence intensity of the compounds. The experimental results were in accordance with the mechanism of hypothesis 1.

Orbital–orbital interaction may exist between 3d orbital of transition metal ions and the ligands (10). The orbital–orbital interaction will be negligible for Mn^{2+} and Zn^{2+} because the third orbital of Mn^{2+} is half filled and that of Zn^{2+} is completely filled. The coordination capacity of Mn^{2+} and Zn^{2+} are relatively small compared with those of $Co^{2+}(3d^7)$, $Ni^{2+}(3d^8)$, and $Cu^{2+}(3d^9)$. Although the third orbital of Fe^{3+} is half filled, the same as Mn^{2+}, exhibiting powerful oxidizing properties, Fe^{3+} showed strong fluorescent quenching on the fluorophores. That is why only the polymer solution exhibited good selectivity upon exposure to Fe^{3+}. The polmer chain curled in the poor solvent. Only Fe^{3+} ions exhibited remarkable fluorescence intensity quenching to the polymer, while the influence of other cations were less pronounced.

The stoichiometry and binding constant of ACNF and GMANF with the metal ion Fe^{3+} were established by use of the Benesi–Hildebrand method. Assuming that the metal ions form 1:1 inclusion complexes with compound ?, Equation 2 is applicable:

$$\frac{1}{F - F_0} = \frac{1}{(F_\infty - F_0)K[M]} + \frac{1}{F_\infty - F_0} \qquad (2)$$

In this approach, a graph of $1/(F - F_0)$ vs. $1/[M]$, was made where F is the observed fluorescence at each concentration tested, F_0 is the fluorescence intensity of analyte in the absence of metal ion, K is the binding constant of the complex and the metal ions, and $[M]$ is the concentration of metal ion. A linear plot is required for this double reciprocal plot in order to conclude 1:1 stoichiometry. If the stoichiometry is 1:1, a linear relationship will be obtained when $1/(F - F_0)$ vs. $1/[M]$ is plotted (15). As shown in Figure 5 A and B, linear relationships were gained for both ACNF and GMANF. This observation suggested that the stoichiometries of the complexes between the fluorophores and the metal ions were 1:1. The association constant K was determined as $1.10 \times 10^7 M^{-1}$ for complex ACNF:Fe and $1.49 \times 10^6 M^{-1}$ for complex GMANF:Fe.

Figure 6 showed the linear relationship between I_0/I of the polymer solution and the concentration of Fe^{3+} in the range of $6.67 \times 10^{-7} - 4.84 \times 10^{-4}$ M. The linear calibration plot can be described by $I_0/I = 1.21 + 1.05 \times 10^4 \cdot [Fe^{3+}]$ and the correlation coefficient was 0.9922.

Thin films of the polymer with intense blue fluorescence were prepared on glass plates by solvent casting. The typical fluorescence spectra of the optical clear thin film has been shown in Figure 2. The time based response data of the polymeric sensor in film state for the response to Fe^{3+}
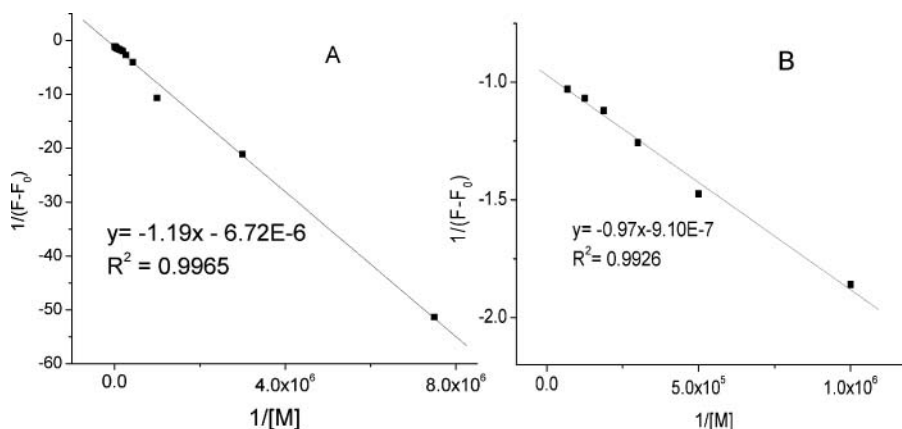
Fig. 5. Benesi–Hildebrand's plots for compounds ACNF: Fe(||||) (A) and GMANF: Fe(||||) (B) complexes.
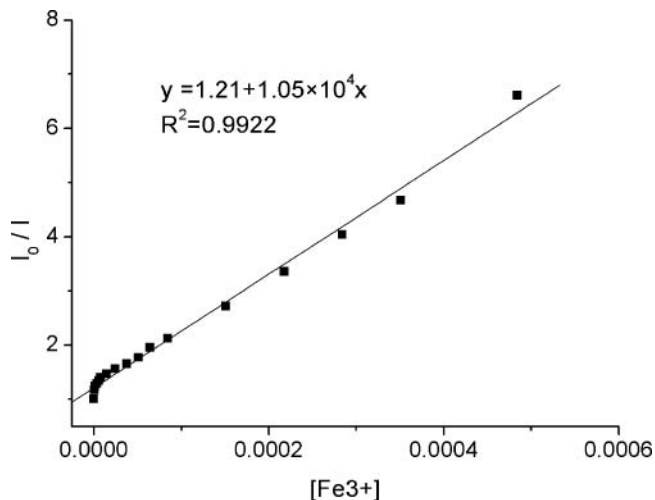
Fig. 6. Response of fluorescence intensity of the polymer solution to Fe^{3+} cations.

was acquired in the concentration range from 4.40×10^{-6} to 5.65×10^{-4} M (Fig. 7). The linear calibration plot can be described by $FI = 1.59 + 6716 \log[Fe^{3+}]$ and the correlation coefficient was 0.99. In addition, the sensor responded rapidly within the dynamic working range with the average response time 40s under injection condition. In view of the selectivity and rapid response ability of the polymer sensor, it would be suggested to use as a new polymeric sensor for water pollution by Fe^{3+} cations.
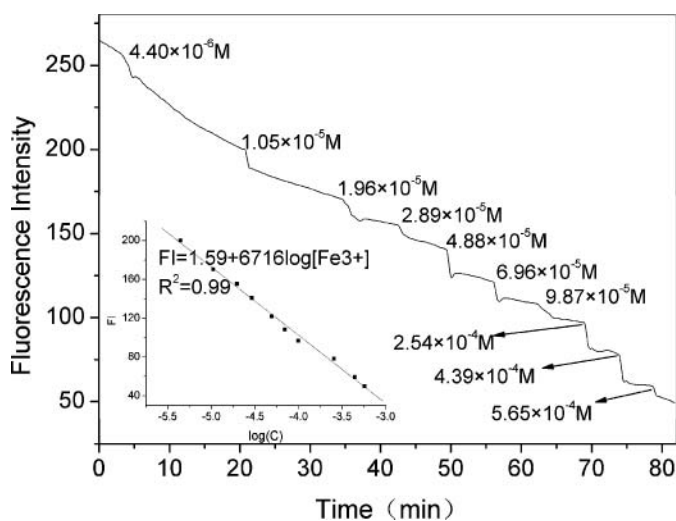

Fig. 7. Time dependent response of the polymer film after injection of certain concentration of Fe^{3+} cations. The inset was the relation of fluorescence intensity of the polymer film to the logarithm of the concentration of Fe^{3+} cations ($\log[Fe^{3+}]$).

4 Conclusions

A novel norfloxacin-containing polymer was synthesized based on two norfloxacin derivatives. The photo-physical properties of the fluorescent compounds and the synthesis polymer in different pH medium and in the presence of different metal cations have been investigated. Compound ACNF and GMANF showed different fluorescent respond to protons in the pH range of 0.5-13.5 because of the different substitution at N(4'). The fluorescent polymer had stronger fluorescence intensity both at pH 2.5 and 12.0, which expanded the application possibility of the polymer compared to the fluorescent compounds. In addition, ACNF, GMANF and the polymer showed fluorescence intensity quenching in different levels to metal cations addition (Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}). However, only the designed fluorescent polymer exhibited fluorescence quenching selectivity to Fe^{3+}. In the film state, the fluorescent polymer sensor exhibited rapid response ability to Fe^{3+}. The results suggested the possibility that this newly synthesized polymer works as a sensor responding to water polluted by protons and Fe^{3+} cations.

References

1. Lee, T.S., Yang, C. and Park, W.H. (2000) *Macromol. Rapid Commun.*, 21, 951.
2. Grabchev, I. and Chovelon, J.M. (2008) *Dyes Pigments*, 77(1), 1–6.
3. Grabchev, I., Sali, S., Betcheva, R. and Gregoriou, V. (2007) *Eur. Polym. J.*, 43, 4297–4305.
4. Grabchev, I. and Guittonneau, S. (2006) *J. Photochem. Photobiol. A Chem.*, 179, 28–34.
5. Grabchev, I., Bojinov, V. and Betcheva, R.S (2001) *J. Appl. Polym. Sci.*, 81, 2463–2470.
6. Navaratnam, S. (2000) *J. Claridge, Photochem. Photobiol.*, 72, 283-290.
7. Park, H.R., Lee, H.C., Kim, T.H., Lee, J.K., Yang, K. and Bark, K.M., (2000) *Photochem. Photobiol.*, 71, 281–293.
8. Park, H.R., Lee, H.C., Kim, T.H., Lee, J.K., Yang, K. and Bark, K.M. (2002) *Photochem. Photobiol.*, 75, 237–248.
9. Albini, A. and Monti, S. (2003) *Chem. Soc. Rev.*, 32, 238–250.
10. Cuquerella, M.C., Miranda, M.A. and Bosca, F., (2006) *J. Phys. Chem. A*, 110, 2607–2612.
11. Valeur, B. Molecular Fluorescence: Principles and Applications. Wiley: Weinheim, 2001.
12. Hu, Y.L., Wang, B.Y. and Su, Z.X. (2007) *International Journal of Polymer Anal. Charact.*, 12, 391–397.
13. Moon, W.S.; Kim, J.C., Chung, K.H., Park, E.S., Kim, M.N. and Yoon, J.S. (2003) *J. Appl. Poly. Sci.*, 90, 1797–1801.
14. Bilski, P., Martinez, L.J., Koker, E.B. and Chignell, C.F. (1996) *Photochem. Photobiol.*, 64(3), 496–500.
15. Rodriguez, M.I., Agbaria, R.A., Warner, I.M. (2005) *J. Fluoresc.*, 15(2), 185–190.