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Synthesis and Solvent Dependent Fluorescence of 4-Amino naphthalene-1-sulfonic acid (AmNS)-Alginate (ALG) Bacterial Polymer

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الملخص

تم تصنيع رقائق البوليمر البكتيرية 4-أمينو النفثالين-1-حمض السلفونيك-ألجينات (AmNS-ALG) وتم دراسة خصائصها الطيفية. تمت مراقبة سلوك المذيبات الطيفية للبوليمر الفلوري في مذيبات ذات قطبية متنوعة باستخدام أطياف الإثارة والانبعاثات الفلورية جنبًا إلى جنب مع قياسات عمر الفلورة. أظهر البوليمر المحضر تجاوب مختلف في انبعاث الفلورة بتغيير قطبية المذيب من قطبية عالية (ماء) إلى قطبية منخفضة (بيوتانول). زادت الإثارة وشدة الانبعاث لـ AmNS-ALG بشكل ملحوظ في الماء مقارنة بالمذيبات الكحولية. يشير قياس العائد الكمي إلى أنه تم الحصول على أعلى قيم للكفاءة الكمية في المزيد من المذيبات القطبية. بالنسبة لـ AmNS-ALG في الماء والبيوتانول، تم تقليل أكبر قيمة عمرية من 11 إلى 7 نانو ثانية. تشير البيانات الطيفية التي تم الحصول عليها إلى أنه يمكن استخدام AmNS-ALG كمستشعر قطبية.

الكلمات المفتاحية: مطيافية الفلورة (التألق)، سلوك المذيبات الطيفية، الخصائص الطيفية، AmNS، البوليمرات البكتيرية.

Abstract

4-Amino naphthalene-1-sulfonic acid-alginate (AmNS-ALG) bacterial polymer flakes were synthesized and their spectroscopic properties were investigated. The solvatochromic behavior of the fluorescent polymer was monitored in solvents of diverse polarity using the excitation and emission fluorescence spectra combined with fluorescence lifetime measurements. The AmNS-ALG exhibited a positive solvatochromism with regard to the change of solvent polarity from high polar (water) to low polar (butanol). The excitation and emission intensities of AmNS-ALG increased markedly in water compared with alcoholic solvents. Measuring the quantum yield indicated that the highest values of quantum efficiency were obtained in more polar solvents. For AmNS-ALG in water and butanol, the greatest lifetime value was reduced from 11 to 7 ns. The obtained spectroscopic data suggest that the AmNS-ALG could be used as a medium polarity sensor.

Keywords: Fluorescence spectroscopy; Solvatochromic behavior; Spectral parameters; AmNS, bacterial polymers.

1. INTRODUCTION

In order to monitor additives in situ, such as alcoholic content in an aqueous system, analytical methods that operate in real-time and in completely safe conditions must be developed. The use of polymer in conjunction with chemical indicators has lately resulted in the development of a new class of polarity sensors known as Solvatochromic sensors (SS) [1, 2]. A fluorescent molecule having possible optical properties such as absorption or fluorescence is covalently attached to a polymeric system in the solvatochromism phenomenon [3]. When an SS is placed in a liquid medium to be investigated, the fluorescent probe undergoes a photophysical reaction and generates an optically detectable modification which is characteristic of the chemical composition of the fluorescent molecule [4, 5]. In an absorption-based SS, for example, the absorption of an input light beam by the fluorescent molecule is recorded after the reflection of the beam on a mirror as an output absorption signal**.** Since alcohol-water mixtures improve the solubility of hydrophobics in an aqueous medium and consequently, these mixtures play a significant role in the examination of the stability of a hydrophobic solution and protein folding [6]**.**

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The polarity gradient of the solution can straightforwardly be sensed by using the well-known solvatochromic effect, which consists of the distinct shift of the longest wavelength absorption band of a fluorescent molecule following a minor difference in the polarity of the solvent[7, 8, 9, 10, 11]. The effect of numerous parameters in addition to possible applications of organic solvatochromism has been widely explored in literature [12, 13, 14, 15, 16, 17, 18, 19]. As a result, we have decided to create an SS based on the solvatochromic effect. Preparing a solvatochromic molecule that can be attached to a polymer-producing polymeric flake**.**

For some biophysical studies, the biomolecule must be labeled with a tiny foreign molecule with distinct photophysical properties that forms covalent or noncovalent bonds with the macromolecule [4, 5, 20, 21, 22]. This fluorescent molecule, referred to as a spectroscopic probe, functions as a sensor for changes in the biomacromolecule, translating them into changes in the photophysical characteristics of the biomacromolecule [23]. In scientific studies, labeling biomacromolecules with fluorescent probes is a frequent procedure. This technology enables the investigation of a wide range of structural characteristics, structural change dynamics, and molecular interactions [24].

In this study, since alginate is non-fluorescent, 4-amino naphthalene-1-sulfonic acid (AmNS) was selected as a fluorescent probe (see Scheme 1). The fluorescently labeled alginate was studied by absorption and fluorescence

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spectroscopy. With the aim of introducing a molecular-level detection of solution polarity, this paper examines the influence of alcoholic chemical composition on the spectral behavior of alginate using fluorescence spectroscopy. An approach was established involving the synthesis of fluorescently labeled ALG, and the use of fluorescence lifetime to monitor the conformational behavior of this biopolymer up on altering the alcoholic solvent. This was achieved using the fluorescent-labeled 4-amino naphthalene-1-sulfonic acid (AmNS), which was covalently bound to the ALG backbone.

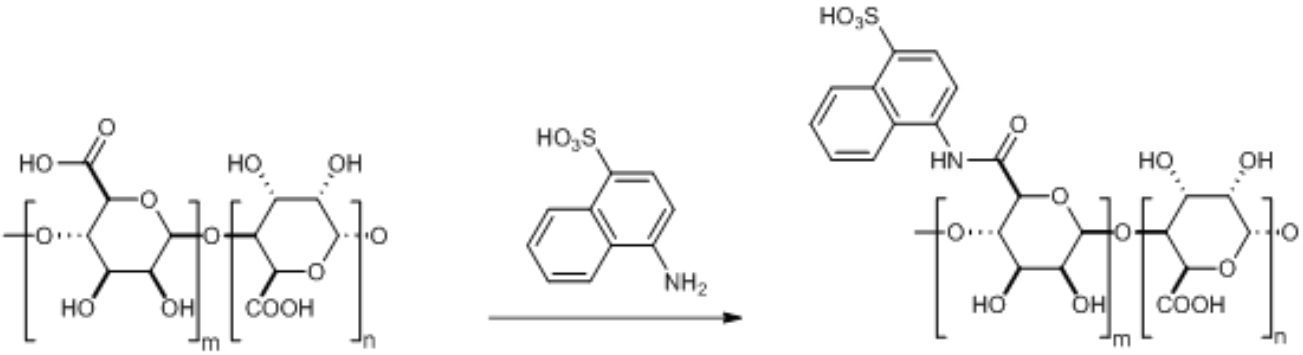
2. EXPERIMENTAL

2.1 Materials

Alginate (Sigma-Aldrich) Methanol (Aldrich), butanol (Aldrich), glycerol (Aldrich), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (Fluka, ≥99.0%) Used as received, without any further purification. N-hydroxysuccinimide (NHS) (Aldrich, 98%). Used as received, without any further purification, Water (double distilled)**,** Hydrochloric acid (Fluka, ≥ 37%), Sodium hydroxide (Sigma-Aldrich, ≥ 97%), Tri ethylamine (Aldrich, 99.5%)**.** All other solvents utilized in this work were of spectroscopic grade. 4-amino naphthalene-1-sulfonic acid (AmNS) (Aldrich, 97%) 4-Amino naphthalene-1-sulfonic acid (AmNS) was purified by extraction with hexane, the residue was detached, and the produced material had a melting point in the range of 300-302 °C, which had been dried under vacuum over $CaCl_2$. Then it was kept in a dark-colored container, in a cold place. The yield of pure AmNS was 74 %.

2.2 Synthesis of AmNS-labelled alginate

Firstly, ALG was purified by dissolving 5 g in 0.5 M NaOH and stirring for about two hours. Then 0.5 M HCl was dropped to pH 2-3 and stirred for a whole day. The formed gel was filtered and washed with ethyl alcohol. The produced solid was then dried in a vacuum oven at 40 °C overnight. Secondly, ALG was fluorescently labelled with AmNS according to a protocol taken from the literature [25]. 18 mmol monosaccharide was mixed with 80 ml water, 30 ml of 1,4-dioxane, 0.15 mmol 4-Amino naphthalene-1-sulfonic acid and 7.8 mmol N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride. The mixture was stirred for 180 minutes and left overnight till an orange solution was observed. The mixture was filtered and washed with acetone until the acetone was colorless. The yellow powder was dried to yield a constant weight of the product.



Scheme 1. Chemical structure of AmNS-labelled alginate, where n is significantly larger than m.

2.3 Ultrafiltration

Typically, ultrafiltration was used to remove substances with low molecular weight. A 300ml stirred cell (Millipore, UK) with 70mm cellulose 1000 and 3000 MWCO filters was used for ultrafiltration at 400 kPa nitrogen pressure. The procedure took about an hour and resulted in a final volume of 20-50ml. The procedure was repeated several times with more solvent, and the solvent was removed from the concentrate by freeze-drying.

2.4 Freeze-drying

Freeze-drying is a technique used to remove residual solvent from a polymer, resulting in a dry powder that can be safely stored. Liquid nitrogen was used to freeze the dissolved polymer. The solvent sublimated and was vacuumed away, leaving behind a dry powder polymer.

2.5 Ultraviolet spectroscopy (UV)

UV spectra were obtained with a Hitachi U-2010 spectrometer to measure the amount of fluorescent label ((mol %)fluorophore) in the polymers. With a scan speed of 400nm/min, the scan ranged from 450 nm to 200 nm. The slits were made at a depth of 2 nm. Fluorophores were tested at various concentrations in spectroscopic grade methanol, whereas polymer solutions containing 10^{-1} wt% of polymer in deionized water and measured at room temperature. The following equation was used to compute the fluorescent label's mol percent [26]:

$$(\text{mol}\%)_{\text{fluorophore}} = \frac{C_{\text{fluorophore}}}{C_{\text{fluorophore}}+C_{\text{Monomer}}} x\,100 \quad (1)$$

Where, $C_{fluorophore}$ and $C_{Monomer}$ are the molar concentration of the fluorescent label and the native monomer, respectively. A more accurate concentration (C) can be determined mathematically from Beer-Lambert's law for the calibration curve as flows [23]:

$$A = \mathcal{E}bC \quad (2)$$

Where A is the absorbance value of fluorophore, $\mathcal{E}$ is the molar absorptivity; b is the bath length of the cell, equal to 1 *cm* and C is the concentration of fluorescent label in solution.

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2.6 Fluorescence Analysis

Fluorescence steady-state measurements were carried out on a Perkin-Elmer LS50 Luminescence Spectrometer or Spectrofluorometer FluoroMax®-4. To detect AmNS the samples were excited at 320 nm and the emission range was 360-510 nm. Excitation scans from 260-380 nm were applied with 420 nm emission wavelength. Emission and excitation slits were set between 2.5 and 4nm depending on the analyzed sample. Ten accumulation scans were made ten times for each reading to ensure smooth spectra. Fluorescence excited state lifetime was carried out via an IBH 5000 system and Edinburgh Instruments 199 Fluorescence Spectrometers, respectively. Samples were excited at 370 nm with the monochromator set to detect fluorescence at 450 nm. The range was 100ns, and 30,000 counts were obtained for each sample in lifetime measurements. A prompt was run after each sample to take into account scattered light from the source during fluorescence analysis. Fluorescence measurements were all made using 10^{-1} wt% polymer solutions.

3. RESULTS AND DISCUSSION

The condition for the development of a solvatochromic sensor made from biopolymer flakes is the attachment of a chromophore like AmNS and generating wavelength scan in which the chromophore moiety has distinguishable peaks compared with native biopolymer. The condition is met here, as shown in Fig. 1, because AmNS-alginate spectra contain distinct bands when compared to alginate, and AmNS-alginate has two peaks at 243 nm and 315 nm. Because of the clear influence of the alginate band, which has a peak at 216 nm and might be assigned to the carbonyl group of ALG, the 243 nm peak was excluded from the computation. For the attached chromophore analyses based on UV spectroscopy, only the absorbance values at 315 nm were considered. It is worth noting that the second peak (315 nm in AmNS-alginate) was shifted to a shorter wavelength compared to AmNS alone in solvent, which has a maximum absorbance at 320 nm for the second peak (see Fig. 2), this supports that a covalent attachment might occur between the chromophore and biopolymer molecule. Fluorescent label content: $(\text{mol }\%)_{\text{AmNS}}$ = 0.96 mol% was calculated by using equation (1), this small value is preferred to prevent excimer formation[23]. Moreover, these analyses suggest that the molar absorptivity of the AmNS group in the ALG is close to that of AmNS chromophore alone. The concentration of AmNS group in 10^{-1}wt% AmNS-alginate solution was calculated from the measured absorbance (A) at 315 nm according to equations (2). The molar absorptivity of the AmNS group, which can be calculated from Beer-Lambert's law for the calibration curve as shown in Fig. 2, a typical linear relationship was plotted with slope equal to 0.107, by applying an equation (2) that gives a molar absorptivity equal to 10700 L $\text{mol}^{-1}\cdot\text{cm}^{-1}$.

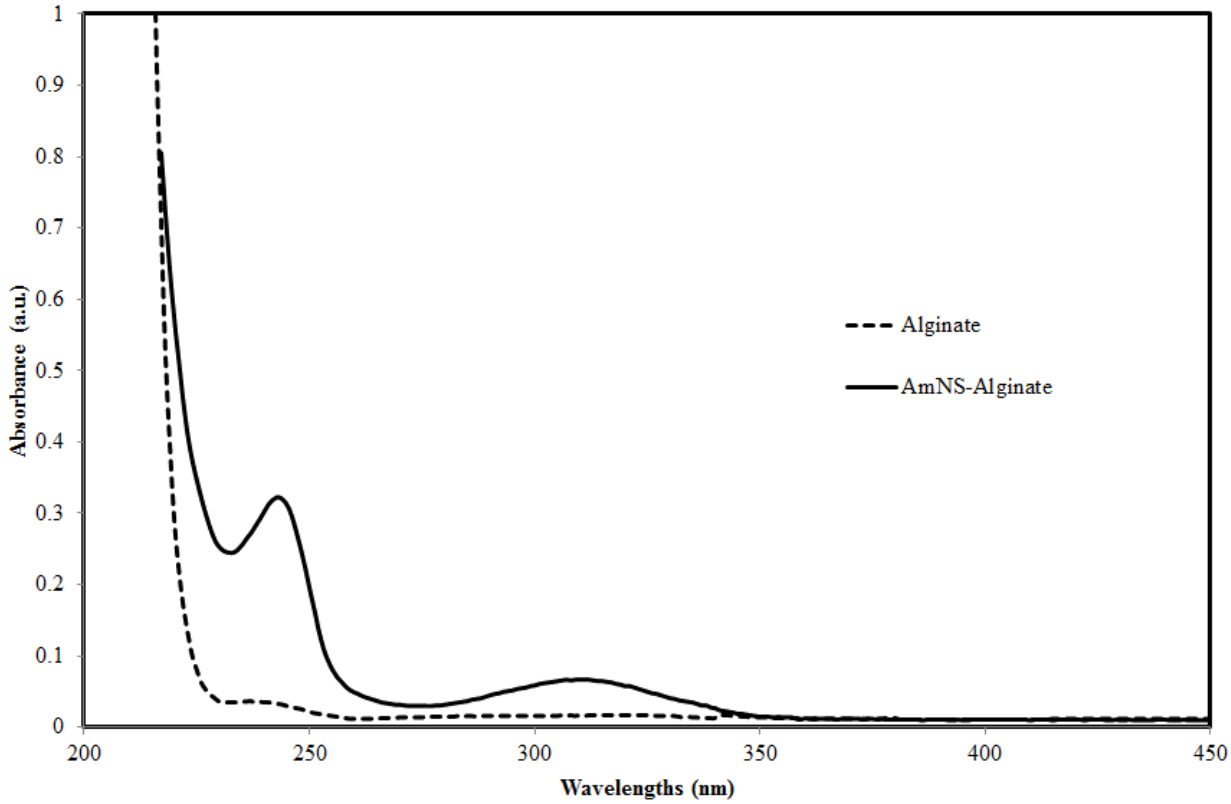


Fig 1. UV-Absorption spectra of 10-1 wt% AmNS-labelled alginate and alginate

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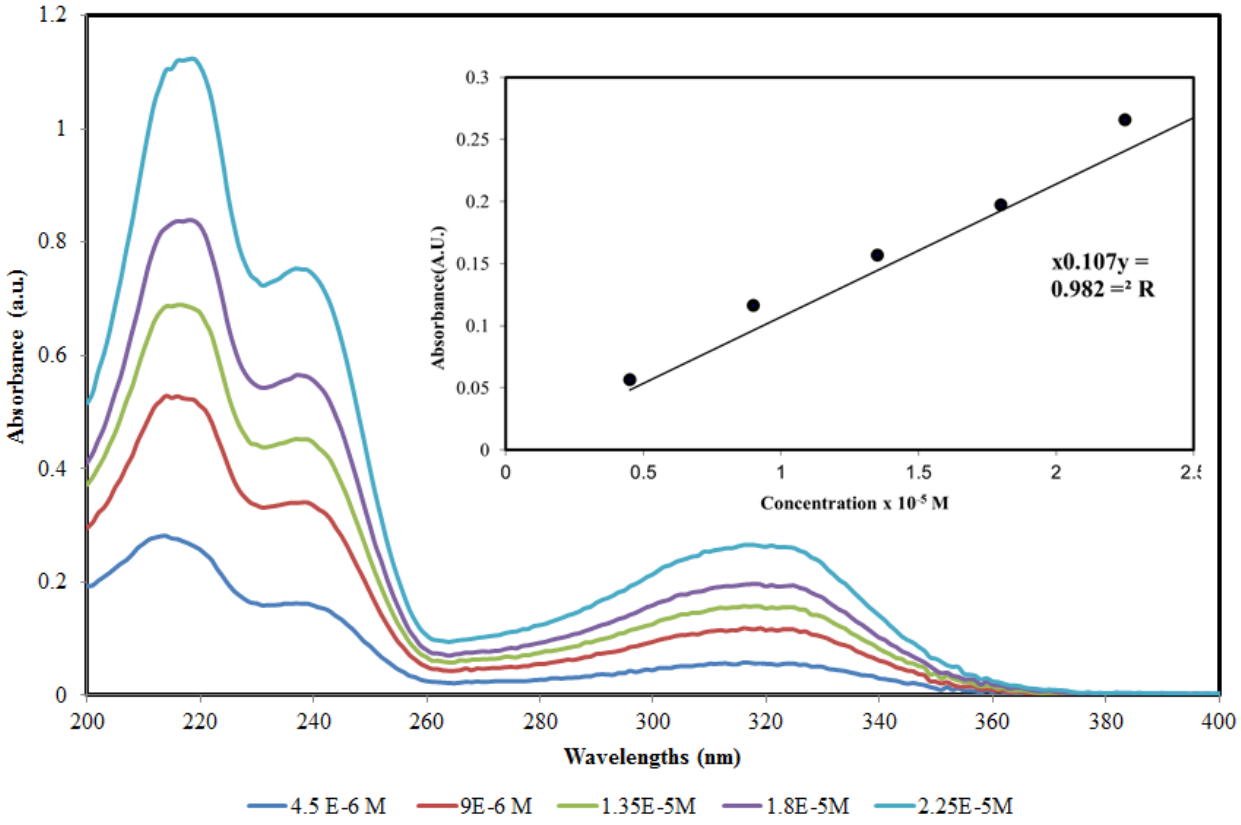


Fig 2. Beer-Lambert's law of AmNS at different molar concentrations in an alcoholic solution. (λmax = 320 nm)

The photophysical behavior of AmNS-ALG under study in terms of fluorescence absorption maxima (λ_{ex}), emission maxima (λ_{em}), Stokes shifts ($\delta\lambda$), and fluorescence quantum yields (Φ) were examined in solvents with different polarities. The fluorescence excitation and emission spectra are depicted in Figs. 3 & 4, and corresponding spectral results are listed in Table 1. The excitation spectra of AmNS-ALG show a broad absorption band in the ultraviolet range, with its maximum excitation wavelength located at 325 nm in water and 429 nm in butanol. This absorption band could be assigned to a π-π^* transition owing to the naphthalene group [27]. Overall, a shift to a shorter wavelength of the excitation band was observed as the solvent polarity decreased as shown in Table 1. While a redshift of the position of the excitation band of AmNS-ALG was observed for glycerol relating to less polar solvents (methanol and butanol). As can be also noted from the data included in Table 1, the effect of solvent polarity is more obvious on the emission bands than on the absorption bands. The fluorescence emission spectra of AmNS-ALG generated by excitation in the absorption band maximum exhibit an emission band in the range 350–550 nm depending on the solvent polarity (Figure 4 and Table 1). Specifically, AmNS-ALG shows a maximum emission at 420 nm in water, which is blue-shifted to 409 nm in butanol. The AmNS fluorophore exhibits very strong emission in alcoholic solvents (methanol, Φ = 0.954 and butanol, Φ = 0.957, while in more polar solvents like water, the quantum efficiency of emission decreases considerably (water, Φ = 0.773 (Table 1).

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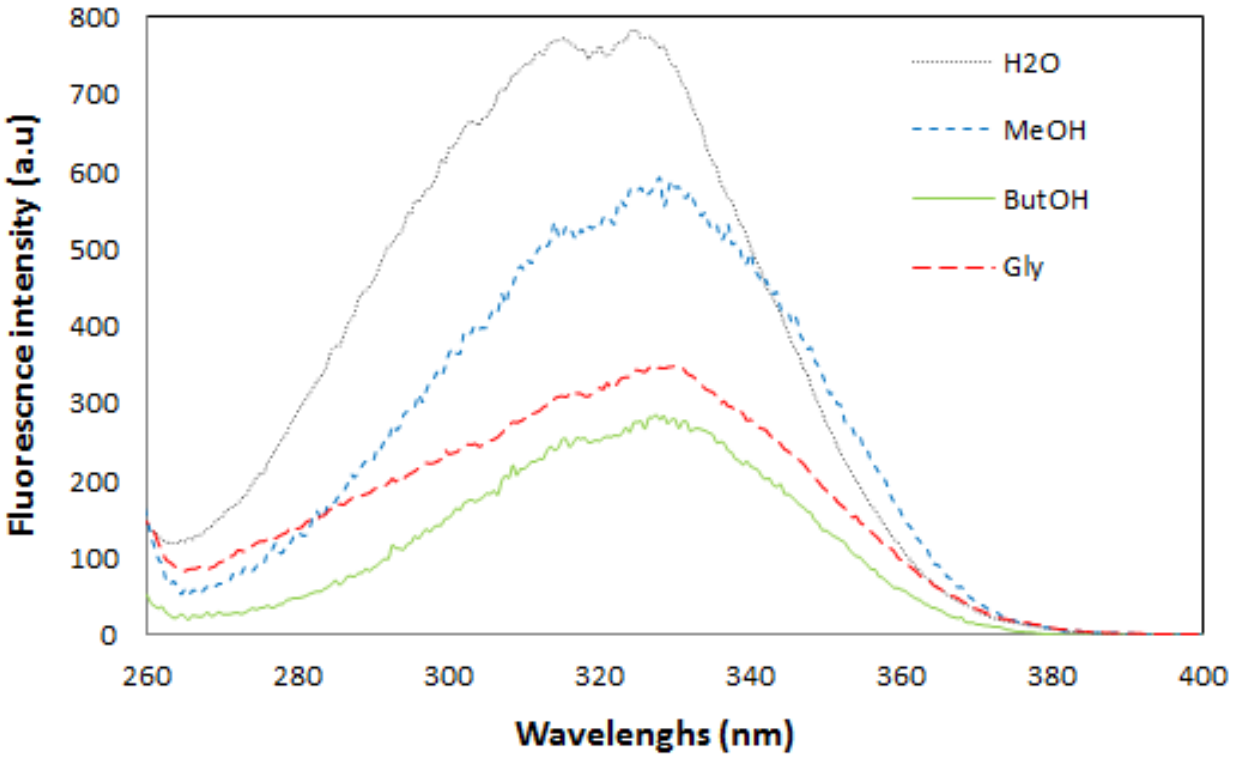


Fig 3. Dependence of fluorescence excitation of AmNS-ALG on solvent polarity. Equal amounts of AmNS-ALG (10-1 wt%) were taken in water (H2O), methanol (MeOH), butanol (ButOH), and glycerol (Gly) and in the fluorescence excitation Scanned for each sample.

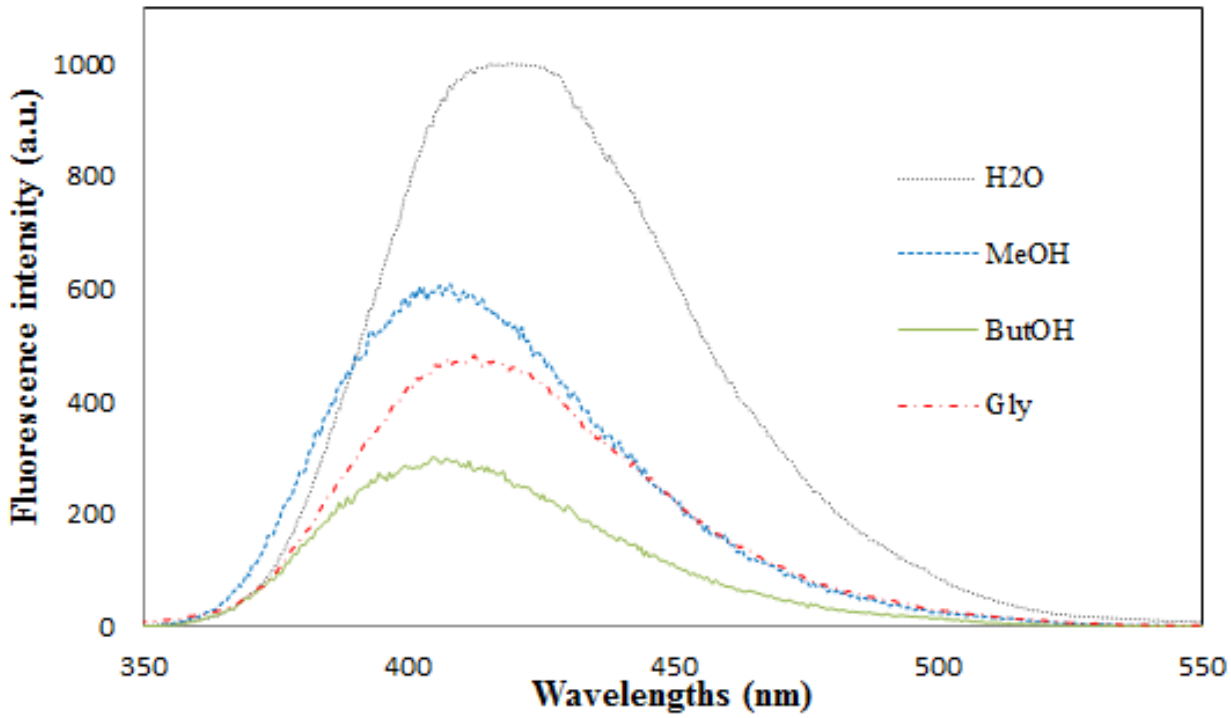


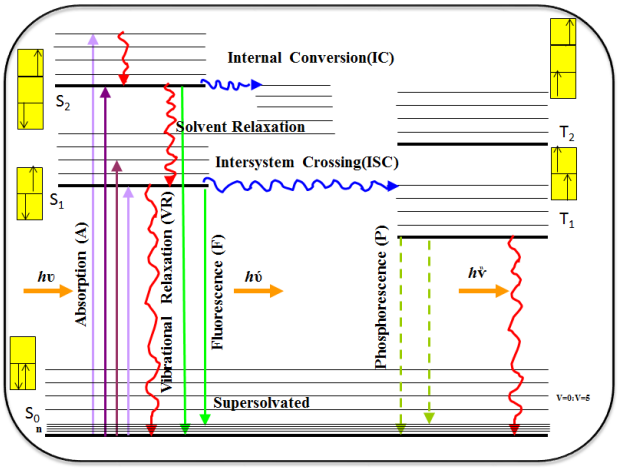
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Table 1. The relation between the relative polarity of solvents with the maximum excitation and emission wavelengths.

Solvent	Relative polarity*	λ_{ex} (nm)	λ_{em} (nm)	$\delta\lambda$ (nm)	ϕ	τ (ns)
Water	1.000	325	420	95	0.773	11.40±0.02
Methanol	0.762	328	408	80	0.954	6.00±0.02
Butanol	0.586	329	409	80	0.746	7.00±0.02
Glycerol	0.812	331	413	82	0.957	7.80±0.02

Obtained results are in agreement with the literature [6, 28] since several chromophores' fluorescence has been studied in a variety of solvents and solvent combinations. Transferring a dye from a non-polar to a polar solvent causes a red shift in the fluorescence emission maxima, an increase in bandwidth, and a swift decline in the quantum yield, while fluorescence absorption is only slightly affected by solvent polarity. Based on a hypothesis presented by Lippert [28] and independently by Mataga [28], the dependency of emission maximum on solvent can be understood [28]. Solvent molecules can relax around the excited state before emission if the dipole moment of the excited state is greater than the dipole moment of the ground state. As a result, the energy of the excited state will tend to decrease, resulting in a red shift in fluorescence emission. This procedure is depicted in Scheme 2.



Scheme 2. A Jablonski transition state diagram showing solvent relaxation and intersystem crossing.

To find out more information about the excited state dynamics of AmNS-ALG, a fluorescence lifetime experiment was carried out on polymer in solvents of different polarities. Demonstrative decay profiles of AmNS-ALG in water, methanol, butanol, and glycerol are presented in Fig. 5. The values of the fluorescence lifetimes generated from exponential decays are listed in Table 1. As can be observed, the lifetime values were markedly influenced by the solvent polarity. All decays were mathematically fitted by a single-exponential decay. The gained lifetime was around 11 ns for AmNS-ALG in the high-polar solvent (Table 1). This value was quenched to 7 ns in a low-polar solvent (butanol).

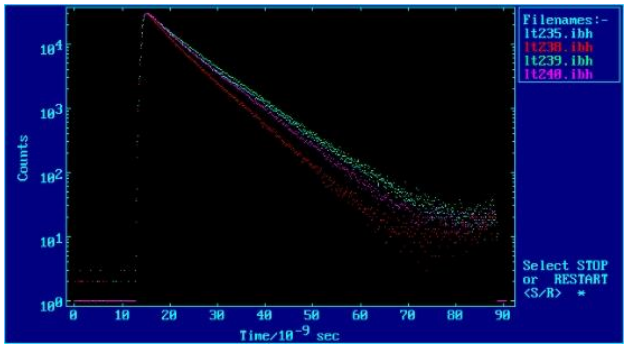


Figure 5. Fluorescence decay lifetimes of AmNS in different solvents.

4. CONCLUSION

Fluorescent 4-Amino naphthalene-1-sulfonic acid-alginate bacterial polymer (AmNS-ALG) was synthesized and its spectral excitation and emission properties were examined. AmNS-ALG had high fluorescence intensity in a high polar solvent (water) and the excitation and emission intensities decreased sharply in less polar solvents (methanol and butanol). When AmNS-ALG was dissolved in water, the quantum yield (ϕ) was steadily quenched compared with methanol and glycerol. The spectral parameters such as fluorescence absorption maxima (λ_{ex}), emission maxima (λ_{em}), and Stokes shifts ($\delta\lambda$) revealed that the solvent polarity had a significant influence on the solvatochromic shifts of the excitation and emission maxima. The fluorescence excited-state lifetimes of AmNS-ALG were noticeably affected by the solvent polarity. The highest lifetime value declined from 11 to 7 ns for AmNS-ALG in water and butanol, respectively. The gained spectroscopic results propose that the AmNS-ALG can act as a possible sensor detecting medium polarity.

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