

Article

Incorporation of Rhodamine into a Host Polymer via In-Situ Generated Isocyanato Group and Application for the Detection of Cu²⁺ Ion

Sai Zhang, Zhe Yao, Wenqiang Qiao * and Zhi Yuan Wang *

State Key Laboratory of Fine Chemicals, Department of Polymer Science & Materials, School of Chemical Engineering, Dalian University of Technology, Dalian 116024, China; zhangsai621@126.com (S.Z.); 13213064961@163.com (Z.Y.)

* Correspondence: wqqiao@dlut.edu.cn (W.Q.); wwjoy@dlut.edu.cn (Z.Y.W.)

Abstract: A rhodamine-based fluorescent polymer P(MMA-co-RB) has been synthesized via an intermediate NCO-containing polymer generated by the Lossen rearrangement reaction. The fluorescent property of P(MMA-co-RB) with regard to metal ions, such as Cu²⁺, Fe³⁺, Cr³⁺, Al³⁺, Zn²⁺, Co²⁺, Sn²⁺ and Ag⁺, was studied by fluorescence emission spectroscopy. The results demonstrate that the fluorescence intensity of P(MMA-co-RB) decreased gradually with an increase of the concentration of Cu²⁺ ion. Furthermore, a test strip made of P(MMA-co-RB) can be used for fast and quantitative determination of Cu²⁺ ion. In the presence of Cu²⁺ ion, the sensory tester undergoes distinct changes in fluorescence intensity and visible color.

Keywords: Lossen rearrangement; rhodamine B; fluorescent polymer; detection of copper ion



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1. Introduction

Copper ion is an indispensable tracing element and heavy metal ion in the human body and plays a vital role in all kinds of living organisms [1–3]. However, excessive levels of Cu²⁺ ion may cause irreparable damages to the ecosystem and human health, such as Wilson's disease and Alzheimer's disease [4,5]. Thus, highly sensitive and selective evaluation of the Cu²⁺ ion content from water or physiological samples is still compulsory in various biological systems and the environment. Up to now, as an important sensory technology, fluorescent sensors for sensitive Cu²⁺ ion detection have been developed based on various fluorophores, such as rhodamine, naphthalimide, tetraphenylethylene, coumarin, quinoline, benzimidazole, pyrazoline and Boron dipyrromethenes [6–13].

Rhodamine dyes have high absorption coefficient and high fluorescence quantum yields [14]. For their excellent photophysical and photochemical properties, they are extensively used as fluorescent probes to detect amino acid, metal ion, anion, hypochlorous acid in lysosomes, alkaline phosphatase and monitoring temperature and pH, etc. [15–27]. To date, all sorts of small molecular rhodamine-based fluorescent probes have been designed for the analysis and detection of metal ions. However, most small molecule probes have some inconvenience, such as they are hard to dissolve in water and make into testing devices [28–30]. In comparison, polymer probes have been demonstrated to be the promising alternatives for detecting Cu²⁺ ion [31–34].

Normally, rhodamine-based polymers can be synthesized via the polymerization and grafting reaction [35–38]. Geng et al. reported a rhodamine-based polymer by copolymerization of a rhodamine 6G derivative containing double bond and comonomer acrylamide (AM), which is identified as a potential prober for Cu²⁺ or Hg²⁺ ions in an aqueous environment [39]. Dhara et al. designed a new block polymer containing pendant rhodamine fluorophores as a Cu²⁺ ion fluorescence sensor in an aqueous solution [40]. The polymer chemosensor was prepared by reversible addition-fragmentation chain transfer (RAFT)

polymerization under harsh conditions, followed by grafting of rhodamine units onto the polymer side chain. In general, all the reported synthetic methods rely on the multi-step synthesis of rhodamine-based monomers for copolymerization or availability of host polymers having a reactive group for chemical grafting with rhodamine dyes. Considering an overall efficiency in making a rhodamine-containing polymer, chemical grafting is a synthetic method choice, given that the host polymer is readily available, its reactive functional group is present and the grafting reaction is simple and highly efficient (e.g., quantitative yield). Therefore, it is highly desirable to find a readily available host polymer for efficient grafting of fluorophores and develop a sensory polymer for detection of Cu^{2+} ion in environmental and biological systems [41–45].

The reactive isocyanate readily reacts with alcohol and amine, so it is commonly used in grafting reactions to prepare functional polymer [46]. In general, phosgenation of primary amines is one of the most important industrially applied methods for the preparation of isocyanates. However, the main drawbacks of this method are the use of highly toxic phosgene and the generation of a large quantity of corrosive hydrochloric acid during the reaction. Therefore, it is necessary to explore milder and more sustainable synthetic approaches for preparing isocyanates. The Lossen rearrangement is deemed to be a significant phosgene-free method to prepare isocyanates, which allows the transformation of hydroxamic acids into the NCO group [47]. However, there is a great deal of literature on the preparation of NCO-containing polymer by using the Lossen rearrangement. The previously reported *N*-acetoxamide (AA) functionalized polymers can be used as such a host for grafting a fluorophore. It has been shown that the AA group can be easily transformed into the reactive NCO group via the Lossen rearrangement reaction [48]. Thus, in this work, we synthesized a rhodamine-based P(MMA-co-RB) polymer for the fluorescence-quenching detection of Cu^{2+} ion. The sensory polymer was synthesized by one-step graft reaction of in-situ generated NCO group in the host polymer with a functionalized rhodamine B (RB-OH). The sensing behavior of this polymer in solution and in a solid state was studied.

2. Materials and Methods

2.1. Materials

Methyl methacrylate (MMA) (purity $\geq 99\%$) (Aladdin, Shanghai, China) was used without further purification. Acetic anhydride (purity $\geq 98\%$) was used from commercially available 4-(dimethylamino)pyridine (DMAP) (purity $\geq 98\%$), *N,N'*-dicyclohexylcarbodiimide (DCC) (purity $\geq 99\%$), Rhodamine B (RB) (purity $\geq 99\%$), dibutyltin dilaurate (DBTDL) (purity $\geq 98\%$), 2,2-azobis(2-methylpropionitrile) (purity $\geq 98\%$), hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$) (purity $\geq 98\%$) and G254 thin-layer chromatography silica gel aluminum plate, purchased from Energy Chemical (Shanghai, China) and used as received without further purification. Polyethylene-polypropylene glycol (F127) (purity, 97%) was used as surface active agent. Ethylene glycol (purity $\geq 99.5\%$), dichloromethane (DCM) (purity $\geq 99.5\%$), sodium hydroxide (anhydrous, 99%), sodium chloride (anhydrous, 99%), diluted hydrochloric acid (purity, 37%) and anhydrous magnesium sulphate (MgSO_4) (anhydrous, 99%) were purchased from Damao Chemical (Tianjin, China) and used as received without further purification. *N,N*-Dimethylformamide (DMF) (purity $\geq 99.5\%$) (Damao Chemical, Tianjin, China), tetrahydrofuran (THF) (purity $\geq 99.5\%$) (Kemiou Chemical, Tianjin, China) and xylene (purity $\geq 99\%$) (Kemiou Chemical, Tianjin, China) were purified by an MBRAUN solvent purifier system (SPS-5). All the other reagents were of analytical grade and used as received without purification. CuBr_2 (purity, 98%), FeCl_3 (anhydrous, 95%), AgNO_3 (purity, 99.8%), $\text{CrCl}_3\cdot 6\text{H}_2\text{O}$ (purity, 98%), $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ (purity, 98%), ZnCl_2 (purity, 98%), AlCl_3 (purity, 98%) and $\text{SnCl}_2\cdot 2\text{H}_2\text{O}$ (purity, 98%) were purchased from Energy Chemical (Shanghai, China) and used as sources for metal cations. Deionized water with a resistivity of $15\ \text{M}\Omega\cdot\text{cm}$ was used from a Milli-Q Aquelix 5 water purification system (Millipore Corp., Billerica, MA, USA).

2.2. Characterization

^1H nuclear magnetic resonance (NMR) spectra of compounds were investigated on a Bruker 400 MHz instrument (Bruker AV400, Bruker, Karlsruhe, Germany) using deuterated chloroform (CDCl_3) as the solvent and tetramethylsilane as the internal standard. Fourier transform infrared spectra (FTIR) were measured by a Shimadzu IRAffinity-1S spectrometer (Shimadzu Corp., Kyoto, Japan) in the range of 400–4000 cm^{-1} using a potassium bromide pellet. The mass spectra were performed on an Agilent 2160–6130 liquid chromatograph-mass spectrometer (Agilent Technologies Inc., Foster, CA, USA). Gel permeation chromatography (GPC) were recorded on a Shimadzu GPC system (Shimadzu Corp., Kyoto, Japan) with an SPD-20A UV-vis detector using polystyrene as standards and THF as eluent at a flow rate of 1.0 $\text{mL}/\text{min}^{-1}$ at 40 $^\circ\text{C}$. The UV-vis spectra were recorded on a Shimadzu UV-3600 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The fluorescence spectra of P(MMA-co-RB)-metal complex in DMF solution and in G254 silica gel aluminum plate was measured at room temperature by HORIBA Scientific PTI QuantaMaster 8000 fluorescence spectra photometer (HORIBA Corp., Kyoto, Japan).

2.3. Synthesis of Rhodamine B Ethanol Ester (RB-OH)

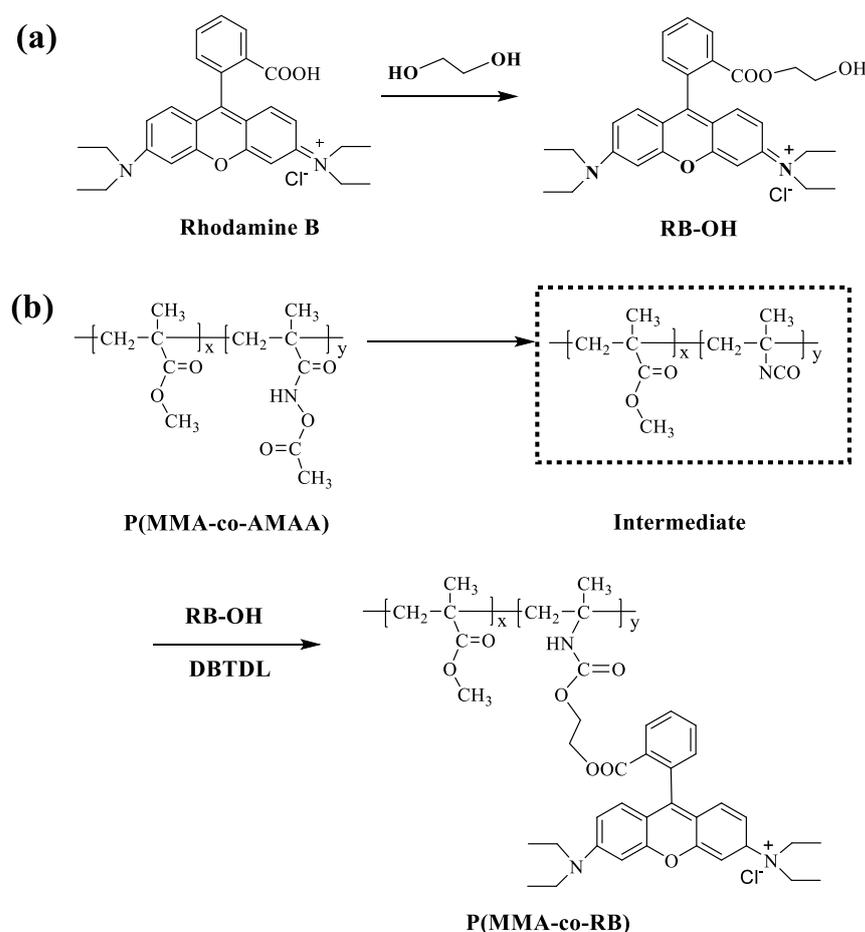
Ethylene glycol (0.62 g, 10.0 mmol), DMAP (0.03 g, 2.5 mmol), DCC (0.52 g, 2.5 mmol) and DCM (20 mL) were added to a 100 mL round-bottomed flask. A solution of RB (0.96 g, 2.0 mmol) in 20 mL of DCM was added dropwise and the mixture was then stirred for 12 h. The reaction solution was purified by diluted hydrochloric acid (3×50 mL). The separated DCM part was dried over anhydrous MgSO_4 and concentrated under reduced pressure. RB-OH was obtained as a purple solid with a yield of 65%.

IR (cm^{-1}): 3320 (OH stretching), 1720 (C=O stretching from ester), 1646, 1630, 1590 (Aromatic ring). ^1H NMR (400 MHz, CDCl_3 , ppm): 8.41–8.39 (br, 1H, Ar-H), 7.76–7.70 (br, 2H, Ar-H), 7.52 (br, 1H, Ar-H), 7.10–7.14 (dd, 2H, Ar-H), 6.91–6.88 (dd, 2H, Ar-H), 6.85–6.84 (d, 2H, Ar-H), 4.13–4.10 (t, 2H, $-\text{CH}_2-$ from ethanol ester), 3.70–3.56 (br, 8H, $-\text{CH}_2-$ from RB), 3.53–3.51 (t, 2H, $-\text{CH}_2-$ from ethanol ester), 0.99–1.33 (t, 12H, $-\text{CH}_3$ from RB). ^{13}C NMR (100 MHz, CDCl_3 , ppm): 165.43–113.73 (19C, $-\text{C}-$ from RB), 96.51 (1C, $-\text{COO}-$ from ethanol ester), 67.49, 59.55 (2C, $-\text{CH}_2$ from ethanol ester), 46.08 (4C, $-\text{CH}_2$ from RB), 12.66 (4C, $-\text{CH}_3$ from RB). MS (EI): Exact mass calculated for $\text{C}_{30}\text{H}_{35}\text{N}_2\text{O}_4$ $[\text{M}]^+$: 487.26, found: 487.3.

2.4. Synthesis of the Rhodamine-Based Polymer P(MMA-co-RB)

Polymer P(MMA-co-RB) was synthesized by the reaction of RB-OH with P(MMA-co-AMAA) (Scheme 1). P(MMA-co-AMAA) was synthesized according to a previously reported procedure [48]. P(MMA-co-AMAA) (0.52 g), RB-OH (0.52 g, 1.0 mmol) and DBTDL (0.003 g, 0.005 mmol) were dissolved in 10 mL of xylene, and the resulting solution was heated at 130 $^\circ\text{C}$ for 4 h and stirred. After the reaction was achieved, the resulting cooled mixture was precipitated in methanol under vigorous stirring and washed several times with methanol. After being dried at 40 $^\circ\text{C}$ under vacuum for 24 h, the objective polymer P(MMA-co-RB) was obtained as a pink solid powder.

IR (cm^{-1}): 3420 (N-H stretching), 1730 (C=O stretching from MMA and urethane bond), 1645, 1625, 1590 (C=O stretching from RB-OH). ^1H NMR (400 MHz, CDCl_3 , ppm): 8.41–8.39, 7.76–7.70 (m, 3H, Ar-H from RB-OH), 7.52–6.84 (m, 2H, Ar-H from RB-OH), 3.60 (s, 3H, O- CH_3 from MMA), 3.53–3.51 (t, 2H, $-\text{CH}_2-$ from ethanol ester), 0.99–1.33 (t, 12H, $-\text{CH}_3$ from RB). GPC (THF): PDIs = 1.65; M_n = 17,300 g mol^{-1} ; M_w = 28,600 g mol^{-1} .



Scheme 1. Synthesis of (a) RB-OH and (b) P(MMA-co-RB).

2.5. Selectivity Measurements of Metal Ion

The solution of P(MMA-co-RB) was prepared by dissolving the P(MMA-co-RB) powder in DMF solvent at a concentration of 5.0 mg mL^{-1} . To evaluate the selectivity, the following cations were utilized: Cu^{2+} , Fe^{3+} , Cr^{3+} , Al^{3+} , Zn^{2+} , Co^{2+} , Sn^{2+} and Ag^+ . The test solutions were prepared using 10 mL solution of P(MMA-co-RB) mixed with 5 mM of metal ions and measured for fluorescence spectra at room temperature.

2.6. Preparation of Sensory Test Strip and Metal Ion Sensing Test

P(MMA-co-RB) (0.20 g) and F127 resin (0.40 g) were dissolved into 10 mL of chloroform and stirred to form a clear solution. The G254 silica gel aluminum plates were cut into $5 \text{ mm} \times 30 \text{ mm}$ and soaked in the above solution for 10 min. After dried in the oven at $60 \text{ }^\circ\text{C}$ for 1 h, the simple test strips were obtained, which displayed a strong fluorescence under a 365 nm UV lamp. The test strips as a sensor were inserted into an aqueous solution of various metal ions (5 mM) for 10 min, and then dried before the fluorescence measurement.

3. Result and Discussion

3.1. Synthesis of RB-OH and P(MMA-co-RB)

The synthetic route of RB-OH and P(MMA-co-RB) is shown in Scheme 1. RB-OH was synthesized using RB as the starting material according to the literature method [49,50]. Its structure was confirmed by IR (Figure S1), ^1H NMR (Figure S2), ^{13}C NMR (Figure S3) and MS analyses (Figure S4).

P(MMA-co-RB) was synthesized from the reaction of P(MMA-co-AMAA) and RB-OH in the presence of DBTDL in xylene at $130 \text{ }^\circ\text{C}$ (Scheme 1b). In fact, the synthesis involves two steps: first and most critically, the formation of the reactive NCO-containing

intermediate polymer by Lossen rearrangement reaction, and subsequent grafting reaction between the intermediate polymer and RB-OH [48]. Thus, RB fluorophore was introduced into the polymer via the urethane bond. IR (Figures S1 and S5) and ^1H NMR (Figures S2 and S6) spectroscopies were used to confirm the urethane linkage between the RB moiety and the polymer side chain. The spectrum of P(MMA-co-RB) shows absorption bands around 3420 cm^{-1} and 1730 cm^{-1} , which can be attributed to the N–H stretching and C=O stretching from the urethane bond. The new characteristic peaks of P(MMA-co-RB) at 1646 cm^{-1} , 1630 cm^{-1} , and 1590 cm^{-1} can be assigned to the aromatic ring stretching of RB moiety. Meanwhile, the ^1H NMR spectra (Figure S6) of P(MMA-co-RB) reveal a series of new peaks from 8.1 ppm to 6.84 ppm, corresponding to the benzene ring region of RB moiety, furthermore demonstrating a successful incorporation of the RB unit into the polymer structure. Gel permeation chromatography (GPC) relative to polystyrene standards was performed to determine the molecular weight of polymer P(MMA-co-RB), the number molecular weight (M_n) of the polymer as $17,300\text{ g mol}^{-1}$ and degree of dispersion (M_w/M_n) as 1.65 (Figure S7).

In the solid state, in comparison with P(MMA-co-AMAA), RB-OH and P(MMA-co-RB) powders under visible and UV light (Figure 1), it can be seen that P(MMA-co-RB) is quite fluorescent. However, the derivatives of rhodamine B usually are not fluorescent in the solid state due to the aggregation-caused quenching (ACQ) effect, thus making simple solid-state sensing very difficult and quite challenging [51,52]. P(MMA-co-RB) powder shows high fluorescent, not only confirming successful grafting of RB onto the polymer host, but also implying a potential sensory application in a solid polymer state rather than in solution.

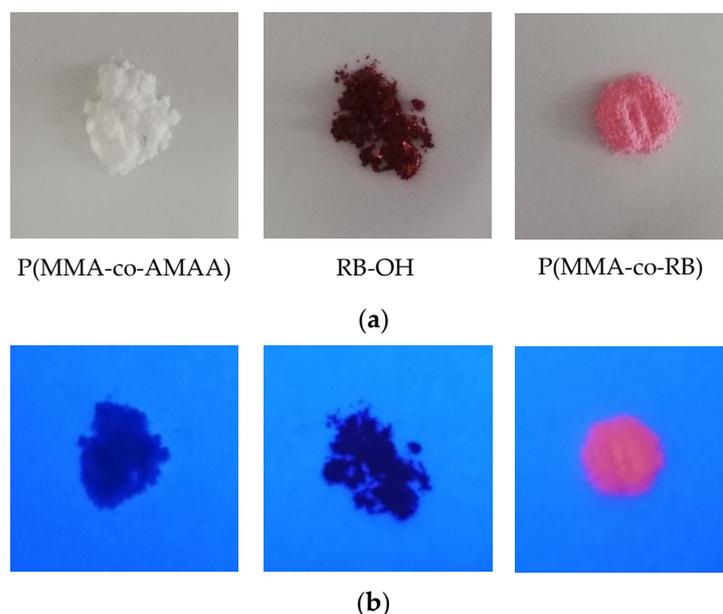


Figure 1. Photographs of P(MMA-co-AMAA), RB-OH and P(MMA-co-RB) under (a) visible light and (b) UV light at 365 nm.

In solution, P(MMA-co-RB) emits an intense orange light, nearly as intensely as RB-OH by visual comparison, with the emission maximum at 560 nm (Figure 2). As expected, P(MMA-co-AMAA) is non-fluorescent and does not absorb at 560 nm wavelength, thus not interfering with the fluorescence property of P(MMA-co-RB) (Figure 3).

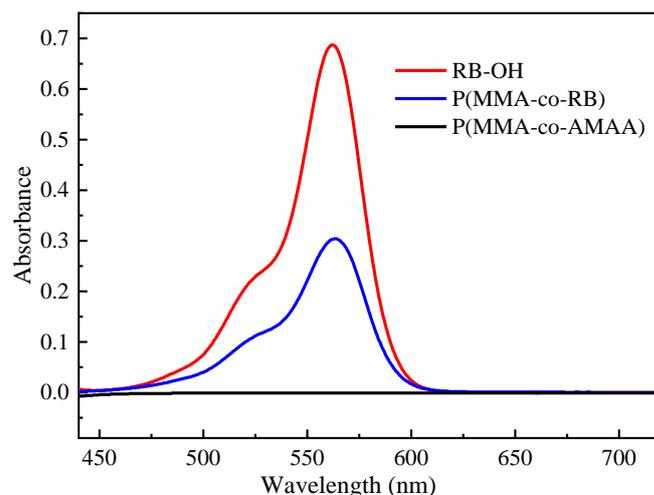


Figure 2. Absorption spectra of P(MMA-co-AMAA) (0.5 mg/mL), RB-OH (0.05 mg/mL) and P(MMA-co-RB) (0.5 mg/mL) in DMF solution.

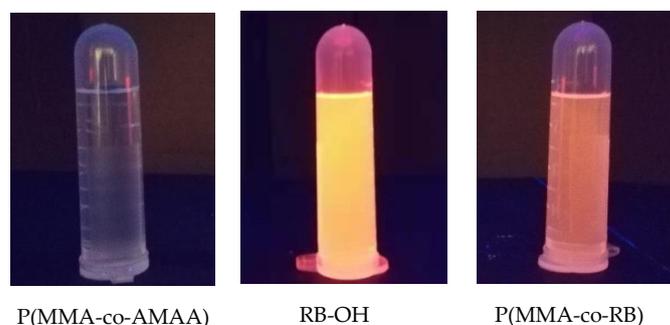


Figure 3. Photographs recorded under UV light 365 nm of P(MMA-co-AMAA) (0.5 mg/mL), RB-OH (0.05 mg/mL) and P(MMA-co-RB) (0.5 mg/mL) in DMF solution.

3.2. Fluorescence Sensing of Different Metal Ions by P(MMA-co-RB) in Solution

The influence of various metal cations (Cu^{2+} , Fe^{3+} , Cr^{3+} , Al^{3+} , Zn^{2+} , Co^{2+} , Sn^{2+} , Ag^{+}) on the fluorescence of P(MMA-co-RB) was investigated. Upon the addition of 5 mM of each of these metal ions, the fluorescence spectra were recorded after 30 min. As shown in Figure 4, the fluorescence of the P(MMA-co-RB) solution was quenched to a different degree by different metal cations. Metal cations such as Al^{3+} , Zn^{2+} , Sn^{2+} , Ag^{+} showed less or no influence on the fluorescence quenching and Fe^{3+} , Cr^{3+} , Co^{2+} only induced a slight fluorescence quenching. However, the addition of Cu^{2+} ion into the P(MMA-co-RB) solution resulted in remarkable fluorescence quenching. These results suggested that the P(MMA-co-RB) solution has a good selectivity for Cu^{2+} ion among the metal cations tested under the same conditions. Clearly, this RB-containing polymer is more sensitive towards Cu^{2+} ion among all the ions tested and thus has a good sensing selectivity.

Furthermore, the fluorescence quenching efficiency ($\text{FQE} = I_0/I$) for the above metal ions was determined accordingly, which is the ratio of the initial maximum fluorescence intensity (I_0) and the maximum fluorescence intensity (I) after addition of metal cations (Figure 5). The results further confirmed a relatively high selectivity towards Cu^{2+} ion.

The fluorescence response of the P(MMA-co-RB) solution with various concentrations of Cu^{2+} ion was also studied (Figure 6). In the range of 0–1.0 mM of Cu^{2+} ion, the FQE of P(MMA-co-RB) changed slightly. Further increasing to 10 mM of Cu^{2+} ion, the FQE increased significantly and the polymer solution showed no fluorescence. Furthermore, with the increase of Cu^{2+} ion concentration, a sensory polymer solution changed the color from pink to grey-green (Figure 7). However, by the naked eye, the detection limit for color change was more than 3.0 mM of Cu^{2+} ion.

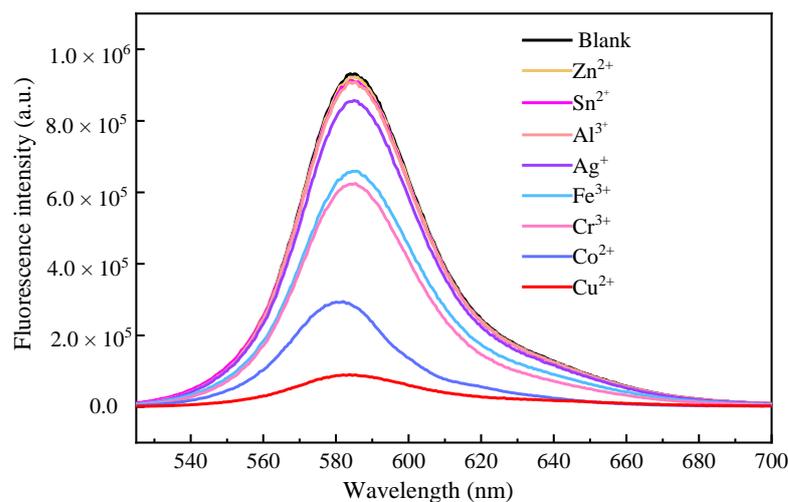


Figure 4. Fluorescence spectra of P(MMA-co-RB) (0.5 mg/mL in DMF, $\lambda_{\text{ex}} = 510$ nm) with different cations (5 mM).

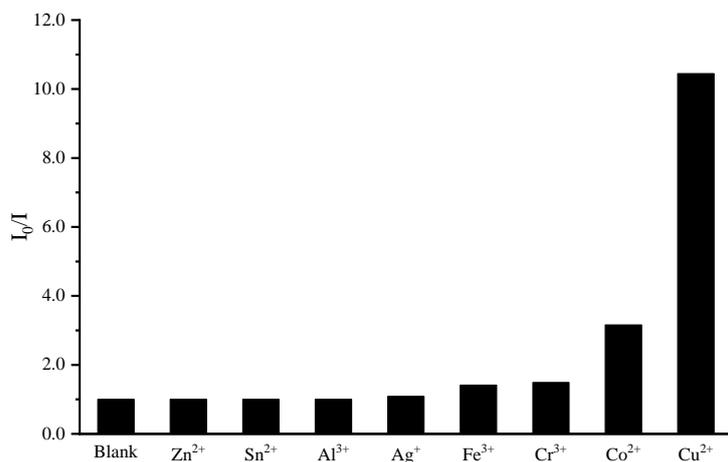


Figure 5. Fluorescence quenching efficiencies of P(MMA-co-RB) (0.5 mg/mL in DMF) versus different metal cations (5 mM).

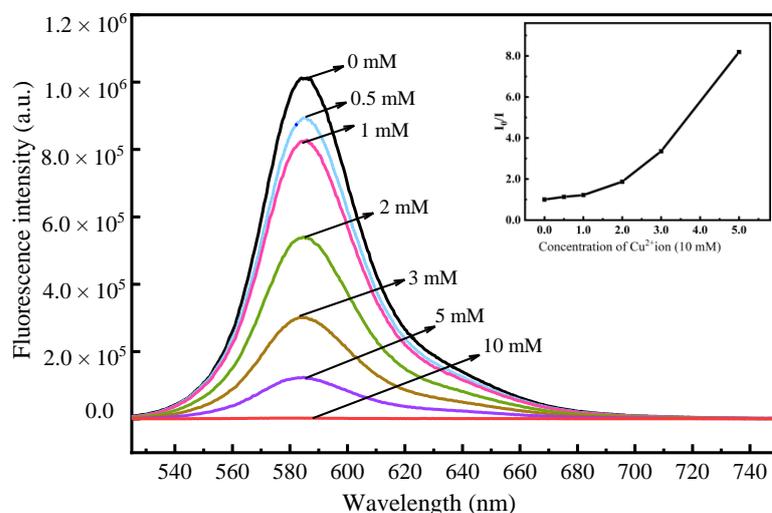


Figure 6. Fluorescence spectra of P(MMA-co-RB) in DMF solution (0.5 mg/mL) upon the addition of Cu^{2+} ion (0, 0.5, 1, 2, 3, 5 and 10 mM, $\lambda_{\text{ex}} = 510$ nm). Inset: fluorescence quenching efficiencies versus the concentrations of Cu^{2+} ($\lambda_{\text{em}} = 585$ nm).

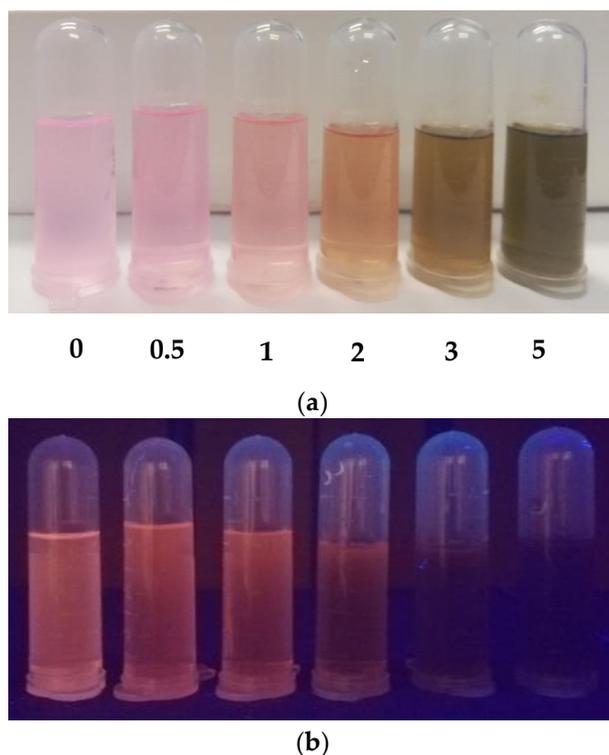


Figure 7. Photographs of P(MMA-co-RB) (0.5 mg/mL) under (a) visible light and (b) UV light at 365 nm after quenching treatment with Cu²⁺ ion (0, 0.5, 1, 2, 3 and 5 mM).

3.3. Fluorescence Sensing of Different Metal Ions by P(MMA-co-RB) in Solid State

Encouraged by the detection performance of P(MMA-co-RB) in solution, we proceeded to investigate the fluorescence sensing of P(MMA-co-RB) in a solid state, in particular using the paper-based test strip. Up to now, many types of paper substrates, such as filter paper, craft paper and chromatography paper, have been employed as base materials for test strips to satisfy different requirements [43]. Among them, chromatography paper has been extensively used for metal ion detection in aqueous solution due to its large aperture and superior absorbing capability. Therefore, a simple test strip was prepared by soaking a silica gel plate in the chloroform solution of P(MMA-co-RB). F127 resin was added to improve the hydrophilicity of the test strip. The selectivity of the test strip for common metal ions was investigated by the fluorescence quenching method. The test strip was placed in an aqueous solution of various metal ions (5 mM) for 10 min and dried in air; then, their emission spectra were recorded (Figure 8). It is obvious that the fluorescence of the test strips was quenched, especially Cu²⁺ ion. The results imply that this strip test is fast, sensitive and convenient for rapid detection of Cu²⁺ ion.

Similar to sensing in solution, as the concentration of Cu²⁺ ion increased, the emission intensity of the test strip decreased gradually (Figure 9). By increasing Cu²⁺ concentrations (0–50 mM), the emission color of the test strips gradually became darker under visible light and as well under illumination of 365-nm UV lamp (Figure 10). However, by the naked eye the detection limit for color change was less than 0.1 mM of Cu²⁺ ion.

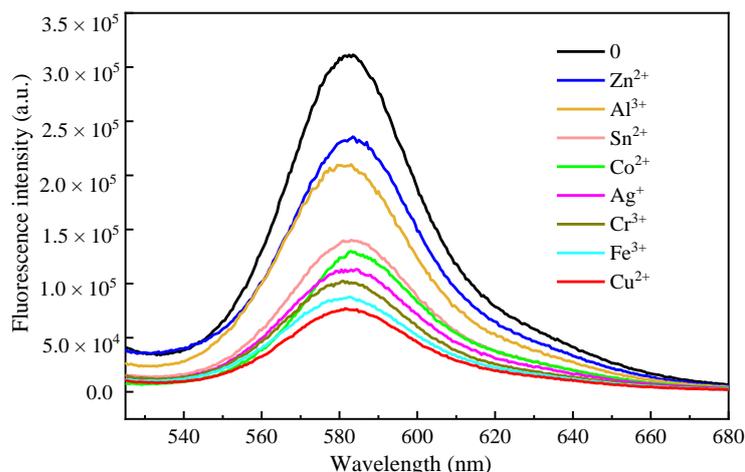


Figure 8. Fluorescence spectra of the test strip for sensing different metal cations (5 mM) in aqueous solution ($\lambda_{ex} = 510$ nm).

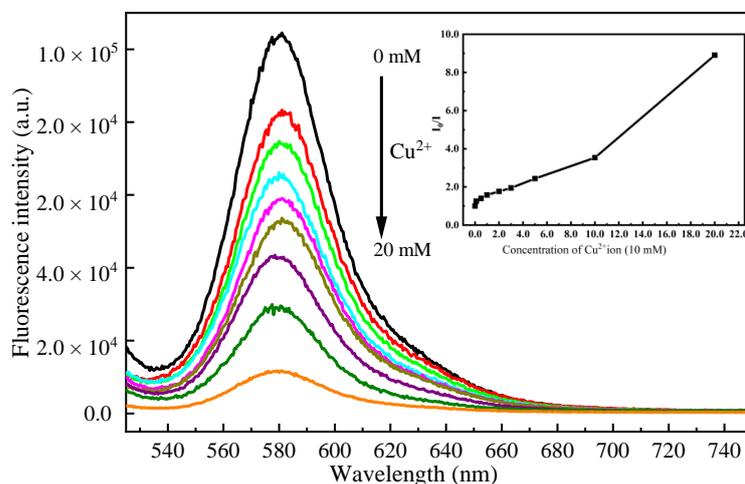


Figure 9. Fluorescence spectra of the test strip for sensing Cu^{2+} ion in aqueous solution (0, 0.1, 0.5, 1, 2, 3, 5, 10 and 20 mM, $\lambda_{ex} = 510$ nm). Inset: fluorescence quenching efficiency versus the concentration of Cu^{2+} ion ($\lambda_{em} = 585$ nm).

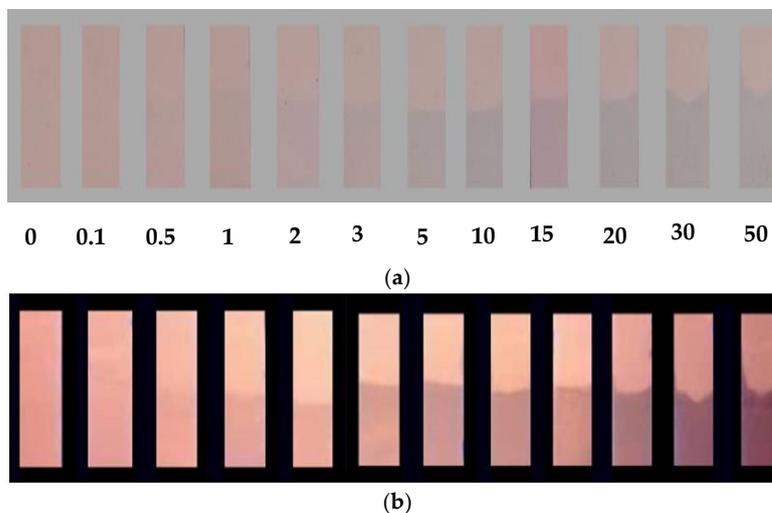


Figure 10. Photographs of the test strips under (a) visible light and (b) UV light at 365 nm after incubation in Cu^{2+} ion aqueous solution (0, 0.1, 0.5, 1, 2, 3, 5, 10, 15, 20, 30 and 50 mM).

4. Conclusions

In summary, fluorescent P(MMA-co-RB) polymer prepared by grafting rhodamine B onto a polymer side chain via the Lossen rearrangement reaction was found to be suitable for fluorescence detection of Cu^{2+} ion. The selectiveness of P(MMA-co-RB) to different metal cations (Cu^{2+} , Fe^{3+} , Cr^{3+} , Al^{3+} , Zn^{2+} , Co^{2+} , Sn^{2+} and Ag^{+}) were also determined. The results showed that this sensory polymer can either be used in solution or in solid state for sensitive and selective detection of Cu^{2+} ion in aqueous or organic solutions. Furthermore, a test strip using P(MMA-co-RB) was demonstrated for fast and sensitive detection of Cu^{2+} ion in water. Overall, this study developed a new synthetic technology of fluorescent chemosensors, which may broaden the scope of research and application of fluorescent chemosensors for detecting metal ions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cryst12060841/s1>, Figure S1: IR spectrum of RB-OH, Figure S2: ^1H -NMR spectrum (400 MHz, CDCl_3) of RB-OH, Figure S3: ^{13}C -NMR spectrum (100 MHz, CDCl_3) of RB-OH, Figure S4: Mass spectrum of RB-OH, Figure S5: IR spectrum of P(MMA-co-RB), Figure S6: ^1H -NMR spectrum (400 MHz, CDCl_3) of P(MMA-co-RB), Figure S7: GPC chromatograms of P(MMA-co-RB).

Author Contributions: Conceptualization: S.Z., W.Q. and Z.Y.W.; Supervision: W.Q. and Z.Y.W.; investigation: S.Z., W.Q. and Z.Y.W.; resources: W.Q. and Z.Y.W.; writing—original draft: S.Z.; writing—review and editing: W.Q. and Z.Y.W.; methodology: S.Z., W.Q. and Z.Y.W.; formal analysis: S.Z. and Z.Y.; data curation: S.Z. and Z.Y.; funding acquisition: W.Q. and Z.Y.W. All authors have read and agreed to the published version of the manuscript.

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References

1. Udhayakumari, D.; Naha, S.; Velmathi, S. Colorimetric and fluorescent chemosensors for Cu^{2+} . A comprehensive review from the years 2013–2015. *Anal. Methods* **2017**, *9*, 552–578. [CrossRef]
2. Gaggelli, E.; Kozłowski, H.; Valensin, D.; Valensin, G. Copper homeostasis and neurodegenerative disorders (Alzheimer's, Prion, and Parkinson's diseases and amyotrophic lateral Sclerosis). *Chem. Rev.* **2006**, *106*, 1995–2044. [CrossRef] [PubMed]
3. Cotruvo, J.A., Jr.; Aron, A.T.; Ramos-Torres, K.M.; Chang, C.J. Synthetic fluorescent probes for studying copper in biological systems. *Chem. Soc. Rev.* **2015**, *13*, 4400–4414. [CrossRef] [PubMed]
4. Turski, M.L.; Thiele, D.J. New roles for copper metabolism in cell proliferation, signaling, and disease. *J. Bio. Chem.* **2009**, *284*, 717–721. [CrossRef]
5. Jiang, R.; Liu, N.; Li, F.; Fu, W.; Zhou, Y.; Zhang, Y. Novel PSMA-coated On-Off-On fluorescent chemosensor based on organic dots with AIEgens for detection of copper (II), iron (III) and cysteine. *Polymers* **2018**, *10*, 786. [CrossRef]
6. Chang, H.; Zhao, X.; Wu, W.; Jia, L.; Wang, Y. A highly sensitive on-off fluorescent chemosensor for Cu^{2+} based on coumarin. *J. Lum.* **2017**, *182*, 268–273. [CrossRef]
7. Tang, L.; Wang, N.; Zhang, Q.; Guo, J.; Nandhakumar, R. A new benzimidazole-based quinazoline derivative for highly selective sequential recognition of Cu^{2+} and CN^- . *Tetrahedron Lett.* **2013**, *54*, 536–540. [CrossRef]
8. Tang, L.; Zhou, P.; Zhang, Q.; Huang, Z.; Zhao, J.; Cai, M. A simple quinoline derivatized thiosemicarbazone as a colorimetric and fluorescent sensor for relay recognition of Cu^{2+} and sulfide in aqueous solution. *Inorg. Chem. Commun.* **2013**, *36*, 100–104. [CrossRef]
9. Liu, Z.; Zhang, C.; Wang, X.; He, W.; Guo, Z. Design and synthesis of a ratiometric fluorescent chemosensor for Cu(II) with a fluorophore hybridization approach. *Org. Lett.* **2012**, *14*, 4378–4381. [CrossRef]
10. Liu, S.; Wang, Y.; Han, J. Fluorescent chemosensors for copper(II) ion: Structure, mechanism and application. *J. Photochem. Photobiol. C* **2017**, *32*, 78–103. [CrossRef]
11. Yu, X.; Xu, J.; Yang, X.; Liu, W.; Qin, W. Synthesis and photochemical properties of BODIPY-functionalized silica nanoparticles for imaging Cu^{2+} in living cells. *RSC Adv.* **2014**, *4*, 23571–23579. [CrossRef]

12. Liu, Y.; Wang, Z.; Qin, W.; Hu, Q.; Tang, B.Z. Fluorescent detection of Cu(II) by chitosan-based AIE bioconjugate. *Chin. J. Polym. Sci.* **2017**, *35*, 365–371. [[CrossRef](#)]
13. Cao, X.; Pang, C.; Xiao, Y.; Xiao, W.; Luo, S.; He, J.; Wang, Z. Preparation of large conjugated polybenzimidazole fluorescent materials and their application in metal ion detection. *Polymers* **2021**, *13*, 3091. [[CrossRef](#)] [[PubMed](#)]
14. Beija, M.; Afonso, C.A.M.; Martinho, J.M.G. Synthesis and applications of Rhodamine derivatives as fluorescent probes. *Chem. Soc. Rev.* **2009**, *38*, 2410. [[CrossRef](#)] [[PubMed](#)]
15. Forbat, E.; Al Niaimi, F. Nonvascular uses of pulsed dye laser in clinical dermatology. *J. Cosme. Derm.* **2019**, *18*, 1186–1201. [[CrossRef](#)]
16. Shen, S.; Chen, X.; Zhang, X.; Miao, J.; Zhao, B. A rhodamine B-based lysosomal pH probe. *J. Mater. Chem. B* **2015**, *3*, 919–925. [[CrossRef](#)]
17. Shen, S.; Huang, X.; Jiang, H.; Lin, X.; Cao, X. A rhodamine B-based probe for the detection of HOCl in lysosomes. *Anal. Chim. Acta* **2019**, *1046*, 185–191. [[CrossRef](#)]
18. Zhang, J.; Zhang, L.; Wei, Y.; Chao, J.; Shuang, S.; Cai, Z.; Dong, C. A selectively rhodamine-based colorimetric probe for detecting copper(II) ion. *Spectrochim. Acta A* **2014**, *132*, 191–197. [[CrossRef](#)]
19. Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. New fluorescent rhodamine hydrazone chemosensor for Cu(II) with high selectivity and sensitivity. *Org. Lett.* **2006**, *8*, 2863–2866. [[CrossRef](#)]
20. Asane, J.; Noginov, M. Concentration dependence of two-photon absorption in PMMA polymeric films doped with rhodamine laser dyes. *J. Opt. Soc. Am. B* **2020**, *37*, 3108–3115. [[CrossRef](#)]
21. Chen, Y.; Chiou, C.; Chen, W.; Jhou, Y.; Lee, Y.; Cheng, C. Rhodamine-ethylenediol, A novel vital fluorescent probe for labelling alkaline phosphatase-rich organelles. *J. Chin. Chem. Soc.-Taip.* **2010**, *57*, 1257–1261. [[CrossRef](#)]
22. Huang, Q.; Zhang, Q.; Wang, E.; Zhou, Y.; Qiao, H.; Pang, L.; Yu, F. A new "off-on" fluorescent probe for Al³⁺ in aqueous solution based on rhodamine B and its application to bioimaging. *Spectrochim. Acta A* **2016**, *152*, 70–76. [[CrossRef](#)] [[PubMed](#)]
23. Lee, M.H.; Wu, J.S.; Lee, J.W.; Jung, J.H.; Kim, J.S. Highly sensitive and selective chemosensor for Hg²⁺ based on the rhodamine fluorophore. *Org. Lett.* **2007**, *9*, 2501–2504. [[CrossRef](#)] [[PubMed](#)]
24. Chang, L.; Gao, Q.; Liu, S.; Hu, C.; Zhou, W.; Zheng, M. Selective and differential detection of Hg²⁺ and Cu²⁺ with use of a single rhodamine hydrazone-type probe in the absence and presence of UV irradiation. *Dye. Pigment.* **2018**, *153*, 117–124. [[CrossRef](#)]
25. Dong, Z.; Tian, X.; Chen, Y.; Hou, J.; Guo, Y.; Sun, J.; Ma, J. A highly selective fluorescent chemosensor for Hg²⁺ based on rhodamine B and its application as a molecular logic gate. *Dye. Pigment.* **2013**, *97*, 324–329. [[CrossRef](#)]
26. Wang, B.; Liu, X.; Hu, Y.; Su, Z. Synthesis and photophysical behavior of a water-soluble coumarin-bearing polymer for proton and Ni²⁺ ion sensing. *Polym. Int.* **2009**, *58*, 703–709. [[CrossRef](#)]
27. Saleem, M.; Abdullah, R.; Ali, A.; Park, B.J.; Choi, E.H.; Hong, I.S.; Lee, K.H. Facile synthesis, cytotoxicity and bioimaging of Fe³⁺ selective fluorescent chemosensor. *Bioorgan. Med. Chem.* **2014**, *22*, 2045–2051. [[CrossRef](#)]
28. Wang, Y.; Chang, H.; Wu, W.; Mao, X.; Zhao, X.; Yang, Y.; Xu, Z.; Xu, Z.; Jia, L. A highly sensitive and selective colorimetric and off-on fluorescent chemosensor for Cu²⁺ based on rhodamine 6G hydrazone bearing thiosemicarbazide moiety. *J. Photoch. Photobio. A* **2017**, *335*, 10–16. [[CrossRef](#)]
29. Xue, D.; Zheng, C.; Qu, S.; Liao, G.; Fan, C.; Liu, G.; Pu, S. A highly selective fluorescent chemosensor for Cu²⁺: Synthesis and properties of a rhodamine B-containing diarylethene. *Luminescence* **2017**, *32*, 652–660. [[CrossRef](#)]
30. Zhang, X.; Sumiya, S.; Shiraishi, Y.; Hirai, T. Effects of alkyl chain length on Cu(II)-selective green fluorescence of rhodamine-diacetic acid conjugates. *J. Photoch. Photobio. A* **2009**, *205*, 215–220. [[CrossRef](#)]
31. Guo, W.; Tang, T.; Lu, S.; Xu, D. Detection of Fe³⁺ using a novel hyperbranched polymeric spectral sensor. *Anal. Methods* **2019**, *11*, 4456–4463. [[CrossRef](#)]
32. Liu, X.; Chen, A.; Wu, Y.; Kan, C.; Xu, J. Fabrication of fluorescent polymer latexes based on rhodamine B derivatives and their reusable films for Fe³⁺ detection. *Dye. Pigment.* **2020**, *182*, 108633. [[CrossRef](#)]
33. Pipattanawarothai, A.; Trakulsujaritchok, T. Hybrid polymeric chemosensor bearing rhodamine derivative prepared by sol-gel technique for selective detection of Fe³⁺ ion. *Dye. Pigment.* **2020**, *173*, 107946. [[CrossRef](#)]
34. Chen, X.; Zeng, W.; Yang, X.; Lu, X.; Qu, J.; Liu, R. Thiourea based conjugated polymer fluorescent chemosensor for Cu⁺ and its use for the detection of hydrogen peroxide and glucose. *Chin. J. Polym. Sci.* **2016**, *34*, 324–331. [[CrossRef](#)]
35. Li, G.; Tao, F.; Liu, Q.; Wang, L.; Wei, Z.; Zhu, F.; Chen, W.; Sun, H.; Zhou, Y. A highly selective and reversible water-soluble polymer based-colorimetric chemosensor for rapid detection of Cu²⁺ in pure aqueous solution. *New J. Chem.* **2016**, *40*, 4513–4518. [[CrossRef](#)]
36. Chang, L.; Gao, Q.; Liu, S.; Luo, D.; Han, B.; Xia, K.; Zhou, C. A single polymer chemosensor for differential determination of Hg²⁺ and Cu²⁺ in pure aqueous media without mutual interference. *Mater. Today Commun.* **2019**, *19*, 148–156. [[CrossRef](#)]
37. Maiti, C.; Banerjee, R.; Maiti, S.; Dhara, D. Water-soluble polymeric chemosensor for detection of Cu²⁺ ions with high selectivity and sensitivity. *Des. Monomers Polym.* **2016**, *19*, 669–678. [[CrossRef](#)]
38. Gao, S.; Li, Z.; Sun, Z.; Wen, J.; Li, F.; Du, X.; Liu, Y.; Tian, Y.; Niu, Z. Single-wavelength excited ratiometric fluorescence pH probe to image intracellular trafficking of tobacco mosaic virus. *Chin. J. Polym. Sci.* **2020**, *38*, 587–592. [[CrossRef](#)]
39. Geng, T.; Wu, D.; Huang, W. Dual turn-on fluorescent chemosensor for Cu²⁺ and Hg²⁺ in aqueous medium based on a water-soluble polyacrylamide containing rhodamine. *J. Polym. Res.* **2015**, *22*, 40. [[CrossRef](#)]

40. Banerjee, R.; Pal, D.S.; Dhara, D. Synthesis of a new rhodamine-containing block copolymer for highly selective and sensitive detection of Cu^{2+} and CN^- ions in aqueous media. *Polym. Int.* **2014**, *63*, 1974–1981. [[CrossRef](#)]
41. Liu, C.; Ning, D.; Zhang, C.; Liu, Z.; Zhang, R.; Zhao, J.; Zhao, T.; Liu, B.; Zhang, Z. Dual-colored carbon dot ratiometric fluorescent test paper based on a specific spectral energy transfer for semiquantitative assay of copper ions. *ACS Appl. Mater. Interfaces* **2017**, *9*, 18897–18903. [[CrossRef](#)] [[PubMed](#)]
42. Guo, Z.; Niu, Q.; Li, T.; Wang, E. Highly chemoselective colorimetric/ fluorometric dual-channel sensor with fast response and good reversibility for the selective and sensitive detection of Cu^{2+} . *Tetrahedron* **2019**, *75*, 3982–3992. [[CrossRef](#)]
43. Sivakumar, R.; Lee, N.Y. Paper-based fluorescence chemosensors for metal ion detection in biological and environmental samples. *BioChip. J.* **2021**, *15*, 216–232. [[CrossRef](#)]
44. Wu, M.; Suo, F.; Zhou, J.; Gong, Q.; Bai, L.; Chen, B.; Wu, Q.; Zhang, C.; Yu, H.; Huang, X.; et al. Paper-based fluorogenic device for detection of copper ions in a biological system. *ACS Appl. Bio Mater.* **2018**, *1*, 1523–1529. [[CrossRef](#)] [[PubMed](#)]
45. Zhou, J.; Wu, Q.; Chen, X.; Qin, X.; Zhang, G.; Wu, M.; Fang, H.; Lu, Y.; Yu, H.; Li, L.; et al. Two-component ratiometric sensor for Cu^{2+} detection on paper-based device. *Anal. Bioanal. Chem.* **2019**, *411*, 6165–6172. [[CrossRef](#)] [[PubMed](#)]
46. Zhu, S.; Li, H.; Zhang, X.; Chen, W.; Feng, L. Synthesis, copolymer composition, and rheological behavior of functionalized polystyrene with isocyanate and amine side groups. *Des. Monomers Polym.* **2015**, *18*, 232–241. [[CrossRef](#)]
47. Thomas, M.; Alsarraf, J.; Araji, N.; Tranoy-Opalinski, I.; Renoux, B.; Papot, S. The Lossen rearrangement from free hydroxamic acids. *Org. Biomol. Chem.* **2019**, *17*, 5420–5427. [[CrossRef](#)]
48. Zhang, S.; Ghosh, K.R.; Yao, Z.; Li, L.; Qin, A.; Qiao, W.; Wang, Z.Y. Formation of reactive isocyanate group-containing polymers via Lossen rearrangement. *Mater. Today Commun.* **2022**, *30*, 103169. [[CrossRef](#)]
49. Liu, J.; Li, A.; Tang, J.; Wang, R.; Kong, N.; Davis, T.P. Thermoresponsive silver/polymer nanohybrids with switchable metal enhanced fluorescence. *Chem. Commun.* **2012**, *48*, 4680–4682. [[CrossRef](#)]
50. Lee, J.; Yang, H.; Park, C.H.; Cho, H.; Yun, H.; Kim, B.J. Colorimetric thermometer from graphene oxide platform integrated with red, green, and blue emitting, responsive block copolymers. *Chem. Mater.* **2016**, *28*, 3446–3453. [[CrossRef](#)]
51. Hong, Y.; Lamab, J.W.Y.; Tang, B.Z. Aggregation-induced emission. *Chem. Soc. Rev.* **2011**, *40*, 5361–5388. [[CrossRef](#)] [[PubMed](#)]
52. Hong, Y.; Lam, J.W.Y.; Tang, B.Z. Aggregation-induced emission: Phenomenon, mechanism and applications. *Chem. Commun.* **2009**, 4332–4353. [[CrossRef](#)] [[PubMed](#)]