VOLUME - VIII, ISSUE - I - JANUARY - MARCH - 2019
AJANTA - ISSN 2277 - 5730 - IMPACT FACTOR - 5.5 (www.sjifactor.com)

5. Interaction of Tyrosine with 7 Methoxy Coumarin Fluoresces Quenching and Thermodynamics Study

Dr. Rajendra Haribhau Satpute
Dept. of Chemistry, Govt. College of Arts & Science, Aurangabad. (M.S.)

Abstract

Tyrosine is the aromatic amino acid and rich sources of foodas chicken, fish, milk, cheese, peanuts, almonds, beans.7-Methoxy Coumarin (7-MOC) has been widely used as fragrance enhancers in many cosmetics products.Natural sources of Coumarin are plants, food products such as fruits, tomatoes, vegetables and green tea.

The fluorescence spectra were recorded in the range of 280-500 nm at excitation wavelength 275 nm.At the different temperatures the values of binding site indicate the presence of only one binding site for 7- methoxy Coumarin on tyrosine. Values of thermodynamic parameters reveals that the binding process is spontaneous and entropy driven. Hydrophobic force and hydrogen bonding are major factor in the interactions.

Key Words: Tyrosine,7-Methoxy Coumarin,fluoresces quenching,thermodynamics.

Introduction

Tyrosine is the aromatic amino acid generally found in proteins and having absorption in near UV region. Its side chain contains hydrophilic hydroxyl group (Fig 1) attached to benzene ring making its chemical properties somewhat ambiguous. The fluorescence of aromatic amino acids (phenylalanine, tyrosine and tryptophan) and there residues incorporated into a peptide or protein chain is subject of extensive studies because of their use as internal probes in conformational analysis [1, 2].

Tyrosine may found in many high protein food products such as chicken, fish, milk, cheese, peanuts, almonds, beans. Tyrosine was first isolated from Casien in 1849. Tyrosine can also be synthesized from the phenylalanine in the body. It is precursor of the neurotransmitters, epinephrine and dopamine, both of them is extremely important since they transmit nerve impulses and prevent depression. In a study using soldiers, tyrosine proved effective in relieving stress and keeping them more alert [3].

It may reduce stress symptoms like headache, tension and fatigue. Tyrosine is used by the cell to synthesize proteins and also acts as an electron donor in the reduction of oxidized chlorophyll in chloroplast i.e. it has an important role in photosynthesis. A deficiency of tyrosine may causes low blood pressure, low body temperature (including cool hands and feet) [4] and unreactive thyroid.

Molecular formula of tyrosine is $C_9H_{11}NO_3$ having molar mass 181.19 g/mol. The solubility of tyrosine in water is about 0.0453 g/100ml. Molecular structure of tyrosine is shown in below figure (1):

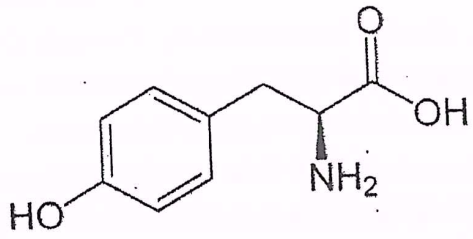

Figure 1: Structure of Tyrosine

7-Methoxy coumarin (7-MOC) has been widely used as fragrance enhancers in many cosmetics products [5]. Coumarin has been obtained in its isolated form from natural sources and some coumarin may synthesize in laboratories [6]. Natural sources of coumarins are plants, food products such as fruits, tomatoes, vegetables and green tea. Specifically 7-MOC have been isolated from various plants such as German chamomiles flowers, flower tops of santolina oblongifolia and leaves of Artemisia druccunculus[7], and it possess anti-inflammatory activity[8]. Coumarins are used in therapeutic ointment preparations [9], also substituted coumarins (i.e. methyl, methoxy or hydroxy) possesses strong hepatoprotective properties [10].

Molecular formula of 7-MOC is $C_{10}H_8O_3$ and IUPAC name is 7-methoxychromen-2-one. Molar mass of 7-MOC is 176.17g/mol and solubility is about 0:133 mg/mol. Molecular structure of 7-methoxy coumarin is given below:

VOLUME - VIII, ISSUE - I - JANUARY - MARCH - 2019
AJANTA - ISSN 2277 - 5730 - IMPACT FACTOR - 5.5 (www.sjifactor.com)

like

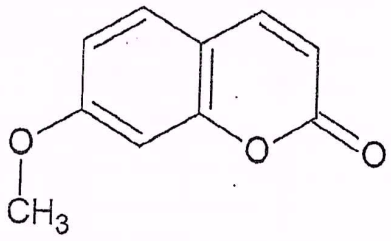

Figure 2: Structure of 7-methoxy coumarin

Efforts were made to investigate the quenching mechanism, binding site, binding mode and evaluation of thermodynamic parameter. From the sign and magnitude of thermodynamic parameters non covalent forces involved in binding were estimated.

Materials and Methods

Apparatus

The fluorescence and fluorescence excitation spectra were recorded on a Jasco-FP-8300 spectrofluorimeter (Jasco, Japan) equipped with a 1 cm quartz cuvette. The width of both excitation and emission slit were set at 5 nm. The absorption spectra of tyrosine and 7- methoxy Coumarin were measured on UV- visible – NIR spectrophotometer (specord 210 plus analytic Jena). The sample masses were accurately weighed using a microbalance(METTLER TOLEDO).

Reagents

Tyrosine,Sodium lauryl sulphate (SDS) and N-cetyl-N,N,N-trimethyl ammonium bromide (CTAB). The stock solutions of TYR and 7-MOC (1×10^{-4}mol dm^{-3}). The fresh surfactant solutions having concentration 1×10^{-2} mol $dm^{-3}$and 1×10^{-3} mol dm^{-3} for SDS and CTAB respectively were used for determining effect of surfactant on fluorescence decay. These values of concentrations are much higher than critical micelle concentrations corresponding to 8×10^{-3} mol dm^{-3} and 8×10^{-4} mol dm^{-3} for SDS and CTAB respectively, to ensure complete micellisation.

The fluorescence spectra were recorded in the range of 280-500 nm at excitation wavelength 275 nm.

Table 1: Solutions of Desired Concentrations are Made as Follows

	Volume of TYR (1×10^{-4}M) ml	Volume of 7-MOC (1×10^{-4}M) ml	Volume of water ml
1	5	-	5
2	5	0.2	4.8
3	5	0.4	4.6
4	5	0.6	4.4
5	5	0.8	4.2
6	5	1.0	4.0
7	5	1.2	3.8
8	5	1.4	3.6
9	5	1.6	3.4
10	-	0.8	9.2

Same procedure was carried out for determining effect of surfactant, only difference here is that instead of water, solutions were prepared in SDS and CTAB medium.

Results and Discussion

UV–visible Absorption Spectra

UV-visible absorption measurement is a common method to explore the structural change [11] and acquire information about extent of interaction. Figure 3 displays UV-visible absorption spectra of Tyr, 7-MOC and their mixture .Tyr exhibits peak at 271nm while 7-MOC shows maximum absorbance at 323nm. Spectra indicate no appearance of any new peak in the mixture. Both the component retains their spectral characteristics in the mixture also. Increase in absorbance indicates the interaction between them.

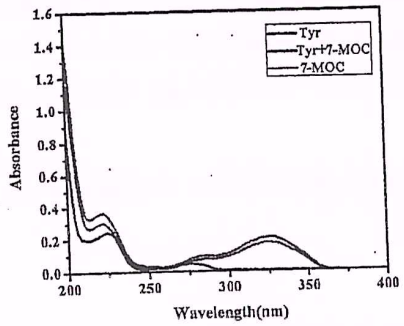

Figure 3: UV- visible absorption spectra of Tyrosine in presence of 7–MOC

VOLUME - VIII, ISSUE - I - JANUARY - MARCH - 2019
AJANTA - ISSN 2277 - 5730 - IMPACT FACTOR - 5.5 (www.sjifactor.com)

Steady State Fluorescence Quenching of Tyrosine by 7-Methoxy Coumarin

A variety of molecular interaction can result in quenching, including excited state reactions, molecular rearrangement, energy transfer, ground-state complex formation and collision quenching. For investigation of the interaction between Tyrosine and 7-methoxy coumarin, the fluorescence emission spectra were recorded upon excitation at 275 nm and 300K. Tyrosine exhibit strong and sharp emission spectra with emission maximum at 303 nm and its fluorescence intensity drops regularly with the addition of increasing concentration of 7-MOC with enhancementin fluorescence intensity of 7-MOC is as depicted in figure 4.

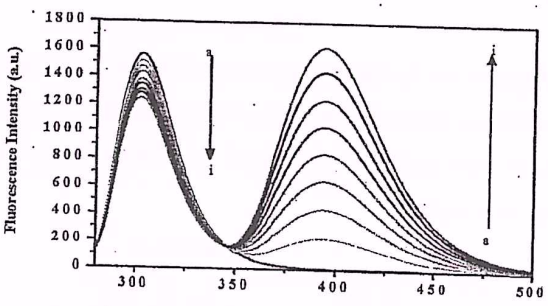

Figure 4: The fluorescence quenching spectra of Tyrosine with increasing concentration of 7–MOC at 300K

Analysis of Quenching Mechanism

Principal Fluorescence quenching mechanisms are dynamic quenching and static quenching or both distinguished by different temperature dependence, viscosity and fluorescence life time [12]. Thus in dynamic quenching bimolecular quenching constant K_{sv} increases whereas in static quenching K_{sv} decreases with increasing temperature.The Stern-Volmer equation is used for analysis.

$$F_0/F = 1 + K_{SV}[Q] \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where, F_0 and F are the fluorescence intensities in the absence and presence of quencher respectively, [Q] is concentration of quencher, K_{sv} is the Stern-Volmer quenching constant. Figure 5 shows the Stern-Volmer plots of quenching of TYR by 7-MOC at different temperatures. The results are in good agreement with the Stern-Volmer equation within the selected concentration range. The plots are linear revealed the occurance of single type of quenching.

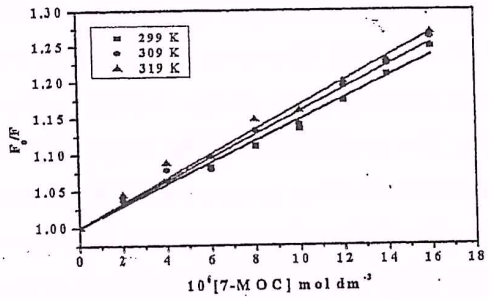

Figure 5: Stern-Volmer plots describing Tyrosine quenching caused by 7–MOC at three different temperatures.

To determine K_{sv} by linear regression of the plot F_0/F against [Q] equation (1) is applied. K_{sv} values at different temperatures along with correlation coefficient R are presented in Table 2. These K_{sv} values were positively correlated with temperature, which indicates that quenching, is dynamic rather than static.

Table 2:

T (K)	K_{sv} (L mol^{-1})	R
299	1.47×10^4	0.99995
309	1.59×10^4	0.99990
321	1.68×10^4	0.99992

3.4 Analysis of Binding Mechanism

When a host molecule independently bind to the guest molecule, the binding constant (K) and number of binding sites (n) can be determined by using following relation.

$$\text{Log } [(F_0\text{-}F)/F] = \log K + n\log[Q] \qquad (2)$$

Values of n and K are determined by plotting graph of log $[(F_0\text{-}F)/F]$ against log [Q], it is shown in figure 6. At different temperatures, values of K and n are determined from the Intercepts and slopes respectively. These values of K and n are depicted in Table 3.

VOLUME - VIII, ISSUE - I - JANUARY - MARCH - 2019
AJANTA - ISSN 2277 - 5730 - IMPACT FACTOR - 5.5 (www.sjifactor.com)

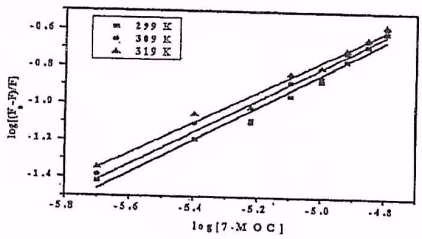

Figure 6: log [(F_0-F)/F] against log [7-MOC] at three different temperatures

It can be seen from the Table 3 that the values of n are approximately equal to unity, this indicates that the single binding sites. The binding constant decreased with temperature it shows the decrease in stability of TYR-7-MOC complex. The unstable compound would be partly decomposed as temperature is raised; therefore the K values are decreased.

Table 3:

Temperature (K)	K($dm^3 mol^{-1}$)	Binding sites N	Correlation coefficient R
299	4.52×10^3	0.89823	0.97938
309	3.87×10^3	0.87794	0.95744
319	2.58×10^3	0.83593	0.98021

Thermodynamic Parameters and Nature of Binding Mode

Four different types of non-covalent interactions exist between fluorophore and quencher during quenching mechanism. They are hydrogen bonding, van der Waals forces, electrostatic and hydrophobic interactions [1]. The main contributing forces of interaction between TYR and 7-MOC are determined from signs and magnitudes of thermodynamic parameters which were determined by using van't Hoff equation [13].

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (3)$$

K, ΔH, ΔS, R and T are the binding constant, enthalpy change, entropy change, gas constant and absolute temperature respectively. Graph of lnK against 1/T is shown in figure 7. Enthalpy change ΔH and entropy change ΔS were calculated from the slope and intercept of the graph .Gibbs free energy change ΔG is calculated by using the equation

$$\Delta G = \Delta H - T\Delta S \qquad (4)$$

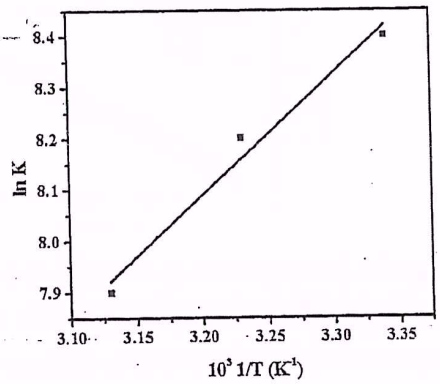

Figure 7: van't Hoff plot for binding of TYR to 7-MOC

The calculated values of thermodynamic parameters are predicted in table 4. Negative values of ΔG indicates that the binding process is spontaneous. According to sign and magnitude of ΔS and ΔH, hydrophobic interaction plays a significant role in the binding between tyrosine and 7- methoxy Coumarin.

Table 4:

Temperature(K)	ΔG (kJmol^{-1})	ΔH (kJmol^{-1})	ΔS (Jmol^{-1}K^{-1})	R
299 309 319	-20.95 -20.99 -21.03	-19.71	4.14	0.95969

Effect of Surfactant

The decrease in quantum yield of the fluorescence from fluorophore induced by variety of molecular interactions with quencher molecules is the fluorescence quenching. From figure 4 we can conclude that, there is successive decrease in fluorescence intensity of TYR with increase in concentrations of 7-MOC in water. The Stern-Volmer plots are linear revealed the occurance of single type of quenching.

Same variation is noticed in fluorescence intensity of TYR with increasing concentration of 7-MOC in SDS and CTAB medium as shown in figure (8 and 9).

VOLUME - VIII, ISSUE - I - JANUARY - MARCH - 2019
AJANTA - ISSN 2277 - 5730 - IMPACT FACTOR - 5.5 (www.sjifactor.com)

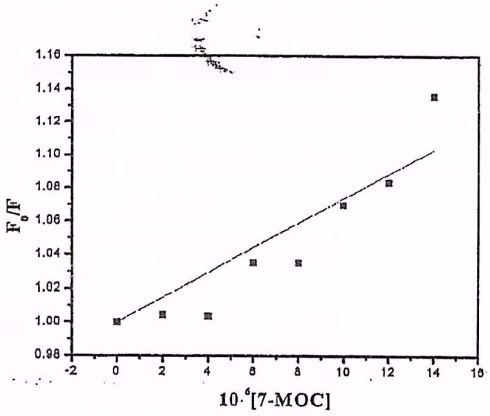

Figure 8: Stern-Volmer plot for TYR-7-MOC system in SDS

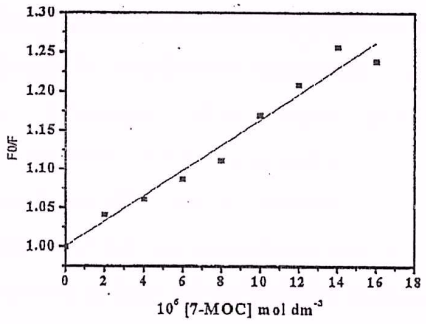

Figure 9: Stern-Volmer plot for TYR-7-MOC system in CTAB

The Stern-Volmer (SV) quenching constant K_{sv} can be determined from the S-V relation [7] i.e.

$$F_0/F=1+K_{sv}[Q] \qquad (7)$$

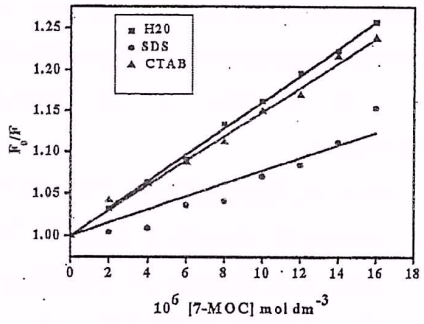

Figure 10: Stern-Volmer plots for TYR-7-MOC system in different surfactant media

Figure 8 (combined SV plot) depicts the S-V plots of fluorescence quenching TYR by 7-MOC in different micellar media and deionized water. In this figure it can be seen that the good linearity is observed for fluorescence intensity quenching in SDS, CTAB and deionized water with S-V quenching constant 7×10^{-3}, 14.85×10^{-3} and 16.09×10^{-3} dm^3/mol respectively.

Table 5:

Medium	Slope K_{sv}	R
Water	16.09×10^{-3}	0.99999
SDS	7.71×10^{-3}	0.99975
CTAB	14.85×10^{-3}	0.99997

Conclusions

The binding interaction between tyrosine and 7-methoxy Coumarin was investigated by spectroscopic method. Temperature dependence of quenching indicates the enhancement of Stern-Volmer quenching constant with temperature confirming the dynamic quenching mechanism. Decrease in binding constant with increase in temperature shows that decrease in stability of TYR-7-MOC complex. At the different temperatures the values of binding site indicate the presence of only one binding site for 7- methoxy Coumarin on tyrosine. Values of thermodynamic parameters reveals that the binding process is spontaneous and entropy driven. Hydrophobic force and hydrogen bonding are major factor in the interactions.

References

1. J. M. Beechem, L. Brand, Ann. Rev. Biochem. 54(1985)43
2. M.R. Eftink, in: C. H. Schulter(Ed.), Methods in Biochemical Analysis, Vol. 35, Protein structure Determination, Wiley, New York, (1991).
3. http://www.anyvitamins.com/tyrosine-info.htm#Deficiency of nutrient.
4. http://www.healthvitaminsguide.com/aminoacids/tyrosine.htm
5. S.P. Felter, J.D. Vassallo, B. D. Carlton, G.P. Daston, Food Chem. Toxicol.44(2006)462..
6. Hoult, J.R.S., Paya, M, Gen pharmacol. 27(1996)713-722
7. Schilcher, H., Imming, P., Goeters, S. (2005)55-76
8. Silvan, A.M., Abad, M.J., Bermejo., P., Sollhuber, M., Villar, A.59(1996)1183-1185
9. Hausen, B.M., Schmieder, M.15(1986)157-163

VOLUME - VIII, ISSUE - I - JANUARY - MARCH - 2019
AJANTA - ISSN 2277 - 5730 - IMPACT FACTOR - 5.5 (www.sjifactor.com)

10. Murat, B.H., Atmaca, M., DenizObay, B., Ozekinchi, S., Tasdemir, E., Ketani, A.63(2011)325-330
11. S.Bi, D.Song, Y. Tian, X. Zhou, Z. Liu, H. Zhang, Spectrochim Acta Part A, 61(2005)629-636
12. U.S. Mote, S.L. Bhattar, S.R. Patil, G.B. Kolekar, Interaction of Fluorescein with Felodipine: A Spectrofluorometric and Thermodynamic StudyJ Solution Chem.38(2009)619–628
13. Y-Z Zhang, X-X. Chen, J. Dai, X-P. Zhang, Y-X. Liu, Y. Liu, J. Lumin, 23(2008)150.
14. H. Zhao, M. Ge, Z. Zhang, W. Wang, G. Wu, Spectrochim Acta Part A, 65(2006)811-817
15. S. EI-Daly, S. A. Azim, H. EI-Daly, K. A. Abu-Zied, J. Chem. Phys. 92(1995)1195.

Govt. College of Arts & Science Aurangabad

PRINCIPAL
Govt. College of Arts & Science
Aurangabad