



理學碩士 學位 論文

A new Avenue for the Discrimination of

Geometrical Isomer (Maleic acid vs Fumaric acid)

using water-soluble polymer

蔚山大學校大學院

化學科

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指導敎授이형일

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Contents

Abstract

Water-soluble copolymers were prepared by reversible addition-fragmentation chain transfer polymerization (RAFT), which is used for successful discrimination between maleic acid and structurally similar fumaric acid. After polymerization, we can get a series of polymeric probes (P1, P2, and P3) that various pendent -R groups (i.e., aldehydes, thiazolidines, and nitriles) containing azo chromophore. In case of P1 (aldehyde group), maleic acid and fumaric acid containing other carboxyl groups afforded the selective colorimetric detection. In case of P2 (thiazolidine ring), maleic acid and fumaric acid did not afforded the selective detection but discriminate from other carboxylic acids. Finally, P3 did not discriminate all carboxylic acids including maleic acid and fumaric acid. These results were influenced by the magnitude of the acid dissociation constant of the polymer probe. In this regard, these pK_a could be important parameters to understand the mechanism of selective analysis sensing.

Introduction

During the past few decades, people have considered that the development of mono- or dicarboxylic acid selective and sensitive optical chemo-sensors devices is industrially and biologically important. ¹ Among the various carboxylic acids, maleic acid and fumaric acid is the most studied as biomolecule because of its association with various research fields such as food processing, medicinal chemistry, and polymer synthesis.^{2, 3} Maleic acid used food additives plays an important role as an inhibitor of Kerbs cycles. On the other hand, fumaric acid derivatives have been used for the treatment of patients with multiple sclerosis or psoriasis.^{4, 5} Despite its biological use, severe accumulation of maleic acid and fumaric acid in the human body results in serious heart disease.⁶ Therefore, it is important to develop reliable and economically viable analytical methods for the detection of these acids. Especially, the colorimetric chemo-sensor is convenient because the detection process can be real time and easily visible by naked eye without the aid of any instrumental setups.^{7, 8}

Various kinds of chemo-sensors for detection of maleic acid or fumaric acid on derived from small organic/organometallic molecules have been investigated, but most suffer from low solubility and structural instability in water, their practical applications limited in environmental and biological circumstances.⁹⁻¹³ To overcome these issues, water-soluble polymers with the small incorporation of receptor moieties via copolymerization have been developed. This allows for structural stability, better biocompatibility than the probes alluded to above, multifunctional sensing capability, separation ability, reusability, and facile device fabrication.¹⁴⁻¹⁷

The differentiation of geometric isomers with chemically and physically similar properties

is one of the most difficult tasks. Much effort has been devoted to achieving the differentiation of Male and Fumaric acids. Among many tasks, Manez and colleagues have developed simple small molecule receptors combined with specific anionic forms that can distinguish fumarate and maleate via form-induced cyclization. However, this probe has a similar colorimetric response to phthalates, so it lacks selectivity.¹⁸ Similarly, Upendar and colleagues reported reversible thiourea-based probes for the detection of maleic acid and fumaric acid, but did not distinguish these two isomers by optical reactions.¹⁹ Most recently, Samanta and colleagues have synthesized Schiff base-derived probes for the colorimetric and fluorescence measurements of maleic acid and fumaric acid. This depends on the protonation and subsequent complexation of the receptor by the target carboxylic acid.²⁰ However, these probes are organic monomolecular and are not water soluble, reducing the probe's suitability for practical applications.

So we designed water-soluble azo-based polymeric probes (P1~P3) that various pendent –R groups (i.e., aldehydes, thiazolidines, and nitriles) to discriminate between maleic acid and fumaric acid in pure aqueous media at physiological pH. In order to obtain a clear picture of the probes' colorimetric response to the target carboxylic acids and the effect that various –R groups had on the electronic structures of the azo-chromophore, the values of the acid dissociation constant (pK_a) of the designed probes were estimated by titration methods and compared with the pK_a values of the target carboxylic acids.

Experimental

Materials.

2,2 - Azobis(isobutyronitrile) (AIBN, Aldrich, 98%) was recrystallized from ethanol prior to use. 4-nitrobenzaldehyde, 4-(dimethylamino)pyridine (DMAP), *N*-(3-dimethylaminopropyl)-N - ethylcarbodiimide hydrochloride (EDC), *N*,*N*-Dimethylformamide (DMF, 99.8%), sodium nitrite, *N*-(2-hydroxyethyl)piperazine-*N* - (2-ethaneslfonic acid) (HEPSE), and 2-dodecylsul fanylthiocarbonylsul fanyl-2-methylpropionic acid (DMP) were purchased from Sigma-Aldrich and used as received. Triethylamine (99%), maleic acid, and tin (II) chloride were purchased from Alfa Aesar. Fumaric acid supplied by TCI was of the highest purity, and it was used as received. Acetic acid, Benzoic acid, Gallic acid, Stearic acid Succinic acid, Terephthalic acid were purchased from general suppliers. And amino acids were purchased from general suppliers and used without further purification. Deionized (DI) water was used in the present sensing studies.

Characterization

¹H NMR spectra of the polymer solutions were recorded in a Bruker Avance 300 MHz, and the data were collected at 298 K using CDCl₃ as solvent. The apparent molecular weight and molecular weight distribution was measured by gel permeation chromatography (GPC, Agilent technologies 1200 series) using a poly(methyl methacrylate) (PMMA) standard, with DMF as the eluent at 30 °C and a flowrate of 1.00 mL/min. The UV-Vis spectra were recorded using a Varian Cary-100 UV-Vis spectrophotometer.

Synthesis

Synthesis of (E)-2-((4-Formyl phenyl) diazenyl) phenyl) (methyl)amino) ethyl acrylate (FPDEA) were prepared as previously reported.

Synthesis of p(DMA-co-FPDEA) (P1). Polymer P1, consisting of 96% dimethylacrylamide (DMA) and 4% aldehyde-terminated azo units ($M_n = 11,000$ Da, polydispersity index =1.05), was synthesized according to the procedure we reported previously.

Sensing studies The P1 solution was prepared (based on the assumption of a 4% incorporation extent of azo units within the polymer chain) so as to provide a 31 μ M concentration in aldehyde moieties. The solution of various carboxylic acids (1.75 x 10⁻¹M) and amino acids (1.0 x 10⁻¹M) were prepared in DI water. The sensing studies were carried out by adding 96 μ L aliquots of the various carboxylic acid solutions to obtain different final concentrations (0.0–8.0 mM) and amino acid into 2.0-mL polymer (P1, P2, and P3) solutions.

Results and Discussion



Scheme 1 Showing of their efficacy in the colorimetric discrimination between maleic acid (Mal) and fumaric acid (Fum) via a series of water-soluble polymeric probes(P1~P3) with three different pendent functional groups. (EDG: electron-donating group; EWG: electron-withdrawing group.)



Figure 1. ¹H NMR spectrum of P1 in CDCl₃.



Figure 2. Gel permeation chromatography of P1.

3.1 Synthesis and characterization of P1

Water-Soluble polymers with three different functional groups synthesized to allow for the selective detection of maleic acid and/or the discrimination between the two structural isomers maleic acid and fumaric acid (scheme 1). As reported previously, P1 with aldehyde group was synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization. To synthesize water-soluble polymer, it was designed to represent high levels of hydrophilic dimethylacrylamide (DMA) units and a small number of azo-receptor units. Successful synthesis of P1 was confirmed by ¹H NMR spectroscopy (Figure 1). The incorporation ratio of P1 was same as the initial feed ratio of [DMA] : [Azo-CHO monomer] = 96:4, which was very close to the initial feed ratio (95:5). The average molecular weight and polydispersity index (PDI) of random copolymers were measured by gel permeation chromatography (GPC) with p (methylmethacrylate) (PMMA) standards (Mn = 11,000g/mol), Mw/Mn = 1.05) (Figure 2).



Figure 3 UV-Vis absorption spectra of an aqueous solution of (a) P1 (31 μ M concentration of aldehyde units), (c) P2 (31 μ M concentration of thiazolidine units), and (e) P3 (31 μ M concentration of nitrile units) upon the addition of maleic acid to various final concentrations (0.0–8.0 mM) in deionized water at pH 6.8. Selectivity bar diagram and photographs of the aqueous solution of (b) P1, (d) P2, and (f) P3 versus 7 different carboxylic acids. Plot of $\Delta\lambda$ max ($\Delta\lambda$ max = $\lambda_0 - \lambda_x$, λ_x is the maximum wavelength with addition of 8Mm of each various carboxylic acid, λ_0 is the absorption maximum of P1, P2 and P3)



Figure 4 Linear regression curve of P1 (31 μ M, deionized water) solution with increasing concentration of maleic acid (0–8 mM final acid concentration) (LOD = 0.42Mm). λ_{max} : wavelength of the absorption maximum; λ_0 : wavelength of the absorption maximum at 0 mM of the target analyte.



Figure 5 UV-Vis absorption spectra of P1 (31 μ M concentration in aldehyde units) with various carboxylic acids (8.0 mM). Ace: acetic acid; Ben: benzoic acid; Fum: fumaric acid; Gal: gallic acid; Ste: stearic acid; Suc: succinic acid; Tere: terephthalic acid.

3.2 Selective colorimetric sensing and discrimination efficiency or inefficiency of maleic acid using P1~P3

The maleic acid-detecting properties of P1 (31 μ M concentration of aldehyde units) were examined by UV-Vis absorption spectroscopy. First, UV-vis titration experiments were conducted in the deionized water containing P1 (pH 6.8). When maleic acid was gradually added to that solution, P1 maximum absorption peak shifted from 460nm to 484nm accompanied by color change from pale orange to red by naked eye. (see the photograph in Figure 3a). The lowest limit of detection (LOD) of P1 toward maleic acid was obtained by linear regression and was 0.42mM (Figure 4). We can evidence the selectivity of the probe (31 μ M) toward maleic acids, a range of carboxylic acids (8mM), such as acetic acid, benzoic acid, terephthalic acid, stearic acid, gallic acid, succinic acid, and fumaric acid, were recorded under conditions identical to those employed for the titration of the probe with maleic acid (Figure 5). None of the other carboxylic acids showed appreciable shifts in the absorption maxima, suggesting the excellent selectivity of P1 toward maleic acid (Figure 3b). And it enables researchers to discriminate between maleic acid and fumaric acid (Figure 1b). P1 has been reported to be an excellent candidate for the selective colorimetric detection of cysteine (over that of the other amino acids) in pure water. When cysteine was gradually added to that solution (0-2.0Mm), P1 maximum absorption peak shifted from 460nm to 430nm accompanied by color change from light orange to yellow by naked eye (Figure 6). The blue-shift of absorption spectra of P1 indicated that Intramolecular Charge Transfer (ICT) effect of azobenzene-aldehyde moieties of P1 was interrupted by the formation of thiazolidine ring with cysteine.²¹⁻²⁵



Figure 6 UV-Vis absorption spectra of P1 with various concentrations of cysteine in the deionized water (Cys, 0-2.0 mM).

When maleic acid was gradually added to that solution, P2 maximum absorption peak shifted from 430 nm to 460 nm, a shift in absorption wavelength made evident by a change in color of the solution from yellow to orange (Figure 3c). Unlike the case of P1, P2 Maximum absorption peak are also changed upon the gradual addition of fumaric acid. P2 maximum absorption peak shifted from 430 nm to 454 nm accompanied by color change from yellow to pale orange (Figure 7). Also similar between these two acids were the heights of the relevant bars in the selectivity diagram, although addition to the P2 solution of the other carboxylic acids tested did not trigger any significant spectral and color changes (Figures 1d and Figure 8). In summary, whereas P2 could be used to selectively detect maleic acid and fumaric acid versus other carboxylic acids, no discrimination between these two acids was achieved. LOD values of P2 toward maleic and fumaric acids were obtained from the liner regression curve and calculated to be 0.953 and 1.01 mM, respectively (Figures 9 and 10).



Figure 7 UV-Vis absorption spectra of P2 with various concentration of fumaric acid in the deionized water (Fumaric acid, 0-8.0 mM).



Figure 8 UV-Vis absorption spectra of P2 (31 μ M concentration in aldehyde units) with various carboxylic acids (8.0 mM). Ace: acetic acid; Ben: benzoic acid; Fum: fumaric acid; Gal: gallic acid; Ste: stearic acid; Suc: succinic acid; Tere: terephthalic acid.



Figure 9 Linear regression curve of P2 (31 μ M, deionized water) solution with increasing concentration of maleic acid (0–8 mM final acid concentration) (LOD = 0.953mM). λ_{max} : wavelength of the absorption maximum; λ_0 : wavelength of the absorption maximum at 0 mM of the target analyte.



Figure 10 Linear regression curve of P2 (31 μ M, deionized water) solution with increasing concentration of Fumaric acid (0–8 mM final acid concentration) (LOD = 1.01mM). λ_{max} : wavelength of the absorption maximum; λ_0 : wavelength of the absorption maximum at 0 mM of the target analyte.

A reaction between the aldehyde groups of P1 and hydroxylamine led to the formation of a polymer with pendent aldoxime groups, which reacted further with mercury(II) ions in water to produce a polymer comprising strong electron-withdrawing nitrile groups (P3) obtained as a consequence of mercury(II)-promoted dehydration.²⁶ The color of the *in situ*-formed P3 solution was pink, and its absorption maximum was located at 510 nm. Upon the gradual addition of maleic acid to this P3 solution (0–8.0 mM final analyte concentration), neither a shift in absorption maximum nor a change in the solution color was observed (Figure 1e). Furthermore, the addition to the solution of the probe of any of the tested carboxylic acids, including fumaric acid, did not trigger any significant spectral and color change (Figure 1f). P3 did not enable us to selectively detect maleic acid versus other carboxylic acids or to discriminate between maleic acid and fumaric acid.



Scheme 2 Selective protonation by carboxylic acids of the β -nitrogen atoms of the azo chromophore of water-soluble polymeric probes appended with various –R groups (P1~P3).

3.3 Probable explanation based on pK_a measurements

In view of the differential detection of maleic acid by the functional groups of each polymer, it was assumed that the proton of the β -nitrogen atom of the azo chromophore by carboxylic acid may be an important factor (Scheme 2). Protonation of the β -nitrogen atoms of the azo chromophore (the azonium form) leads to an increase in the negative mesomeric (–M) effect of the chromophore, which in turn strengthens the push–pull effect (Scheme 3). As a result, the polymeric probes' absorption maximum red-shifted in the presence of carboxylic acids.²⁷



Scheme 3 Compare to push-pull effects by negative mesomeric (–M) effect of chromophore.

We can control the extent of protonation of the β -nitrogen atoms by the choice of the –R group (i.e., aldehyde, thiazolidine, or nitrile). As the electron withdrawing effect of the pendant -R group increases, the basicity of the β -nitrogen atom decreases. In addition, it can be seen that as the basicity of the β -nitrogen atom decreases, the tendency of quantization by the surrounding carboxylic acid decreases. The extent of protonation of the azo chromophore is dependent on the acid dissociation constant (p K_a) values of the probe and carboxylic acids, and these p K_a could be important parameters to understand the mechanism of selective analyte sensing.

In this context, the pK_a values of P1, P2, and P3 were evaluated using the following equation

$$log\left[\frac{(A_{HA} - A_i)}{(A_i - A_{A^-})}\right] = pH_i - pK_a \qquad \dots equ. (1)$$
$$log\left[\frac{(A_{HA} - A_i)}{(A_i - A_{A^-})}\right] = 0$$
$$pH_i = pK_a$$

Here, A_{HA} and A_A^- represent the absorbance of the acidic and basic solutions of the polymeric probe at the absorption maximum, respectively. A_i represents the absorbance of the polymeric probe solution at intermediate pH_i values.



Figure 11 Change in the UV-Vis absorption spectra of the aqueous solution of (a) P1, (b) P2, and (c) P3 following the continuous variation of the solution's pH.

To determine the pK_a values of P1, P2, and P3, a small amount of HCl was added to the probe solution to change the pH (Figure 11). The values of solutions at different pH were plotted against pH using the log $[(A_{HA} - A_i)/(A_i - A_A)]$ equation to calculate the pK_a value based on Equation 1. P1 was measured to be 2.84(Figure 12a) higher than maleic acid (1.9) but lower than other carboxylic acids including fumaric acid (3.1). As a result, the β -nitrogen atom of the azo chromophore of P1 was selectively protonated by maleic acid, and the distinction between maleic acid and fumaric acid provided by P1 was observed. On the other hand, the pK_a value of P2 was calculated to be 3.64 (Figure 12b), which is higher than both maleic acid and fumaric acid but lower than other carboxylic acids. This means that the β -nitrogen atom of the azo chromophore of P2 was difficult to discriminate between maleic acid and fumaric acid. Finally, in the case of P3 aqueous solution, when the pH was changed we can't show UV-Vis spectral changes. It was not possible to calculate the result of the pK_a value. The nitrile group of P3 is a very strong electron withdrawing group, which suggests that the protonation of β -nitrogen atoms of azo chromophores may be much lower than that of P1 and P2. Therefore, the protonation of β -nitrogen atoms in the azo chromophore of P3 was not sensed by any of the other carboxylic acids, including maleic acid and fumaric acid. So we didn't discriminate between analyte maleic acid and maleic acid fumaric acid.



Figure 12 The values of the pK_a (a) P1 and (b) P2 using the log $[(A_{HA} - A_i) / (A_i - A_a^-)]$ equation. (A_{HA}: absorbance of the acidic solution of the probe at the absorption maximum; A_A⁻: absorbance of the basic solution of the probe at the absorption maximum; A_i: absorbance of the probe's solution at intermediate pH_i values)



Figure 13 The pK_a values of P1, P2, P3, and of various carboxylic acids.

Conclusions

In conclusion, water-soluble copolymer of DMA-*co*-FPDEA with a low polydispersity index was synthesized by RAFT and called P1. P1 can get various –R groups (aldehydes, thiazolidines, and nitriles) containing azo for selective colorimetric sensing of maleic acid and/or discrimination between maleic acid and its structurally similar fumaric acid. According to the -R group (aldehyde, thiazolidine, or nitrile) the degree of protonation of the β -nitrogen atom was controllable. The protons of β -nitrogen atoms of azo chromophores by pK_a and maleic acid (1.9), fumaric acid (3.1) and other carboxylic acids of each polymer are believed to be important factors depending on the functional groups of each polymer. The degree of protonation of azo chromophores depends on the acid dissociation constant (pK_a) values of the probe and carboxylic acid, and these pK_a may be important parameters to understand the mechanism of selective analyte detection.

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국문 요약

지난 수십년 동안 많은 사람들은 모노 카르복실산 또는 디 카르복실산을 선택적으로 화학 감시 장치의 개발이 산업적으로나 생물학적으로 중요하다고 생각했습니다. 다양한 카르복실 산중에서 말레산과 푸마르산은 식품가공, 의약, 화학 및 중합체 합성과 같은 다양한 연구 분야와의 연관성 때문에 가장 많이 연구된 생체 분자입니다. 말레산의 경우 식품 첨가물에 사용되며 Kerbs cycles의 억제제로 중요한 역할을 합니다. 또한, 푸마르산의 경우 다발성 경화증 또는 건선 환자의 치료에 사용되어 왔습니다. 생물학적 사용에도 불구하고 인체에 말레산과 푸마르산이 과도하게 축적되면 심각한 심장병이 발생하게 됩니다. 따라서, 이들 산의 검출을 위한 신뢰성 있고 경제적으로 실행 가능한 분석 방법을 개발하는 것이 중요합니다. 분석 방법 중 화학 반응을 기반으로 실시간으로 검출할 수 있는 유기물기반 화학센서가 개발되어왔습니다. 이들은 높은 감도와 선택성을 가지고 있습니다. 특히, 비색법을 이용한 화학 센서는 검출 과정을 눈으로 쉽게 관찰할 수 있기 때문에 편리합니다.

저 분자인 유기물, 유기금속물질에서 유래된 다양한 종류의 화학 센서가 연구되어왔지만 구조적인 불안정성과 물에 대한 용해도가 낮기 때문에 환경과 생물학적으로 실제 조건에서의 적용이 제한적입니다.

이러한 문제를 극복하기 위해, 저분자인 공중합을 통한 수용체 부분의 작은 혼입을 갖는 수용성 중합체가 개발되었습니다. 이를 통해 구조적 안정성, 생체 적합성 향상, 다기능 감지 기능, 분리 기능, 재사용성 및 손쉬운 장치 제작이 가능합니다.

화학적 및 물리적 특성이 유사한 기하 이성질체의 차별화는 가장 어려운 작업 중 하나입니다. 다른 카르복실 산에 대한 말레산의 선택적 검출 및 성공적인 차별을 위해

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다양한 -R 기 (알데히드, 티아 졸리 딘, 나이트릴)를 가진 아조계를 포함한 수용성 중합체 (P1 ~ P3)를 만들었습니다. P1 (알데히드기)의 경우, 다른 카르복실기를 함유하는 말레 산 및 푸마르산은 선택적인 비색 검출을 제공 하였습니다. P2 (티아 졸리 딘 고리)의 경우, 말레 산 및 푸마르산은 선택적인 검출을 제공하지 않았지만 다른 카르 복실 산과 구별되었습니다. 마지막으로, P3 (나이트릴)은 말레 산 및 푸마르산을 포함한 모든 카르 복실 산을 구별하지 못하였습니다.

이러한 연구 결과는 -R 그룹 (알데히드, 티아졸리딘 또는 니트릴)이 전자 흡인 효과가 증가함에 따라 β- 질소 원자의 염기도는 감소함을 알 수 있었습니다. 또한, β- 질소 원자의 염기도가 낮아질수록 주변 카르 복실 산에 의한 양자화 경향이 감소 함을 알 수 있었습니다. 이는 아조발색단 (아조 늄 형태)의 β- 질소 원자의 양성자 화는 발색단의 음의 mesomeric (-M) effect을 증가시켜 푸시-풀 효과를 강화시켰습니다. 각 중합체의 작용기에 의한 말레산의 성공적인 검출의 관점에서 카르복실 산에 의한 아조 발색단의 β- 질소 원자의 양성자가 중요한 인자 일 수 있다고 보여집니다. 또한, 특정 -R그룹을 선택하여 β- 질소 원자의 양성자 화 정도를 제어 할 수 있습니다.

설계된 산 해리 상수 (pK_a)의 값 적정 방법으로 각 중합체의 pK_a 값을 계산하고 표적 카르 복실 산의 pK_a 값과 비교 하였습니다. 결과적으로 아조 발색단의 양성자 화 정도는 프로브 및 카복실산의 산 해리 상수 (pK_a) 값에 의존하고, 이들 pK_a는 선택적 분석 물 감지의 메커니즘을 이해하는 중요한 파라미터 일 수 있습니다.

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