MAJOR PROJECT

ABSORPTION AND EMISSION STUDIES ON THE INTERACTION OF RIBOFLAVIN WITH SYNTHETIC POLYMERS

A Major Project Report submitted in partial fulfilment for the award of the degree of

MASTER OF TECHNOLOGY (M. Tech.)

In

POLYMER TECHNOLOGY

Submitted by

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[2K14/PTE/19]

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DECLARATION

I, **Sadaf Azeez**, hereby certify that the work which is being submitted in this major project report entitled "**Absorption and emission studies on the interaction of riboflavin with Synthetic polymers**" in the partial fulfilment for the award of the degree of Master of Technology (Polymer Technology) at **Delhi Technological University** is an authentic record of my own work carried out by me under the supervision of **Dr. Ram Singh** (Assistant Professor, Department of Applied Chemistry, Delhi Technological University, Delhi.

I, further declare that the project report has not been submitted to any other Institute/University for the award of any degree or diploma or any other purpose whatsoever. Also it has not been directly copied from any source without giving its proper reference.

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CERTIFICATE

This is to certify that the M.Tech. major project entitled entitled “**Absorption and emission studies on the interaction of riboflavin with Synthetic polymers**” submitted by **Sadaf Azeez**, Roll Number 2K14/PTE/19, for the award of the degree of **“Master of Technology in Polymer Technology”** is a record of bonafide work carried out by her. She has worked under my guidance and supervision and has fulfilled the requirements for the submission of the project report.

To the best of our knowledge and belief the content therein is his own original work and has not been submitted to any other university or institute for the award of any degree or diploma.

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Sadaf Azez

Contents

ABBREVIATIONS

S. No.	Symbol	Meaning
1.	DMF	Dimethyl formamide
2.	DMSO	Dimethyl sulphoxide
3.	PEG	Polyethylene glycol
4.	PVA	Polyvinylalcohol
5.	PMMA	Polymethyl methacrylate
6.	H_2O	Water
7.	nm	Nanometer
8.	cm	Centimeter
9.	HOMO	Highest occupied molecular orbital
10.	LUMO	Lowest unoccupied molecular orbital

ABSTRACT

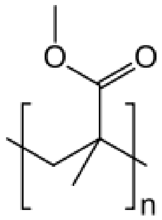
Protein-protein interactions and drug-protein interactions are an important field in medical sciences. The impact and strength of the drug-protein interactions play significant role in the biological activity of the drugs. The non-covalent association between the drug and protein molecules always provides stability to the drug action. In a similar way, the interaction between the drug and its carrier molecules are also plays important role in the efficient delivery and action of the drug molecules. The polymers are promising molecules due to their surface, bulk and improved pharmacokinetic properties. In the present work, we have tried to study the interaction between polymer and riboflavin to understand their stability under different micro environment. The polymers like poly(methyl methacrylate) (PMMA), poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) have been utilized as biomedical materials due to their biocompatibility and polar nature.

CHAPTER 1

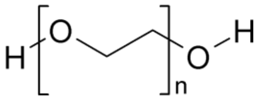
INTRODUCTION

Protein-protein interactions and drug-protein interactions are an important field in medical sciences. The impact and strength of the drug-protein interactions play significant role in the biological activity of the drugs.[1] The interaction depends on the non-covalent associations between the drug and protein molecules. Another important aspect is the interaction between drug and its carrier molecules. Drug along with its carrier constitute an excellent drug delivery system. Due to the advancement in the technology, macromolecules like polymers are becoming an inseparable part of the drug delivery systems.[2] The polymers are promising molecules due to their surface, bulk and improved pharmacokinetic properties.

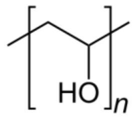
Polymers like poly(methyl methacrylate) (PMMA, **1**), poly(ethylene glycol) (PEG, **2**) and poly(vinyl alcohol) (PVA, **3**) have been utilized as biomedical materials due to their biocompatibility and polar nature.[3] Growing number of recent publications have shown an increasing interest in the applications of these polymers as a drug carrier. They are the carrier of many drugs like antibiotics and antioxidants *via* different routes of administration.[3]



PMMA, **1**

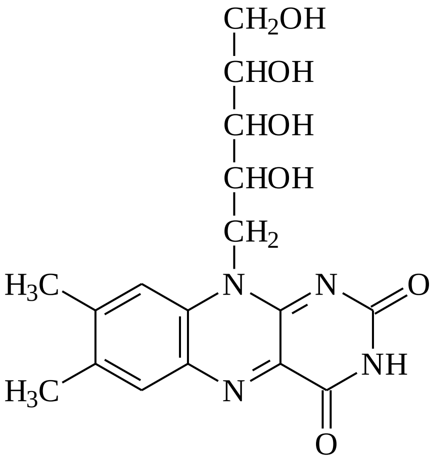


PEG, **2**

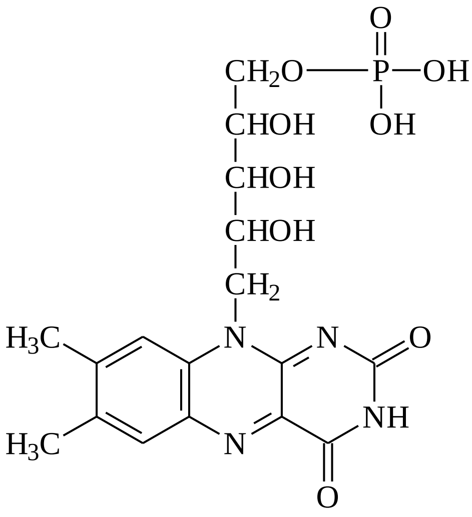


PVA, **3**

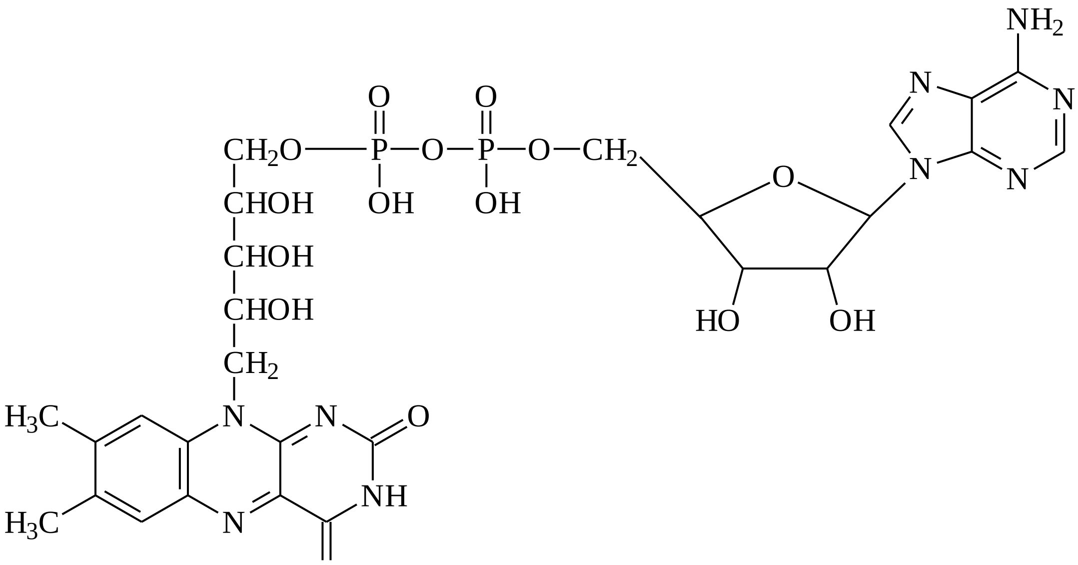
The term ‘flavin’ refers to the yellow chromophoric and redox active prosthetic group of a class of respiratory enzymes occurring widely in animals and plants, namely the flavoproteins.[4] The flavin cofactors riboflavin, FMN (flavin mono nucleotide) and FAD (flavin adenine dinucleotide) (Figure 1) are involved in the catalysis of a wide variety of biological redox reactions.[5] The hydrogen bonding between heteroatoms of flavin coenzymes and amino acid residues of the apoproteins at the flavoenzyme active sites promotes the reactivities of flavoenzymes.[6] Vitamin riboflavin (**1**) is a member of isoalloxazine group of compounds. This work deals with the absorption and emission studies on the interaction of riboflavin with synthetic polymers such as PEG, PMMA and PVA.



Riboflavin



FMN



FAD

Figure 1. Chemical structures of Riboflavin, FMN and FAD

1.1 PEG (polyethylene glycol)

Polyethylene glycol (PEG) is a polymer of ether molecule having applications from industrial manufacturing to medicine. It is also known as polyethylene oxide (PEO) or polyoxyethylene (POE), depending on its molecular weight. The chemical formula of PEG is commonly expressed as $H{-}(O{-}CH_2{-}CH_2)_n{-}OH$. PEG are prepared by polymerization of ethylene oxide. The different types of PEG and PEO having different molecular weights find different applications, and having different physical properties (e.g. viscosity) because of its chain length effects, but its chemical properties are nearly identical.[7]

PEG is obtained by the interaction of ethylene oxide with water, ethylene glycol, or ethylene glycol oligomers. This reaction is basically catalyzed in the presence of acid or base. Ethylene glycol and its oligomers are used as a starting material for the production of PEG instead of water, because they can form the polymers having low polydispersity and the length of the Polymer chain depends on the ratio of the reactants.

$$HOCH_2CH_2OH + n\,(CH_2CH_2O) \rightarrow HO\,(CH_2CH_2O)_{n+1}H$$

In the overall reaction mechanism catalysts like sodium hydroxide (NaOH), potassium hydroxide (KOH), or sodium carbonate (Na_2CO_3) are used to prepare low-molecular-weight polyethylene glycol.[8] PEG is widely used in medical, chemical, biological etc.[9]

1.2 PVA (Polyvinyl alcohol)

Poly (vinyl alcohol) is a water soluble synthetic polymer. Its formula is $[CH_2CH(OH)]_n$. It is used in textiles, paper making, and a variety of coatings. PVA is white, colourless and odourless polymer. It is occasionally supplied as beads or as solutions in water. It is used to increase viscosity in pharmaceuticals and as a lubricant and protectant in ophthalmic preparations. PVA is frequently found in over-the-counter eye redness and eye lubricant, eye drops.[10] Polyvinyl alcohol is the lubricant, and works by given moisture to the eye. PVA is approved to be one of few vinyl polymers that can have superior biodegradation rate. This is possible due to the presence of hydroxyl groups which is the condition of hydrophilic nature of the material.[11]

PVA is prepared by an emulsion polymerization. This is prepared by first polymerizing vinyl acetate, and the follow-on poly vinylacetate is converted to the PVA. Other precursor polymers are sometimes used, with formate, chloroacetate groups instead of acetate. The conversion of the polyesters is usually conducted by base-catalysed transesterification with ethanol.[7]

This polymer enhances the delivery of active pharmaceutical ingredient in a broad range of formulations. Its PVA foils are used in agriculture to deliver pesticides, herbicides, fertilizers, and fungicides. In addition, this is also helpful to coat seeds that are ready for germination under adequate humidity conditions and temperature.[11].

In tissue engineering, PVA is applied as scaffolds for tissues regeneration, mainly for cartilaginous tissue regeneration.[11] The regeneration process of these tissues is ceased at some moment because of more difficult access to oxygen and nutritive substances. This can be prohibited by forming new blood vessels in a place of tissue regeneration. Matrices made from PVA are used for tissue engineering.[11]

1.3 PMMA (Polymethyl methcrylate)

PMMA is a synthetic resin formed from the polymerization of methyl methacrylate monomer. This is a rigid and transparent plastic. This is used as a alternate for glass in products such as skylights, illuminate signs, shatterproof windows, and aircraft canopies.[9] PMMA is regularly produced by emulsion polymerization, bulk polymerization, and solution polymerization. Generally, radical initiation is used for polymerization.[11]

PMMA is a strong, lightweight and transparent plastic with an outstanding outdoor life period and good strength. This is amorphous by nature, due to the presence of bulky side group. This swells and dissolves in many organic solvents.[13] PMMA is used to create attractive signboard and durable lenses for automobile lighting. This in a purified form used as the matrix in laser dye-doped organic solid-state gain media for tunable solid state dye lasers.[14]

In semiconductor research and production, PMMA aids as a resist in the electron beam used in lithography process. A solution consisting of the polymer in a solvent is used to spin coat silicon and other semiconducting and semi-insulating wafers with a thin film. Patterns on this can be made by an electron beam, deep UV light, or X-rays. Exposure to PMMA creates chain scission within the chains, allowing for the selective removal of exposed areas, making it a positive photoresist. PMMA's advantage is that it allows for very high resolution patterns to be made. Smooth PMMA surface can be easily nanostructured when treated by oxygen radio-frequency plasma. Nanostructured PMMA surface can be easily smoothed by vacuum ultraviolet irradiation.[14]

CHAPTER 2

MATERIALS AND METHODS

To perform and study the interaction between riboflavin and synthetic polymers, the compounds have been obtained from the following reputed companies:

Riboflavin ($C_{17}H_{20}N_4O_6$, M_w = 376.36 $gmol^{-1}$) was purchased from Merck.

Polymers were purchased from Sigma Aldrich.

- **a.** Poly(ethylene glycol) [(PEG), $H(OCH_2CH_2)_nOH$, M_n =20000 $gmol^{-1}$]
- **b.** Poly(vinyl alcohol) [(PVA), M_w 89,000-98,000 $gmol^{-1}$]
- **c.** Poly(methyl methacrylate) [(PMMA) M_w ~350,000 $gmol^{-1}$]

Solvents like dimethyl formamide (DMF), dimethyl sulphoxide (DMSO), hexane, water etc were of analytical grade and purchased from Indian companies and used without further purifications.

Absorption spectra were recorded at 25^oC with a parkin Elmer - lambda -750.

Fluorescence spectra were recorded at 25^oC with horiba jobing yyon fluorolog-3.

Sample Preparations

Interaction studies of riboflavin with poly(vinyl alcohol) (PVA)

1 mg of PVA was dissolved in 10 mL of water.

The stock solution of riboflavin was prepared in the concentration of 10^{-4} M (0.376 mg in 10 mL of water.

Sample 1: 10 mL of riboflavin was added to 1 mL of PVA solution.

Sample 2: 10 mL of riboflavin was added to 2 mL of PVA solution.

The absorption and emission spectra were recorded at 364 nm and 444 nm of wavelength.

Interaction studies of riboflavin with poly(ethylene glycol) (PEG):

The fluorescence spectra of riboflavin of concentration 10^{-4} M were recorded in the presence of increasing contents of PEG (1 mg dissolved in 10 mL of water) at temperature 25^{o}C. The absorption and emission spectra were record at 372 nm and 445 nm of wavelength.

Sample No.	**Amount of Riboflavin (mL)**	**Amount of PEG (mL)**
3	10	1
4	10	2
5	10	3
6	10	4
7	10	5

Interaction studies of riboflavin with poly(methyl methacrylate) (PMMA):

The stock solutions were prepared by dissolving 1 mg of PMMA in 10 mL of water. Riboflavin stock solution concentration was 10^{-4} M in water

Sample No.	**Amount of Riboflavin (mL)**	**Amount of PMMA (mL)**
8	10	1
9	10	2
10	10	3
11	10	4
12	10	5

***Interaction studies of riboflavin with polymers in different solvent media*:**

Riboflavin is dissolve in different solvents system such as DMF, DMSO and hexane (0.001 mg of riboflavin in 10 mL of the solvent).

The polymer solutions were made by dissolving 1 mg of polymer in 10 mL of solvents.

The samples were prepared by taking 10 mL of Riboflavin solution in different amount of polymers.

Sample No.	**PEG (mL)**	**PMMA (mL)**	**PVA (mL)**
13	1	-	-
14	2	-	-
15	-	1	-
16	-	2	-
17	-	-	1
18	-	-	2

***Interaction studies of riboflavin with PVA under different pH conditions*:**

The stock solution of PVA was made by dissolving 1 mg of PVA in 10 mL of water.

The stock solution of riboflavin was made by dissolving 0.001 mg of riboflavin in 10 mL of water.

The studies were done at pH 1 acidic, pH 7 neutral and pH 14 basic conditions.

Characterization and Measurement techniques used

The following characterization and measurement techniques have been used in our experiment.

Ultraviolet–visible spectrophotometer: This is an absorption spectroscopy in the ultraviolet-visible spectral region (200 to 700 nm). This uses light in the visible and UV ranges. Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite the electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb.[15] This is based on the Lambert's Beers Law.

Fluorescence spectroscopy: This is a electromagnetic spectroscopy which analyses fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecule of certain compounds and causes them to emit light. In fluorescence, the molecule is first excited by absorbing a photon, from its ground electronic state to vibrational states in the excited electronic state. Collisions with other molecules cause the excited molecule to lose vibrational energy until it reaches the lowest vibrational state of the excited electronic state. This process is often visualized with a Jablonski diagram.[16]

Fluorescence spectral data are generally show by emission spectra. A fluorescence emission spectrum is a plot of fluorescence intensity versus wavelength (nanometers) or wavenumber (cm^{-1}). Emission spectra are widely and depend upon chemical structure of flurophore and the solvent in which it is dissolved.[17]

The energy of emission is typically is less then that of absorption. Fluorescence typically occurs at lover energies or longer wavelength. The fluorescence lifetime and quantum yield are most important characteristics of a fluorophore. Quantum yield is the number of emitted photon relative to the number of absorbed photo.[16]

Fourier Transform Infrared Spectroscopy (FTIR): FTIR is used to obtain an infrared (IR) spectrum of a solid, liquid or gaseous sample. An FTIR spectrometer at the same time collects spectral data in a wide spectral range. This confers an important advantage over a dispersive spectrometer which process intensity over a narrow range of wavelengths at a time. FTIR has made dispersive infrared spectrometers nearly obsolete, opening up new applications of infrared spectroscopy.[18] This is based on Hookes Law and plotted transmittance (%) versus wave number.

CHAPTER 3

RESULTS AND DISCUSSION

Riboflavin (vitamin B_2) is a tricyclic molecule possessing hetero atoms in the ring. Hence, this is a heterocyclic compound. This is a biomolecule and present as cofactor in the flavoenzymes.[19,20] This is attached with the proteins through non-covalent interactions mainly hydrogen bonding (Figure 2). We have tried to mimic this by replacing the protein part with polymers possessing those atoms that can form hydrogen bonds with riboflavins *in vitro*. To understand the interactions, absorption and emission studies have been done by mixing the solutions of riboflavin and polymers under study.

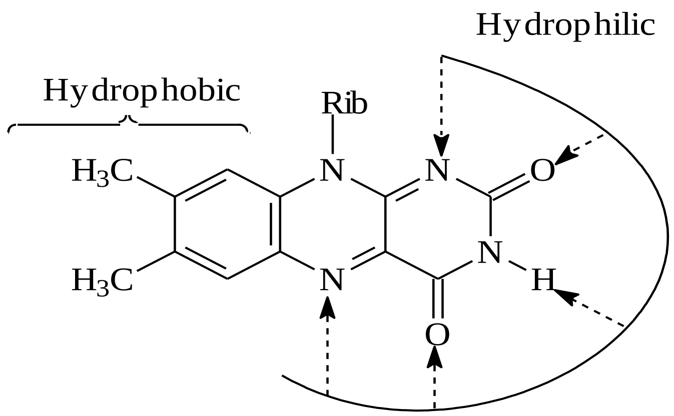


Figure 2. Interaction of riboflavin with protein.

3.1 Emission studies of riboflavin and polymers in different solvents

Florescence spectral data are generally show by emission spectra. A fluorescence emission spectrum is a plot of fluorescence intensity versus wavelength (nanometers, λ_{max}, nm) or wavenumber (cm^{-1}). Emission spectra are widely and depend upon chemical structure of flurophore and the solvent in which it is dissolved. To understand the interaction of riboflavin with polymers, the stock solutions have been made by dissolving 1 mg polymer in 10 mL water and riboflavin in the concentration of 10^{-4}M. The florescence spectral data was collected at the excitation wavelength of 460 nm. The observation has been recorded and compiled in table 1.

Table 1. **Emission studies of riboflavin and polymers in different solvents**

Different solvents*	Riboflavin		PVA (S1)		PEG (S3)		PMMA (S8)	
	λ_{max} (nm)	intensity	λ_{max} (nm) (Sample code)	intensity	λ_{max} (nm) (Sample code)	intensity	λ_{max} (nm) (Sample code)	intensity
Water	522	117571	522 (S1)	97260	522 (S3)	107877	522 (S8)	14801
DMF	509	181098	511	84933	511	117743	509	153537
DMSO	511	68649	512	51031	512	56091	511	66744
Hexane	510	108691	511	89090	515	75880	510	109117

*Concentration of riboflavin, 10^{-4}M; 1 mg of polymer dissolved in 10 mL water
Sample 1 (S1): 10 mL of riboflavin was added to 1 mL of PVA solution
Sample 3 (S3): 10 mL of riboflavin was added to 1 mL of PEG solution
Sample 8 (S8): 10 mL of riboflavin was added to 1 mL of PMMA solution

With the similar concentrations, the other emission spectral data were also obtained (Figures 3-8).

3.2 Absorption spectral studies of riboflavin and polymers in different solvents

The Ultraviolet–visible (UV-visible) spectroscopy is also known as the absorption spectroscopy. Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to get excited and the excited electrons jumps from the HOMO to the LUMO. The stock solutions were made by dissolving 1 mg polymer in10 mL solvents and mixed in different amounts with the riboflavin solution (10^{-4}M). The observations are compiled in table 2.

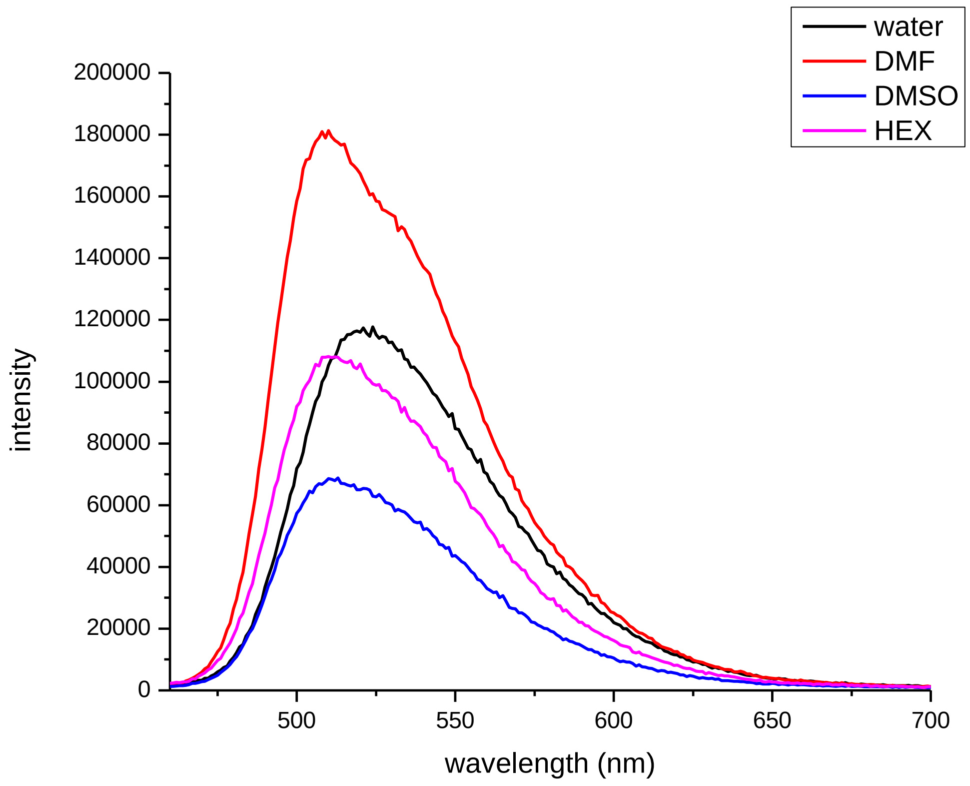


Figure 3. Emission spectra of riboflavin in different solvents

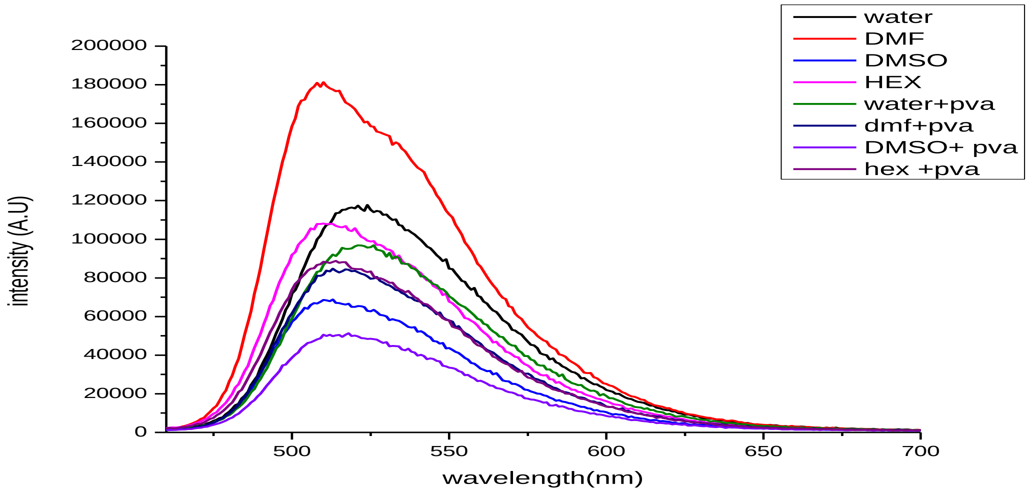


Figure 4. Emission spectra of riboflavin in different solvents & interaction with PVA

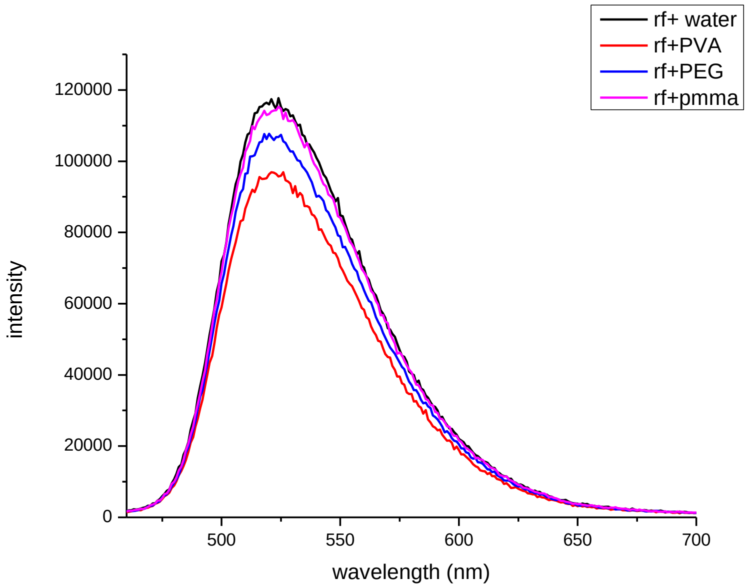


Figure 5. Florescence spectra of riboflavin and polymers in water

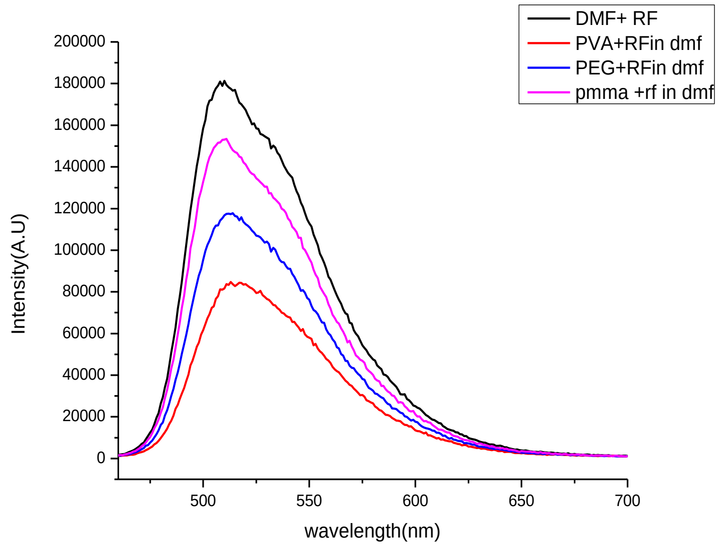


Figure 6. Florescence spectra of riboflavin and polymers in DMF

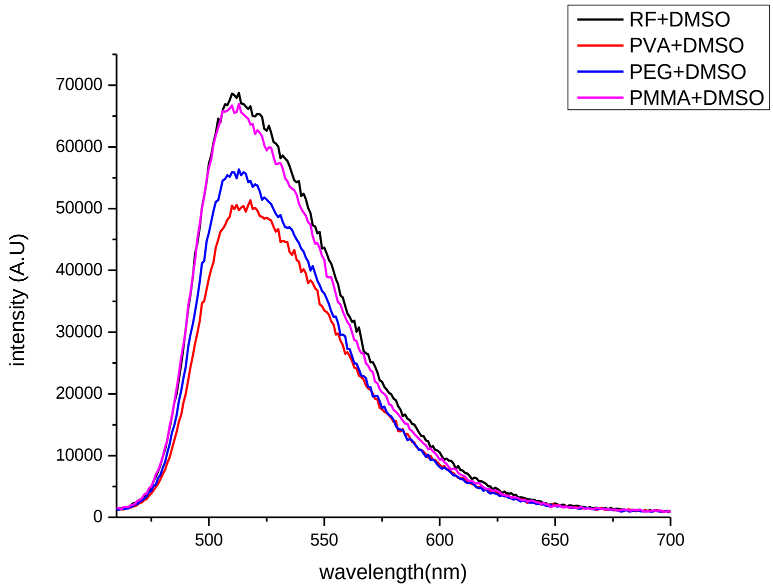


Figure 7. Florescence spectra of riboflavin and polymers in DMSO

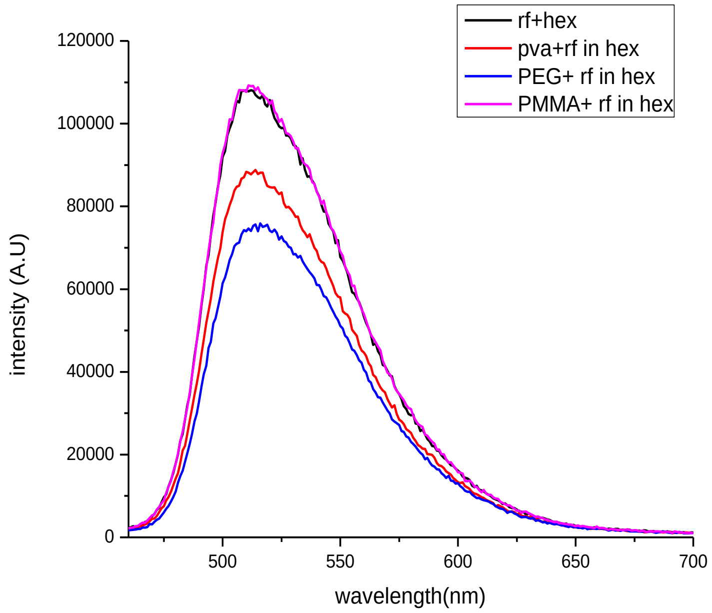


Figure 8. Florescence spectra of riboflavin and polymers in hexane

Table 2. Absorption spectra of riboflavin with different polymer in different solvents

	Water		DMF		DMSO		Hexane	
	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs
Riboflavin	364	1.5234	332	1.0860	341	1.5234	341	0.2790
	444	1.2182	383	1.1284	382	1.3378	385	0.2206
			449	0.0341	447	0.9591	448	0.0472
PVA (1 mL)	364	1.3739	342	0.9542	347	1.3051	343	0.3060
	444	1.0958	383	0.8893	382	1.1461	385	0.2251
			449	0.0465	447	0.7762	448	0.0522
PVA (2 mL)	364	1.2160	347	0.7757	350	1.1259	343	0.3936
	444	0.9735	383	0.6550	382	0.0968	385	0.3245
			449	0.0403	447	0.6324	448	0.0599
PEG (1 mL)	364	1.1452	340	1.0983	340	0.4004	341	0.3271
	444	1.1455	383	0.9758	383	0.3441	385	0.2677
			449	0.0589	449	0.3010	448	0.0269
PEG	364	1.3460	340	1.0669	342	0.3837	341	0.3287

(2 mL)								
	444	1.0587	383	0.9237	383	0.3352	385	0.2707
			449	0.0532	449	0.2447	448	0.0305
PMMA (1 mL)	364	1.5374	330	1.4309	339	0.4146	341	0.4532
	444	1.2450	381	1.2905	383	0.3643	385	0.3654
			449	0.0781	449	0.2797	448	0.0464
PMMA (2 ML)	364	1.5350	330	1.3526	339	0.4134	336	0.7851
	444	1.2501	381	1.2034	383	0.3572	385	0.6133
			449	0.0718	449	0.2453	448	0.2447

This has been observed that the absorption spectra of riboflavin recorded in water shows 2 bands at λ_{max} of 364 nm and 444 nm. This is due to the polar nature of water. When the spectra were recorded in aprotic and nonpoler solvents, 3 band were observed at λ_{max} of 332, 383, 449 nm. The spectra are given in figures 9-17.

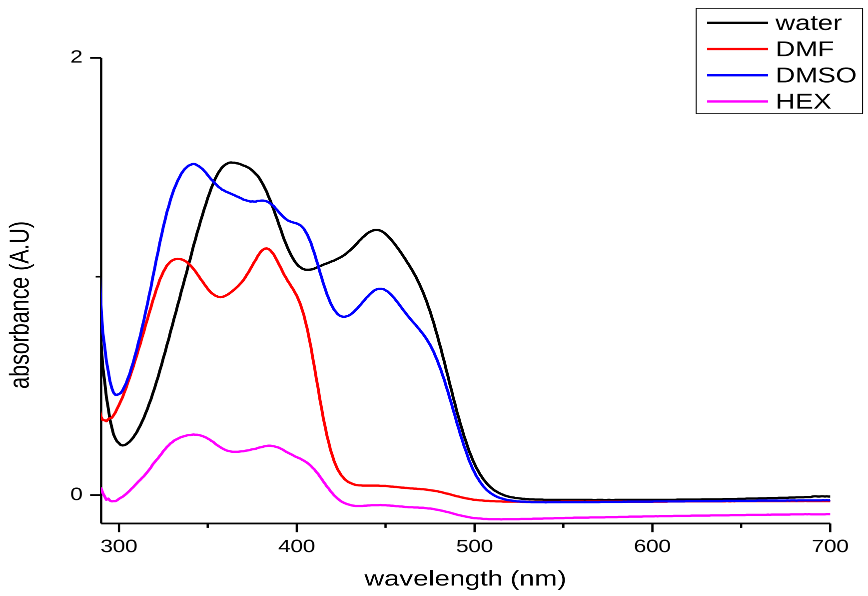
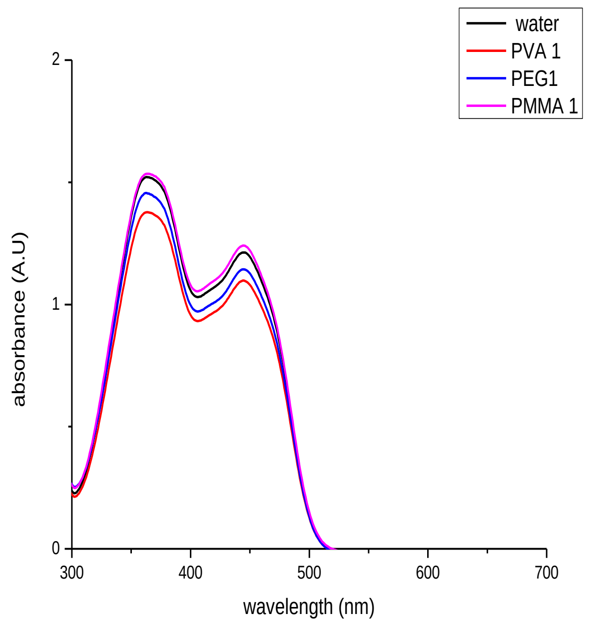


Figure 9. Absorption spectra of riboflavin in different



solvents

Figure 10. Absorption spectra of riboflavin and polymers in water

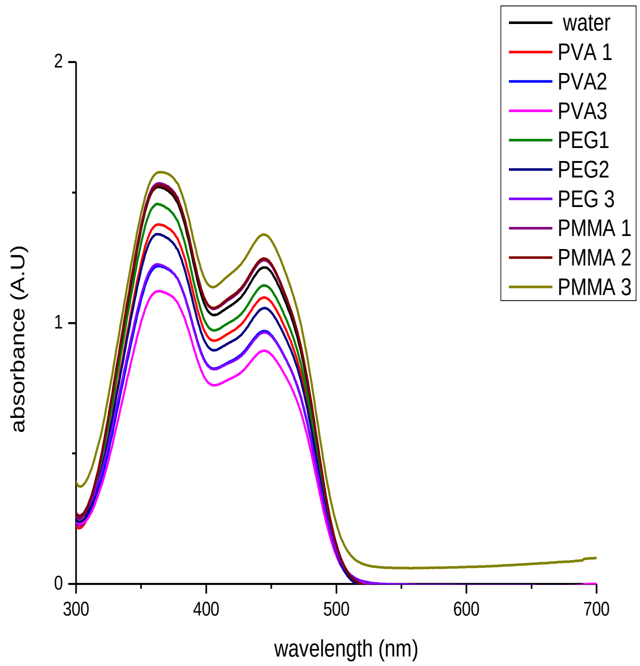


Figure 11. Absorption spectra of riboflavin and different amounts of polymers in water

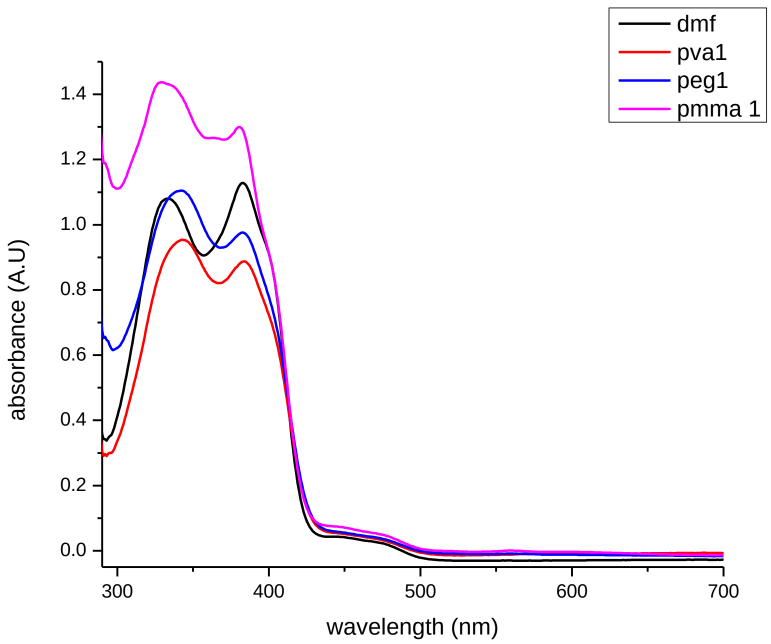


Figure 12. Absorption spectra of riboflavin and polymers in DMF

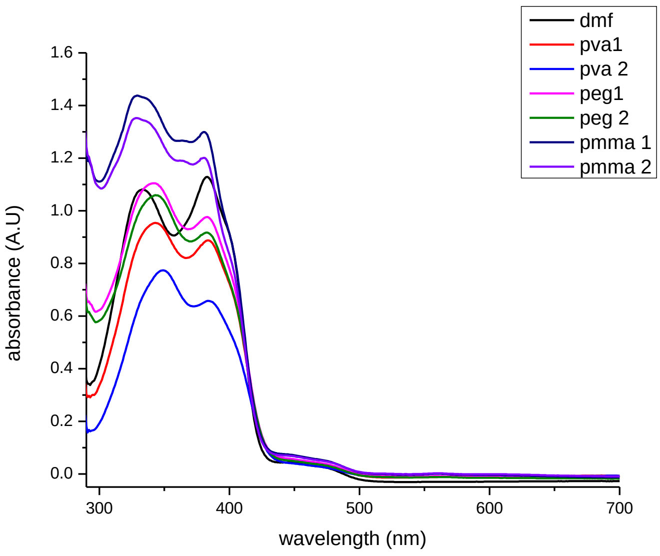


Figure 13. Absorption spectra of riboflavin and different amounts of polymers in DMF

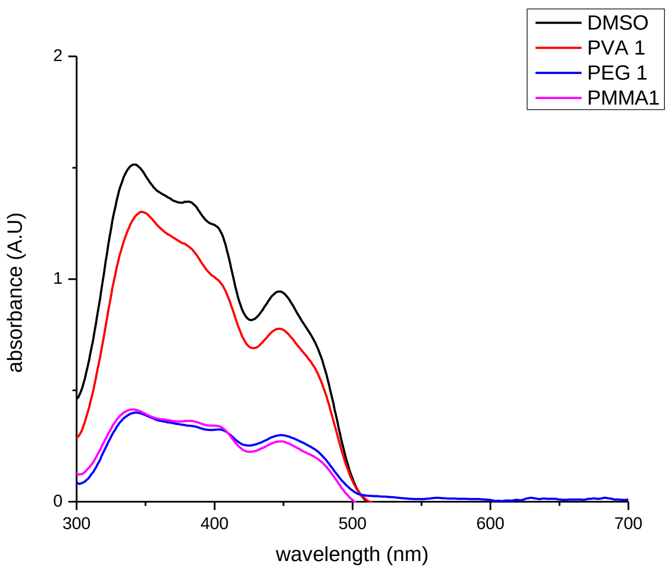


Figure 14. Absorption spectra of riboflavin and polymers in DMSO

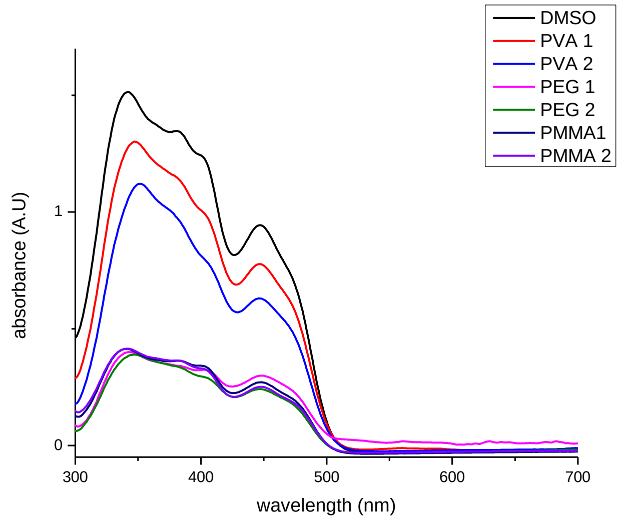


Figure 15. Absorption spectra of riboflavin and different amounts of polymers in DMSO

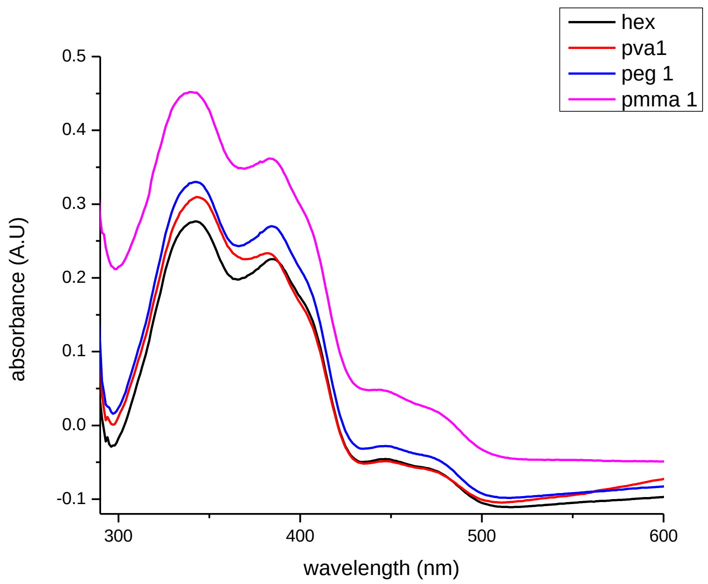


Figure 16. Absorption spectra of riboflavin and polymers in hexane

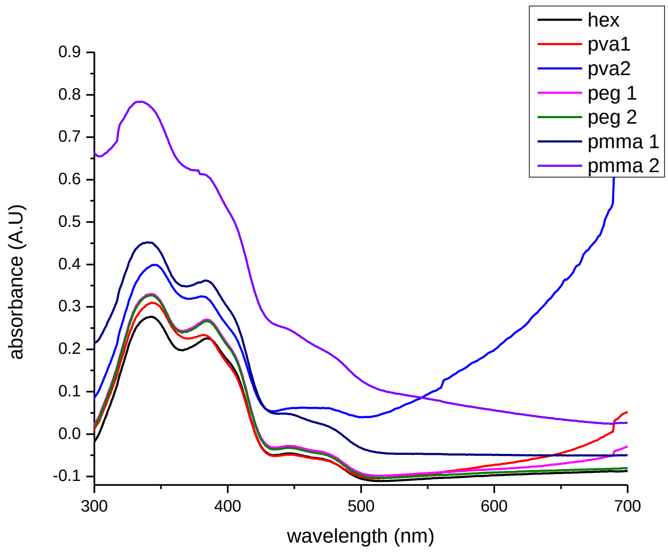


Figure 17. Absorption spectra of riboflavin and different amounts of polymers in hexane

The absorption spectra were also recorded with different amounts of PEG to understand the concentration dependent interaction between riboflavin and PEG. The observations have been summarized in table 3 and spectra are given in figures 18.

Table 3. Absorption spectra of riboflavin interact with PEG in different concentration

Riboflavin sol* (mL)	PEG sol* (mL)	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs
10 ml	0 ml	372	0.2623	445	0.3016
10 ml	1 ml	372	0.2636	445	0.2931
10 ml	2 ml	372	0.2330	445	0.2612
10 ml	3 ml	372	0.2036	445	0.2320
10 ml	4 ml	372	0.1916	445	0.2129

10 ml	5 ml	372	0.1891	445	0.2103

*Concentrations: Riboflavin 0.001 mg in 10 ml water and polymer 1 mg in 10 ml of water

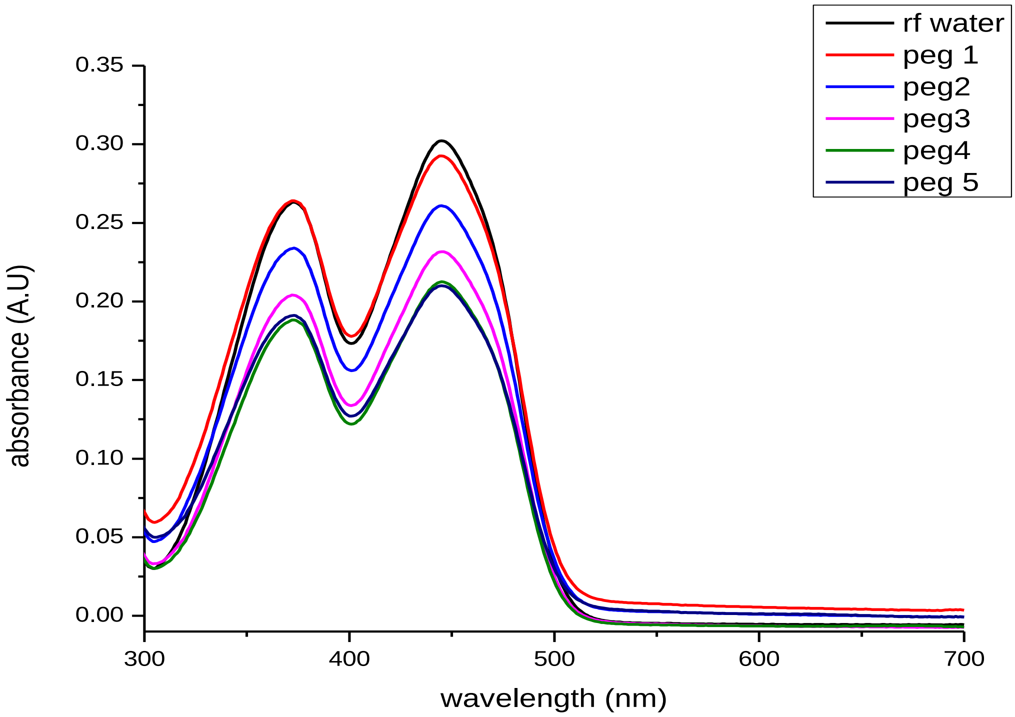


Figure 18. Absorption spectra of riboflavin and different concentration of PEG in water

Table 4. Absorption spectra of riboflavin in different pH

	pH 1		pH 7		pH 13	
	λ_{max} (nm)	abs	λ_{max} (nm)	abs	λ_{max} (nm)	abs
riboflavin	374	.5082	358	.2392	351	.1591
	444	.5272	444	.1534	444	.1129
PVA 1	373	.3823	358	.1534	351	.1385
	444	.3931	444	.1075	444	.1001
PVA 2	373	.3164	358	.1401	351	.1420
	444	.3222	444	.0833	444	.1002

PVA 3	373	.2520	358	.1231	351	.1200
	444	.2564	444	.0762	444	.0845

1 mg polymer in 10 ml water

Concentration of riboflavin = 10^{-4} M

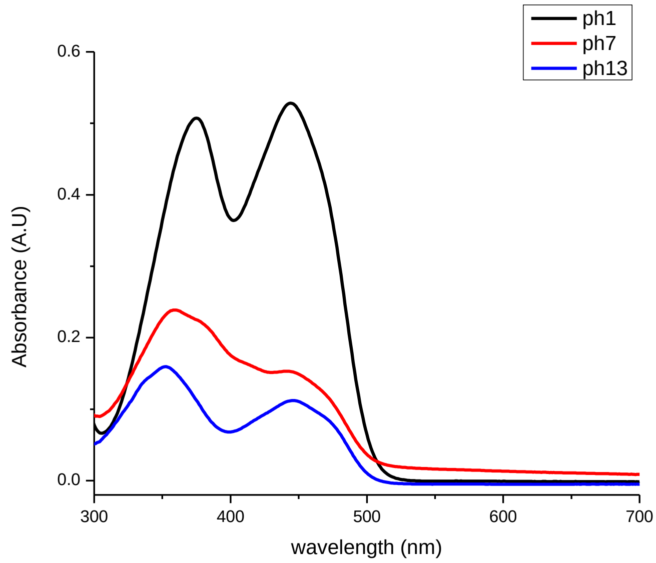


Figure 19. Absorption spectra of riboflavin in different pH ie 1, 7 and 13

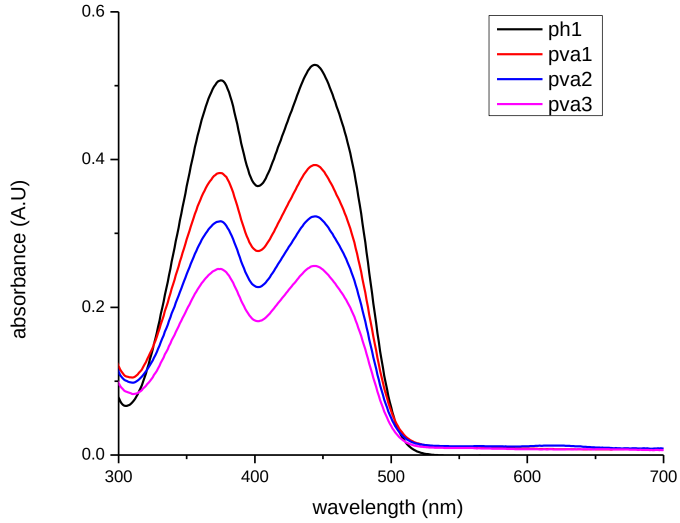


Figure 20. Absorption spectra of riboflavin and PVA at pH 1

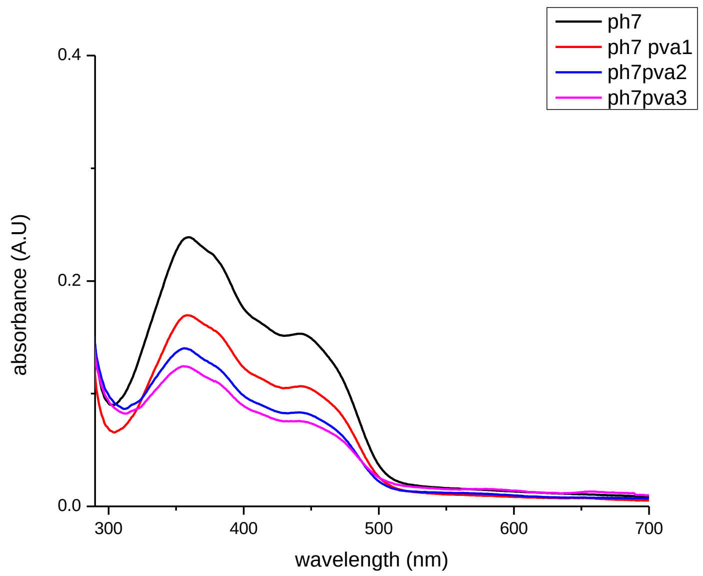


Figure 21. Absorption spectra of riboflavin and PVA at pH 7

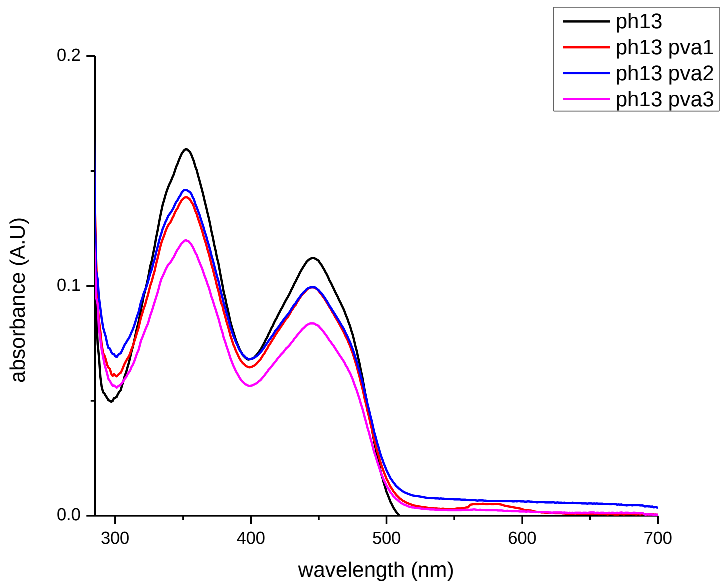


Figure 22. Absorption spectra of riboflavin and PVA at pH 13

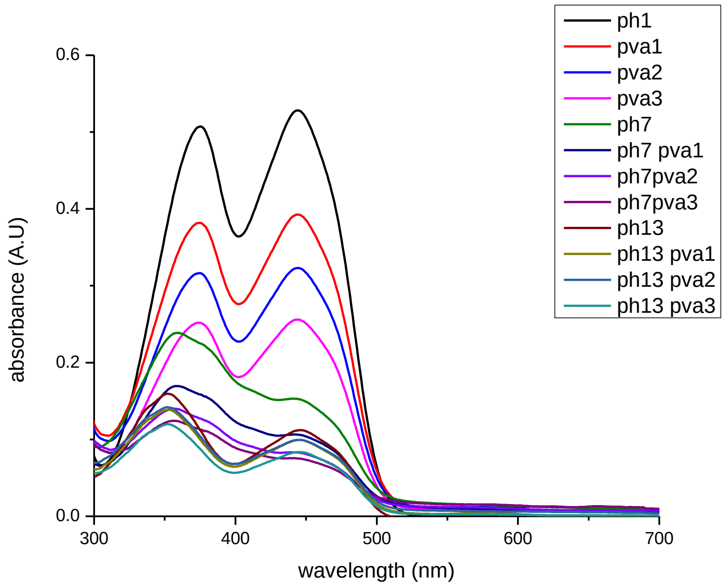


Figure 23. Combined absorption spectra of riboflavin and PVA at different pH

3.3 Emission spectra of riboflavin in different pH & interaction with polymers

The emission spectra were recorded at the excitation wavelength of 440 nm. The summarized observation are given in table 5 and spectra in figure 24 and 25.

Table 5. Emission spectra of riboflavin in different pH & interaction with polymer

	Riboflavin in water		Riboflavin with PVA		Riboflavin with PEG	
	λ_{max} (nm)	Intensity	λ_{max} (nm)	intensity	λ_{max} (nm)	intensity
pH 1	518	140027	519	127090	520	133384
pH 7	516	28014	519	20663	518	20934
pH 13	517	51570	517	41431	518	42829

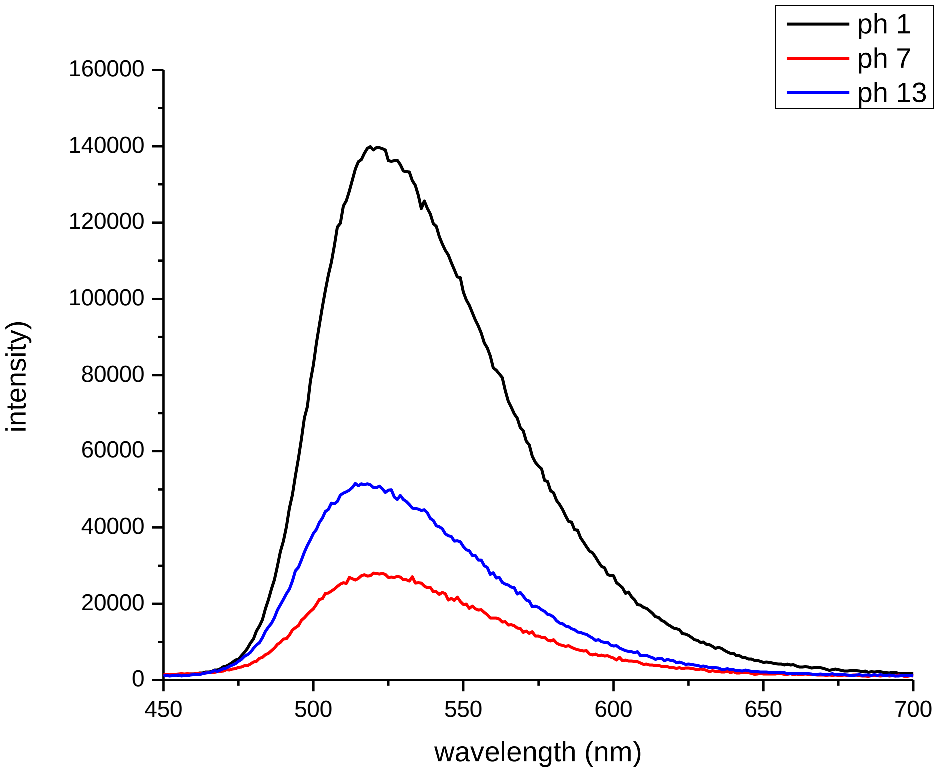
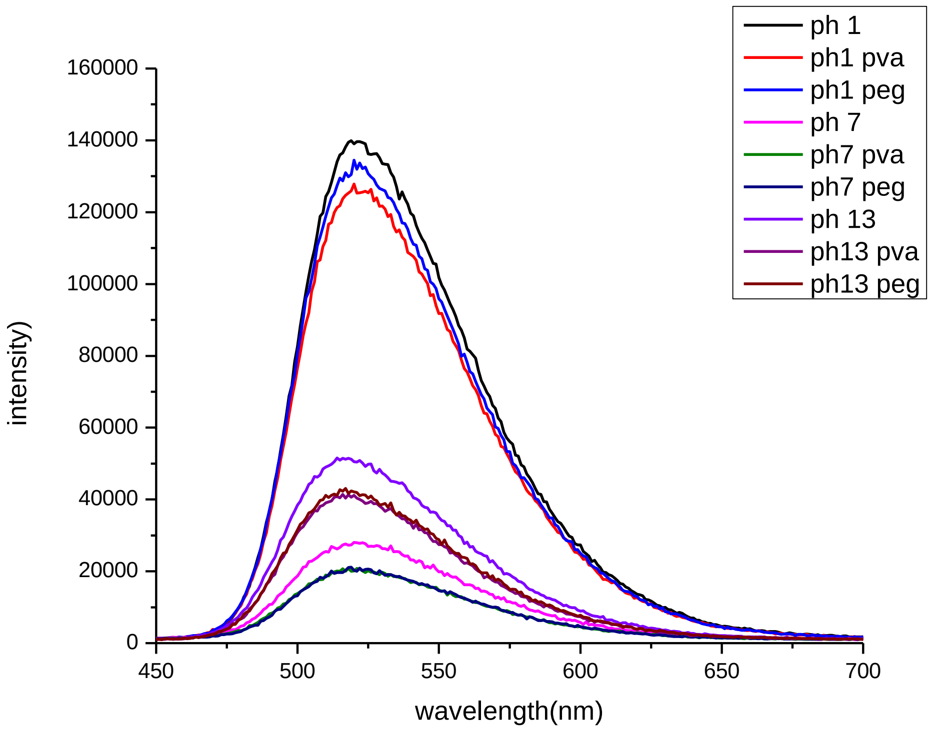


Figure 24. Emission spectra of riboflavin in different



pH

Figure 25. Emission spectra of riboflavin and polymers at different pH

CHAPTER 4

CONCLUSIONS

1. The absorption spectra of riboflavin in water show two bands at 364 nm and 444 nm.
2. The absorption spectra of riboflavin in aprotic and nonpoler solvents show three bands at 332, 383, 449 nm.
3. It has been observed that increasing the pH decrease the wavelength. The observed values at pH 7 was 358 and 444 nm, and at pH 13 was 351 and 444 nm.
4. In the fluorescence spectra of riboflavin at different pH, the increasing content of PVA leads to decrease in absorbance.

CHAPTER 5

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