Mapana – Journal of Sciences
2025, Vol. 24, No. 1, 41-50
ISSN 0975-3303 | https://doi.org/10.12723/mjs.72.3

Fluorescence Quenching Analysis of 6MNPM Molecule by Steady State Method

Srinath*, Dayanand Lalasangi*, Vijayalaxmi Mallayya*,
S. M. Hanagodimath*

Abstract

The current work uses aniline as a quencher in a variety of solvents to investigate the room-temperature fluorescence quenching of a physiologically active fluorescent probe, namely "6-Methoxy-4-(4-nitro-phenoxy methyl)-chromene-2-one (6MN PM) coumarin molecule". To understand its behaviour in various media, several solvents with different dielectric constants and refractive indices have been used. Spectroscopy techniques and time-correlated single photon counting were used to describe the absorption spectra, emission spectra, and lifespan value of a molecule. From fluorescence quenching analysis, we find that the S-V curve shows a linear dependence in given solvents with various dielectric values. It is shown that quenching responses are dynamic; many forms of quenching have been identified, and the relevant parameters have been assessed.

Keywords: Quenching, Steady-state, Stern-Volmer equation, Fluorescence lifespan, Activation energy.

1. Introduction

Coumarins are members of the benzopyrone class. They are made up of a pyrone ring and a benzene ring attached. In the UV/Vis spectrum, coumarins make good fluorescent probes. Fluorescence quenching investigations reveal several photophysical features of organic molecules, including well-known spectroscopic properties that shed light on membranes, proteins, and macromolecular assemblies. In bioresearch and other domains, organic fluorophores are highly useful. They are commonly used to mark target molecules and are employed as chemical and biological sensors [1-3]. Scientific observations and research investigations demonstrate coumarins' outstanding medicinal and therapeutic effects. This leads to the use of coumarin derivatives in pharmacological and therapeutic applications, such

* Department of PG Studies and Research in Physics Gulbarga University, Kalaburagi – 585 106, Karnataka, India; srinathmore@gmail.com

as chemical sensors [10], antioxidants [4], anticancer [5], anti-neoplastic [6], antibacterial [7, 8], and antineuronal degenerative [9]. Furthermore, derivatives based on coumarins have superior charge transfer characteristics and find application in molecular electronics [11]. Nevertheless, there are no studies on the fluorescence quenching of the "6-Methoxy-4-(4-nitro-phenoxy methyl)-chromene-2-one" (6MNPM) molecule in the literature. As the quencher ratio changes in various solvent media with the solute, the acquired data provide crucial information on how the quencher affects the emission intensity of the 6MNPM molecule. Understanding the physical and chemical system of the molecules and providing some crucial information that can assist in understanding the molecules are the objectives of the fluorescence quenching of coumarin composites. Molecular rearrangements, energy transfer, ground-state complex formation, collisional quenching, and other chemical interactions are the root causes of fluorescence quenching [12]. The quenching reaction can also be influenced by the quencher's composition and the polarity of the various solvent mediums [13]. Stern-Volmer kinetics are used to quantify quenching efficiency and various quenching responses [14], and the nature of S-V plots helps to understand the quenching mechanism. They can be positive or negative deviations, or they could be straight lines. Examining the quenching of fluorescence in heterocyclic organic compounds has become a crucial spectroscopic technique for studying biological and metabolic processes. The molecule fluorescence intensity decreases due to resonance energy transfer, chemical rearrangements, and interactions between excited and ground states. CCl_4, aniline, bromobenzene, metal ions, and other well-known quenchers were used to investigate the quenching in the solution media [15].

Experimental Details

Materials and methods

The molecule 6MNPM was prepared using the literature method [16]. The compound structure of 6MNPM is presented in Fig. 1. At room temperature, the fluorescence quenching experiment was carried out in solvents with varying dielectric constant. All the solvents are of spectroscopic grade. Acetone, acetonitrile, toluene, tetrahydrofuran, and dioxane were used to perform the experiment, and all the solvents were procured from S.D. Fine Chemicals Ltd. India. The experiment was carried out at the fixed solute concentration of 1×10^{-6} M. The concentration of the quencher varies in the ratio of 0.02 M, i.e., the higher concentration is at 0.1 M, and the lowest concentration is at 0.02 M in the different dielectric constants of the solvents.

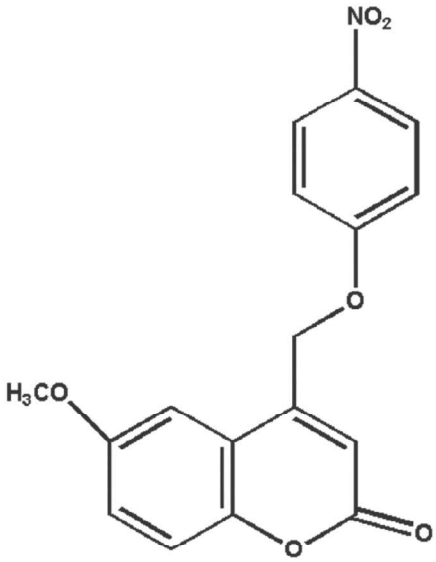


Fig. 1: 6MNPM Molecule molecular structure.

Characterisation Methods

Absorption Spectra

Using a deuterium lamp light source and an identical beam Hitachi UV-Vis Spectrophotometer (model: U-3310), the absorption spectra of the 6MNPM molecule were recorded, and a 0.5 nm wavelength accuracy. Every time, a 5 mm quartz cuvette cell was used to collect a newly prepared sample. Before collecting data, the equipment was calibrated.

Fluorescence Spectra

A spectrophotometer has a spectral range of 200–800 nm, and it is placed perpendicular to the sample geometry. The bandwidth of the instrument was kept at 1 nm, and it was used to record the fluorescence spectra of the 6MNPM molecule in various solvents. The instrument was powered by a 500 volt, and a Xenon lamp was used as the light source.

Fluorescence Lifetime

To measure the fluorescence lifetime (τ) of the molecule the Time-Correlated Single Photon Counting (TCSPC) spectrometer (Model: F-7000) was used. The diode laser source is employed with an excitation wavelength of 330 nm, and the instrument specifications are tuned to the lifespan range of 10^{-12} - 10^{-2}s. It includes a photomultiplier tube detector, which detects light in the 200 - 750 nm range. At room temperature, we set up an emission wavelength of 443 nm with and without a quencher to record the lifespan decay curve of the 6MNPM molecule.

Results and Discussion

The usual absorption and emission spectra of 6MNPM molecule were registered at room temperature to investigate the quenching process. The absorption and emission spectra of 6MNPM molecule in various solvent conditions are shown in Fig. 2 (a) and (b). As the concentration of quencher (aniline) increases, a slight decrease in the absorption spectra and a significant decrease in the fluorescence spectra's intensity are seen, respectively. Additionally, it is noted that the band maxima and spectra shape have not changed significantly in fluorescence spectra as a result, the current system does not show exciplex formation. The absorption spectra of the molecule explain that the aniline at a higher concentration (0.10M) shows a wavelength of 290 nm approximately. It explains that aniline (quencher) dominates the system rather than the solute at that concentration. In addition, a lifespan decay curve was evaluated in various solvents, with and without quencher. The lifespan decay curve of the 6MNPM molecule in the specified solvents is displayed in Fig. 3.

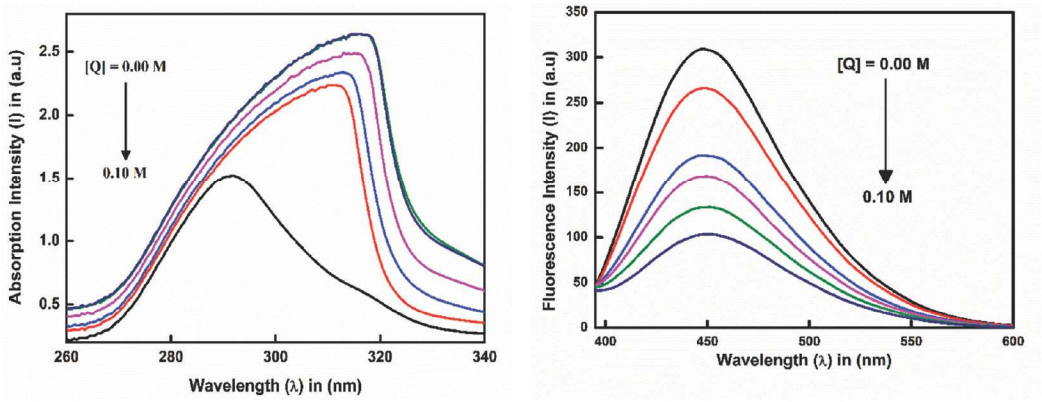


Fig. 2: Absorption and fluorescence spectrum of 6MNPM molecule with different concentrations of aniline in acetonitrile.

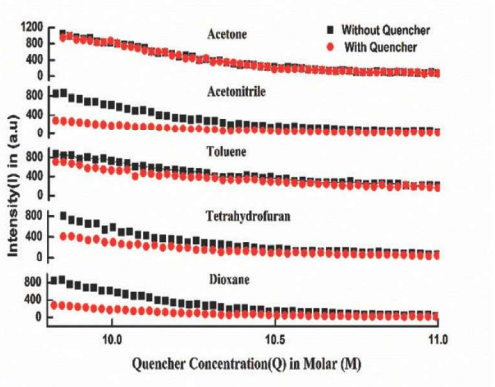


Fig. 3: The fluorescence decay profile of 6MNPM molecule.

Stern - Volmer (S-V) Plots

"The S-V plot has been generated for 6MNPM molecule in a steady state to understand the nature of quenching involved in the quenching process". In every single solvent, it is linear. Table 1 demonstrates the intensity of fluorescence of the specified molecule concerning the steady-state quencher concentration in various solvents. The K_{sv} values obtained using the following Eq. (1), are displayed in Table 2.

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \qquad 1$$

Table 1: Fluorescence intensity (I) of the 6MNPM molecule as a function of quencher (aniline) concentration [Q].

Quencher Concentration [Q] in molar (M)	Acetone		Acetonitrile		Toluene		Tetrahydrofuran		Dioxane	
	I_o	I_o/I	I_o	I_o/I	I_o	I_o/I	I_o	I_o/I	I_o	I_o/I
0.00	404	-	309	-	130	-	583	-	330	-
0.02	346	1.16	266	1.16	101	1.28	463	1.25	280	1.17
0.04	310	1.30	193	1.60	088	1.47	395	1.47	270	1.22
0.06	290	1.39	168	1.83	080	1.60	321	1.81	251	1.31
0.08	253	1.59	154	2.00	075	1.73	300	1.94	224	1.47
0.10	210	1.92	144	2.14	068	1.91	281	2.07	195	1.69

Table 2: The following variables are measured for the 6MNPM molecule in various solvents:

steady-state value of S-V constant (K_{sv}), quenching rate parameter (K_q), frequency of encounter (K_d), probability (p), and activation energy for diffusion (E_d) and activation energy (E_a).

Solvents	$K_{sv} M^{-1}$	K_q $(M^{-1}s^{-1})$ 10^9	K_d $(M^{-1}s^{-1})$ 10^9	p	E_d (kcal mole^{-1})	E_a (kcal mole^{-1})
Acetone	08.62	04.61	23.70	0.19	3.56	3.22
Acetonitrile	11.75	06.09	20.10	0.30	2.90	1.88
Toluene	07.60	01.01	04.18	0.24	2.66	2.59
THF	10.56	07.04	16.80	0.41	2.45	0.74
Dioxane	06.23	01.27	08.85	0.14	3.06	4.05

THF- Tetrahydrofuran

When measuring fluorescence intensity under steady-state conditions, equation (1) is helpful. It is also known as the Stern-Volmer equation. Where $K_{sv} = K_q \tau_o$ was obtained from a linear S-V plot using Eq (1), K_q symbolising steady-state bimolecular quenching rate parameters. The S-V curve in the present situation shows linearity in various solvents. The 6MNPM molecules' steady states are seen in Fig. 4.

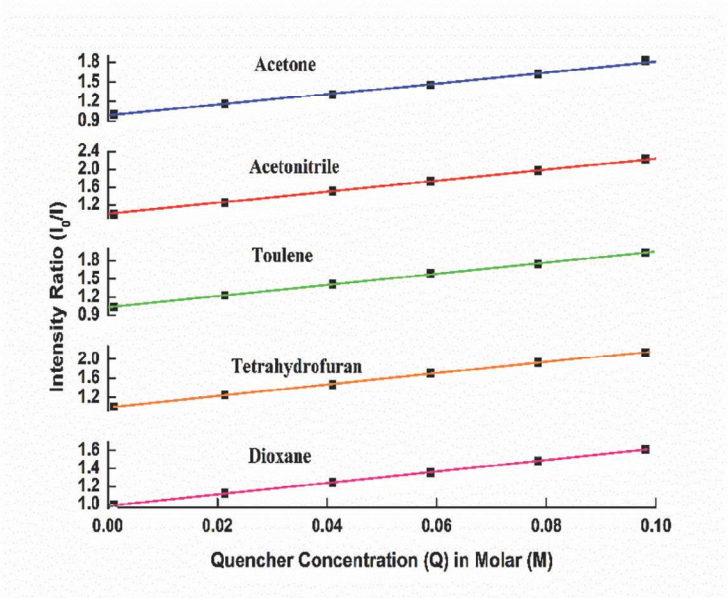


Fig. 4: S-V plots of 6MNPM molecule.

It demonstrates that quenching reactions are only governed by one sort of quenching mechanism [17–21]. For the quenching process with rate parameter 'K_q' to take place, a quencher molecule and an excited solute molecule must first "encounter." Equation (2) is often used to express the steady-state quenching rate.

$$K_q = K_d p \qquad 2$$

when the quenching processes are completely controlled by diffusion (i.e. when p = 1), the frequency of encounters is 'K_d', and the molecule quenching probability per encounter is mentioned as 'P'. The nature of the solute largely determines the value of 'P' for a particular solvent. Consequently, the solvent cannot be the exclusive cause of a quenching reaction.

'P' is highly dependent on the kind of solvent for a given solute. The change in the constants 'K_q' and 'K_d' relative to the solvent's reciprocal viscosity (η) suggests that material diffusion plays a substantial part in the reaction of quenching. The dispersion of material is not the only component; quenching may also be influenced by the activation mechanism. In the steady state of the 6MNPM molecule, several parameters have been determined using Equation (2) and are provided in Table 2. It has been noted that 'P' has a value smaller than unity. It highlights the solute's and the quencher molecule's collisional quenching. 'K_d' is estimated using Equation (3), which is provided as follows [22].

$$K_d = 4\pi N' D R \left[1 + \frac{R}{(2D\tau_0)^{1/2}}\right], \qquad 3$$

where (D) is the mutual diffusion coefficient, (R) is the encounter distance, and (τ_0) the solute's lifespan without quencher. The diffusion coefficient of the solute and the quencher are determined by the Stokes-Einstein number [23].

$$D = \frac{KT}{a\pi\eta R}, \quad 4$$

where the Stokes-Einstein number (a), the viscosity of the medium (η), the absolute temperature (T), and the Boltzmann constant (K). As Edward [24] suggests, the encounter distance may be computed. The ratio of $\frac{K_q}{K_d}$ determined by utilising Eq. (4). It is always smaller than unity, indicating that the diffusion process does not fully regulate the quenching response. Table 3 shows the value of K_{sv}, diffusion coefficient (D), viscosity (η), and lifespan (τ_0) of solute without a quencher along with these values, the quencher radius (R_Q) and the solute radius (R_S) [25] of 6MNPM molecule are provided in the following Table 3.

Table 3: Fluorescence Lifespan (τ_o), Slope (K_{SV}), and Diffusion Coefficient (D) for 6MNPM Molecule.

Solvent	Lifespan (τ_o) in (ns)	Dielectric Constant (ε)	Viscosity η (cP)	K_{SV} M^{-1}	Dy (cm^2s^{-1}) 10^{-5}	D_Q (cm^2s^{-1}) 10^{-5}	**D** (cm^2s^{-1}) 10^{-5}
Acetone	1.87	20.7	0.31	08.62	1.62	2.28	3.90
Acetonitrile	1.93	37.5	0.37	11.75	1.36	1.91	3.27
Toluene	7.50	7.58	1.81	07.60	0.27	0.39	0.66
THF	1.50	2.38	0.46	10.56	1.09	1.54	2.63
Dioxane	4.90	2.25	0.83	06.23	0.60	0.85	1.46

THF- Tetrahydrofuran, R(6MNPM) = R_S+R_Q = 6.80 Å

For the specified compounds, the encounter distance (R) is found to be smaller than the kinetic distance (r). This suggests that processes have occurred within the sphere of action. The activation energy for diffusion in a liquid system can be found using the following equation (5) for the steady state [26-28].

$$E_a = E_d + RT\ ln\ ln\left[\frac{1}{P} - 1\right], \quad 5$$

The diffusion process is important in the quenching mechanism if the activation energy for diffusion is greater than the activation energy (E_d > E_a), then the system is dominated by the diffusion process. If the value (E_d < E_a) is high, then diffusion might not be enough to regulate the quenching response. Activation energies (E_a) have been determined by using Eq. (5) and are given in Table 3; for the 6MNPM molecule, we observed that the

activation energy for diffusion (E_d) is smaller than the activation energy (E_a) of dioxane quenching when compared to other solvents in which quenching takes place. This indicates that the quenching reaction of a molecule is controlled by both the activation process and the dispersion of the material.

Conclusion

In this work, we conclude on a few parameters based on the above discussion. The presence of a quencher efficiently quenches the absorption and emission intensity of the 6MNPM molecule. Using the steady-state approach, it is discovered that the Stern-Volmer plot is linear. It demonstrates the dynamic nature of the quenching response. In emission spectra, there is no secondary band emission for the steadied molecule in the higher wavelength areas. This suggests that the current work does not include the production of exciplexes. "The kinetic distance (r) is found to be greater than the encounter distance (R)". As a result, responses that quench take place inside the action of the sphere. Since there is just a collision between the solute and the probability of quenching of each encounter (P) has the value of less than one for the quencher in each solvent. For the present work, diffusion energy (E_d) has a higher hand in dioxane as compared to the activation energy (E_a). In the 6MNPM molecule, the quenching reaction is dominated by the material diffusion, as well as by the activation process. Hence, the presented work could be used for further application-oriented work in the field of medicine as a drug carrier; the given material could be useful for developing a sensing appliance in many industries.

Acknowledgement: The authors are thankful to M. S. Ramayya Institute of Technology, Bengaluru, for providing the fluorescence characterisation facility and to the University Science Instrumentation Center, Gulbarga University, Kalaburagi, for the essential experimental work.

Author Contributions: Srinath More prepared the materials, collected the data and conducted the analysis. S.M. Hanagodimath conceptualised the work and designed the manuscript. Dayanand Lalasangi and Vijayalaxmi Mallayya gave important feedback and suggestions regarding the work. All the authors approved the final draft of the manuscript.

Funding The authors declare that no funds or grants were received during the preparation of this manuscript.

Data Availability In the current investigation, no datasets were created or examined.

Declarations

Competing Interests The authors declare no competing interests.

References

[1]. Margaret H, Sherlock J, James, Kaminski, 1988 J. Med. Chem. 31 1221-2108.

[2]. Liu C Y, Lin C M, Chem Y J 2012 Toxicol. Appl. Pharm. 259 (2) 219-226.

[3]. Christos A, Mitsos, Alexandros L Zografos 2003 J. Org. Chem. 68 (11) 4567-4569.

[4]. Hoult JR, Paya M (1996) Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. General Pharmacology: The Vascular System 27:713-722.

[5]. Matos M J, Vina D, Vazquez-Rodriquez S, Uriarte E, Santana L (2012). Focusing on new monoamine oxidase inhibitors: differently substituted coumarins as an interesting scaffold. Curr Top Medical Chem 12:2210-2239.

[6]. Matos J, Vazquez-Rodriquez S, Santana L, Uriarte E, Fuentes-Edfuf C, Santos Y, Munoz-Crego A (2013) Synthesis and structure-activity relationship of novel antibacterial agents. Molecules 18:1394-1404.

[7]. Morsy S A, Farahat A A, Naser M N A, Tantawy AS (2017) Synthesis, molecular modeling and anticancer activity of new coumarins containing compounds. Saudi Pharmaceutical Journal 25:873-883.

[8]. Xia L, Wang Y, Hung W, Qian H (2013) Research advance of anticancer drugs with coumarin structures. Chenese Journal of New Drugs 22:2392-2404.

[9]. Munir S, Shah A A, Shahid M, Ahmad M S, Shahid A, Rajoka MSR, Akash MSH, Akram M, Khurshid M (2020) Antiangiogenesis potential of phytochemicals for the therapeutic Management of Tumours. Curr Pharm Des 25:265-278.

[10]. Song Y, Chen Z, Li H (2012) Advances in coumarin-derived fluorescent chemosensors for metal ions. Curr Org Chem 16:2690-2707.

[11]. Fujian Huang, Fan Xia (2018) Fluorescence sandwich assays for protein detection, biosensors based on sandwich assays, 29-45.

[12]. Lackowicz J R, (2006) Principle of fluorescence spectroscopy, 3rd edition. Plenum Press, New York.

[13]. Lackowicz J R, (2006) Principle of fluorescence spectroscopy, 3rd edition. Plenum Press, New York.

[14]. Varsha V Koppal, Raveendra M Melavanki, Raviraj A Kusanur, and Ninganagouda R Patil (2018) Bimolecular fluorescence quenching reactions of the biologically active coumarin composite 2-acetyl-3H-benzo[f] chromen-3-one in different solvents, Luminescence, 1-7.

[15]. Nad S, Pal H (2000) Electron transfer from aromatic amines to excited coumarin dyes: fluorescence quenching and picosecond transient absorption studies. J Phys Chem A 104:673-680.

[16]. Mahantesha Basanagouda, Manohar V Kulkarni, Deepak Sharma, Vivek K Gupta, Pranesha, P Sandhyarani, Vijaykumar P Rasal. J Chem Sci (2009) 121:485-495.

[17]. Nagaraja D, Melavanki RM, Patil NR (2013) Fluorescence quenching of 6BDTC by aniline in different solvents. Cana J Physics 91:966-970.

[18]. Koppal VV, Patil PG, Melavanki RM, Kusanur RA, Patil NR (2019) Solvent effect on the relative quantum yield and preferential solvation of biologically active coumarin derivative. Macro mol Symp 387:1800210-1800212.

[19]. Patil NR, Melavanki Rm, Kapatkar SB, Chandrashekar K, Umapathy S (2011) Fluorescence quenching of biologically active carboxamide by aniline and carbon tetrachloride in different solvents using Stern-Volmer plots. Spectrochim Acta A Mol Bio Mol Soectrosc 79: 1985-1991.

[20]. Andre JC, Niclause M, Ware WR (1978) Kinetics of partly diffusion-controlled reactions. I. Transient and apparent transient effect in fluorescence quenching. Chemical Physics 28:371-377.

[21]. VV Koppal, Raveendra Melavanki, Raviraj Kusanur, NR Patil (2022) Analysis of fluorescence quenching of coumarin derivative under steady state and transient state methods. Journal of Fluo (2021) 31:393-400.

[22]. Wilemski G, Fixman M (1973) General theory of diffusion-controlled processes reactions. Journal of Chem Phy 58:4009-4019.

[23]. Yugerabide J, Dillon MA, Burton M (1964) Kinetics of diffusion-controlled processes in liquids. Theoretical consideration of luminescent systems: quenching and excitation transfer in collision. Journal of Chemical Physics 40:3040-3052.

[24]. Edward J T (1970) Molecular volumes and the stokes-Einstein equation. Journal of Chemical Education 47:261-270.

[25]. Sharma VK, Mohan D, Sahare PD (2007) Fluorescence quenching of 3-methyl-7-hydroxyl coumarin in the presence of acetone. Spectrochim Acta A 66:111-113.

[26]. Williamson B, La Mer VK (1948) The Kinetics of activation-diffusion controlled reactions in solution; the temperature dependence of the quenching of fluorescence. Journal of American Chemical Society 70:717-721.

[27]. Omnath Patil, P.K. Ingalagondi, Shivaraj Gounhalli, and S.M. Hanagodimath. 2020. Dipole moments and quenching of fluorescence of a new coumarin dye. *Canadian Journal of Physics*. **98**(8): 761-769. https://doi.org/10.1139/cjp-2019-0517.

[28]. Srinath, Patil, O., Devar, S. *et al.* Estimation of Electric Dipole Moment by Solvatochromism, Computational Method, and Study of the Effect of Solvents by Preferential Solvation of 6 – Methoxy—4—(4—Nitro—Phenoxy Methyl)—Chromen—2—One (6mnpm). *J Fluoresc* (2024). https://doi.org/10.1007/s10895-024-03955-8