Review article

Spectroscopic studies of naproxen and tryptophan immobilized in polyvinyl alcohol

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Abstract: *Hydrogels are cross-linked hydrophilic polymer networks that find application in biomedical and pharmaceutical industries due to their unique properties. Polyvinyl alcohol is a synthetic polymer, potential biomaterial blended with various materials and compounds, that can provide new options for drug delivery systems. Determination of photochemical properties of naproxen and tryptophan immobilized in polyvinyl alcohol and two other media: water and cyclohexane was performed using UV-Vis absorption and fluorescence methods. The influences of the media used on the spectral properties of naproxen and tryptophan have been shown.*

Keywords: *PVA, polyvinyl alcohol, polymer, naproxen, tryptophan, spectroscopic, fluorescence, absorption.*

Introduction

Spectroscopic studies are central and fundamental to the elucidation of the structure and dynamics of microheterogenous systems, e.g. biological macromolecules, colloid particles, liquid crystals, and polymers [1]. Biodegradable polymers have become increasingly important in biomedical and

pharmaceutical fields and have found many applications as active pharmaceutical ingredients in effective drug delivery. Polyvinyl alcohol (PVA) (Figure 1) is a synthetic polymer which presents many unique properties. PVA hydrogels have attracted many fields of medicine because of its high porous structure, good mechanical as well as bio-tribological properties. PVA is also nontoxic, highly hydrophilic with long-term thermal and pH stability. Thus, PVA gels can provide unconventional form of drug dosage and can be used as artificial cartilage or even repairing tissues [2-11].

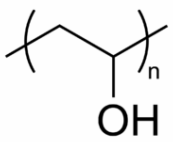

Figure 1. Polyvinyl alcohol unit

Naproxen [(+)-(S)-2-(6-methoxynaphthalen-2-yl)propanoic acid] (Figure 2) is used universally as a drug treatment for pain, fever and inflammation caused by conditions such as rheumatoid arthritis or osteoarthritis. It is a non-steroidal anti-inflammatory drug (NSAID) causing inhibition of COX isoenzymes which are responsible for prostaglandins production. Prostaglandin synthesis, in response to an inflammation, arises from *de novo* induction of cyclooxygenase-2 expression by inflammatory cytokines and other noxious stimuli. Therefore, pharmacological inhibition of COX-2 isoenzyme either selectively or non-selectively helps in reducing inflammation [12,13].

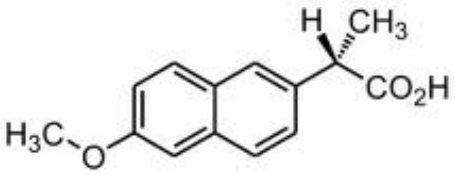

Figure 2. Naproxen structure

The intrinsic amino acid tryptophan is widely used as a tool in studies of protein structure, conformation changes, dynamics, intrinsic fluorescence and functions due to its spectral properties. Tryptophan with its indole chromophore demonstrates high sensitivity to interactions and changes of the environment [1,14,15].

Spectroscopic properties of abovementioned chemicals should be precisely studied and characterized to gain a better understanding of their interrelationships related to proteins, therefore investigations introduced in our study were designed in simple model system [1]. By varying the polarity of the samples using distinct media it is possible to determine whether tryptophan or naproxen are undergoing ($n \rightarrow \pi^*$) or ($\pi \rightarrow \pi^*$) shifts. Therefore two different solvents water and cyclohexane were used for naproxen and tryptophan samples in comparison to PVA [16].

Experimental

Materials

Naproxen and PVA (87-90% hydrolyzed with average molecular weight 30,000-70,000) were purchased from Sigma. Chromatographically homogeneous L-tryptophan was supplied by Roanal, Hungary. All reagents were at least analytical grade and were used without further purification. Concentrations given are concentrations of added reagents.

Equipment

The absorbance of all samples was measured using a Nicolet Evolution 300 UV-Vis spectrophotometer (resolution 1nm; A range 0-6) from Thermo Electron Corporation. The measurements of the steady-state fluorescence were done using a spectrofluorometer Fluoromax-2 (2000 signals/noise) from Jobin Yvon-Spex with the excitation wavelengths of 318 and 332 nm for solutions/films containing naproxen and 295 and 280 nm for the solutions/films containing tryptophan. The measurements of the fluorescence decays were done using a time-correlated single-photon-counting method (Edinburgh Instruments OB-920) with excitation wavelengths of 295 nm and emission wavelengths of 350 nm for films/solutions containing tryptophan and naproxen. A hydrogen nanosecond flash lamp with a repetition rate of 40 kHz was used as the excitation source. Fluorescence decays data was analyzed using F-900 Spectrometer Software. PVA gels were prepared on the 10 mm quartz slides, solutions were placed into 10 mm quartz cuvettes respectively and except as otherwise stated temperature was kept constant at 25°C.

Methods

Preparation of PVA gel

Polyvinyl alcohol (1.75 g) was weighed into a beaker and dissolved in distilled water (50 ml) with stirring at room temperature. The solution was heated using a heating mantle to 80°C and stirred for 1 hour. The solution was cooled with stirring to 40°C then transferred to a polyethylene (PE) plate containing a quartz slide. The solution was left for 3 days to allow the film to form. The film was removed from the plate and cut to obtain the quartz slide with the thin PVA film on the surface. All PVA gels were prepared according to protocol, developed and implemented on the basis of research and literature [7,17-19].

Preparation of PVA gel containing naproxen

Polyvinyl alcohol (1.75 g) was weighed into a beaker and dissolved in distilled water (50 ml) with stirring at room temperature. The solution was heated using a heating mantle to 80°C with stirring. Half of the solution was disposed of and replaced with a 0.0005 M stock solution of naproxen dissolved in ethanol (1 ml) and water (24 ml). The resulting solution was stirred for 1 hour at 80°C then cooled with stirring to 40°C. The solution was poured into a PE plate containing a quartz slide and left for 3 days to allow the film to form. The film

was removed from the plate and cut to obtain the quartz slide with the thin PVA film on the surface. The above procedure was repeated with a 0.00005 M stock solution of naproxen.

Preparation of PVA gel containing tryptophan

Polyvinyl alcohol (1.75 g) was weighed into a beaker and dissolved in distilled water (50 ml) with stirring at room temperature. The solution was heated using a heating mantle to 80°C with stirring. Half of the solution was disposed of and replaced with a 0.0005 M stock solution of tryptophan dissolved in water (25 ml). The resulting solution was stirred for 1 hour at 80°C then cooled with stirring to 40°C. The solution was poured into a PE plate containing a quartz slide and left for 3 days to allow the film to form. The film was removed from the plate and cut to obtain the quartz slide with the thin PVA film on the surface. The above procedure was repeated with a 0.00005 M stock solution of tryptophan.

Preparation of the solutions of naproxen in cyclohexane and water and tryptophan in cyclohexane and water

Naproxen (0.003 g, 0.0005 M) was dissolved in ethanol (1 ml) and water (24 ml) in a volumetric flask. Naproxen (0.003 g, 0.0005 M) was dissolved in cyclohexane (25 ml) in a volumetric flask. This method was repeated for the lower concentration of naproxen (0.0003 g, 0.00005 M) in water and in cyclohexane. The above procedure was repeated with tryptophan respectively.

Results and Discussion

Absorption spectra

Electronic spectra of PVA enable us to identify groups of absorption bands that correspond to the presence of conjugated bonds. PVA backbones consist of carbon-carbon bonds and hydroxyl groups therefore, in the initial stage of heating we should obtain two types of products: polyenes and polyenones. The PVA film spectrum shown in Figure 3 and 4 has one broad absorbance band ~280 nm and one weak band or inflection at ~330 nm. First one is already assigned to the structure of $(CH=CH)_2$-CO- and (CH=CH)-CO- and second one $(CH=CH)_4$-CO-. Heat treatment of PVA results in rapid formation of polyene segments and leads to intensity evolution. π electron systems formed over creation of the principal macromolecule chain are assigned to the abovementioned types and may be differentiated by analysis of peak locations or their intervals [19,20].

Tryptophan in PVA shows three peaks: 273, 281, 290 nm for concentration $5x10^{-4}$ M and 271, 280, 290 nm for concentration $5x10^{-5}$ M. The absorption spectra for tryptophan in water (Figure 4) show maxima peaks at 272, 277 and 287 nm for concentration $5x10^{-4}$ M and 272, 278, 288 nm for $5x10^{-5}$ M. In cyclohexane four maxima peaks are observed at 273, 281, 317 and 331 nm and 273, 281, 316, 330 nm for concentrations $5x10^{-4}$ M and $5x10^{-5}$ M, respectively. The difference in concentration of tryptophan and/or solvent environment slightly affects the position of maxima peaks as well as the intensity of absorbance.

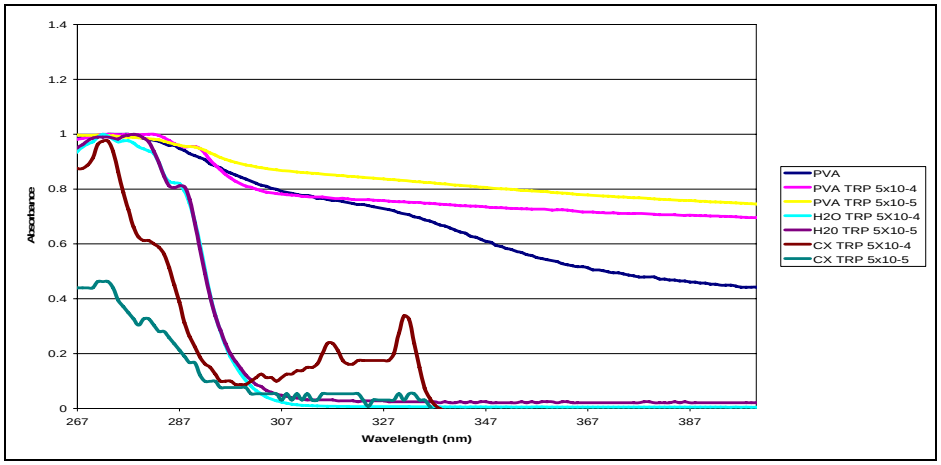

Figure 3. The normalized data absorption spectra of PVA, tryptophan ($5x10^{-4}$M) and ($5x10^{-5}$M) in PVA, water and cyclohexane

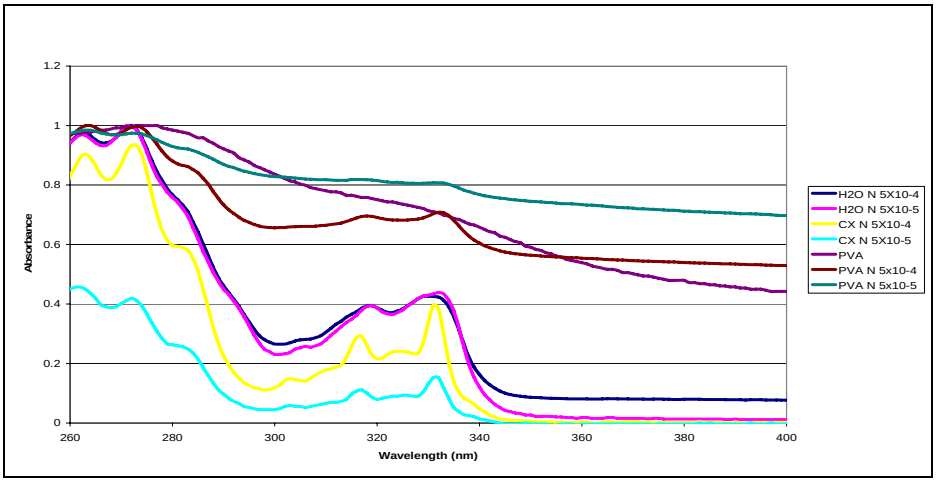

Figure 4. The normalized data absorption spectra of PVA, naproxen ($5x10^{-4}$) and ($5x10^{-5}$) in PVA, water and cyclohexane

In Figure 4 the spectrum for naproxen in PVA shows four peaks with maxima at 264, 273, 318 and 333 nm same for both concentrations. For naproxen in cyclohexane we can observe maximum peaks at 262, 272, 317 and 331 nm for both concentrations. In Figure 6 the spectrum for naproxen in water shows four peaks with maxima at 263, 272, 318, 330 nm for concentration $5x10^{-4}$ M and 262 nm, 271 nm, 319 nm and 332 nm for concentration $5x10^{-5}$ M. The peak at 264/263 nm differs from the literature values which do not include this maximum [21]. The concentration of naproxen and/or solvent environment does not significantly affect the position of maxima peaks.

Fluorescence spectra

In Figure 5 and 6 the spectrum for PVA shows a maximum at 405 nm which corresponds to the literature value [19]. The steady state fluorescence emission spectra for tryptophan in PVA show a maximum peak at 337 nm for concentration $5x10^{-4}$ M and 330 nm due to lower concentration of $5x10^{-5}$ M. The spectrum for tryptophan in water shows maxima at 356 nm for both concentrations. For tryptophan in cyclohexane two peaks at 348 and 355 nm are observed for concentration $5x10^{-4}$ M as well as 341 and 356 nm for concentration $5x10^{-5}$ M.

Emission spectra for naproxen in PVA show a maximum peak at 352 nm for both concentrations, which is comparable with the literature value [21]. The difference between concentrations of naproxen in PVA does not affect the position of maxima peak but it does affect the intensity of absorbance. Emission spectra for naproxen in water at concentration $5x10^{-4}$ M shows maximum peak at 352 nm and for $5x10^{-5}$ M there is a maximum peak at 354 nm; emission spectra for naproxen in cyclohexane shows maximum peaks at 348 nm and 356 nm in both concentrations.

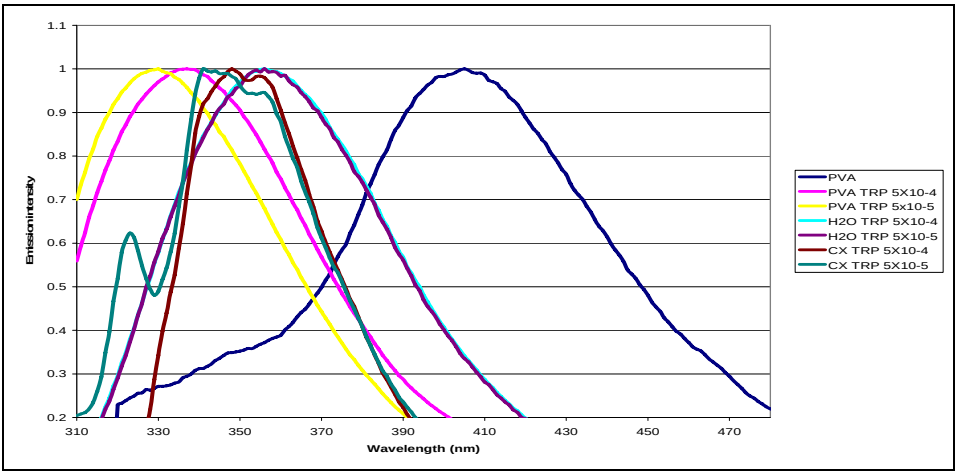

Figure 5. The normalized data emission spectra of PVA, tryptophan ($5x10^{-4}$M) and ($5x10^{-5}$M) in PVA, water and cyclohexane

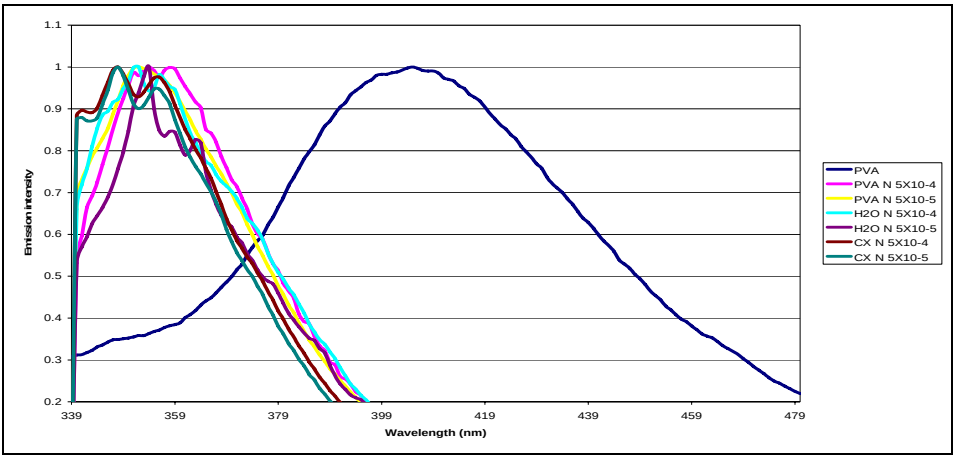

Figure 6. The normalized data emission spectra of PVA, naproxen ($5x10^{-4}$M) and ($5x10^{-5}$M) in PVA, water and cyclohexane

Table 1. Summary of λ maxima of absorption and emission spectra for PVA, tryptophan, and naproxen in PVA, water and cyclohexane

Sample	λ max [nm]	
	UV-Vis	Fluorescence
PVA	277, 330	405
PVA - NAP $5x10^{-4}$	264, 273, 318, 333	352
PVA - NAP 5x10-5	264, 273, 318, 333	352
H2O - NAP 5x10-4	263, 272, 319, 330	352
H2O - NAP 5x10-5	263, 271, 319, 332	354
CX - NAP 5x10-4	263, 273, 317, 332	348, 356
CX - NAP 5x10-5	263, 273, 317, 332	348, 356
PVA - TRP 5x10-4	273, 281, 290	337
PVA - TRP 5x10-5	271, 280, 290	330
H2O - TRP 5x10-4	272, 277, 287	356
H2O - TRP 5x10-5	272, 278, 288	356
CX - TRP 5x10-4	273, 281, 317, 331	348, 355
CX - TRP 5x10-5	273, 281, 316, 330	341, 356

Time-resolved fluorescence

Time resolved fluorescence spectroscopy measurements are used to investigate the structure, molecular interactions and motions as well as dynamics of molecules e.g. proteins, nucleic acids, membranes or photosynthetic systems. This technique illustrates the environment of a fluorophore and its lifetime and allows for monitoring overall motions. Fluorescence data and investigation of dynamic events and local environment are illustrated by decay patterns characterized by one or more lifetimes [22,23].

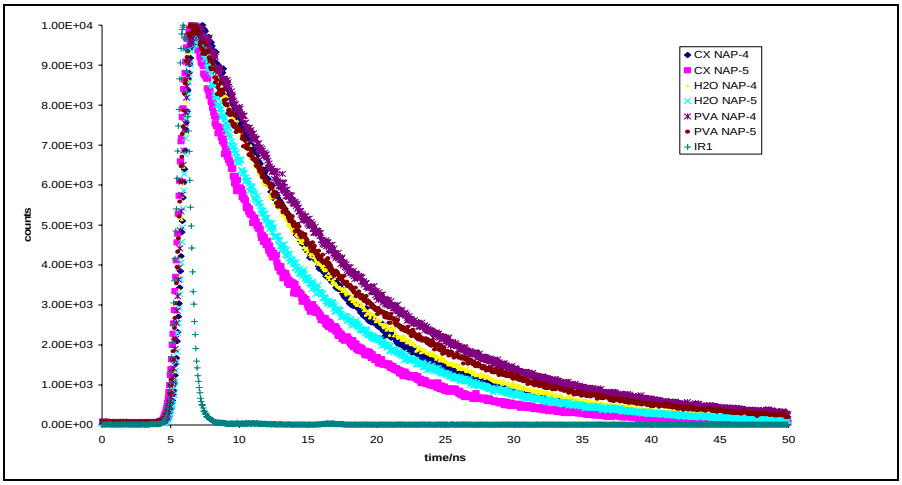

Figure 7. Fluorescence time decay for naproxen ($5x10^{-4}$ and $5x10^{-5}$) in water, cyclohexane and PVA

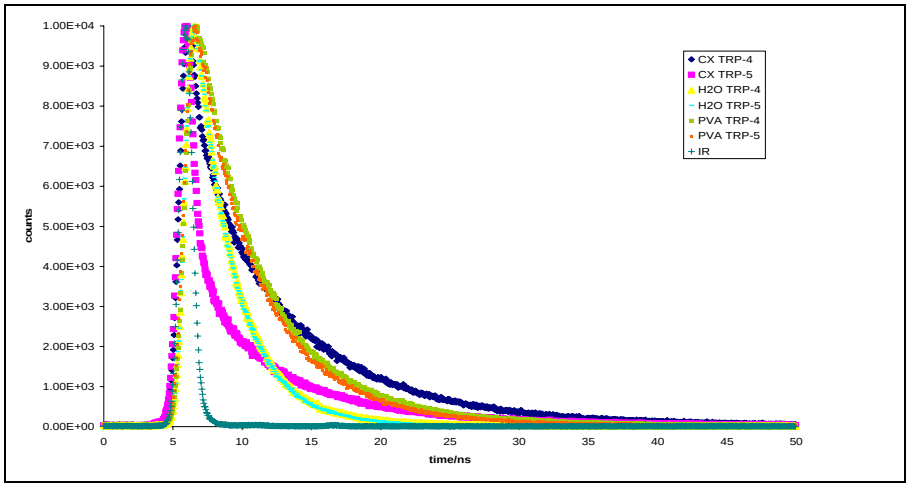

Figure 8. Fluorescence time decay for tryptophan ($5x10^{-4}$ and $5x10^{-5}$) in water, cyclohexane and PVA

Lifetimes were analyzed though deconvolutions of time-resolved decays of fluorescence intensity observed for excited state [24].

PVA itself is a short time component. First component τ_1 as well as average lifetimes apparently depend on concentration. τ_1 and τ_2 values as well as average time decay for naproxen in cyclohexane are comparable with naproxen in water. The τ values for the tryptophan solutions made with water and cyclohexane are also consistent. The kinetics of the fluorescence decay of PVA, tryptophan in water $5x10^{-4}$ M and in cyclohexane $5x10^{-4}$ M and $5x10^{-5}$ M are tri-exponential whereas the fluorescence decay of PVA-tryptophan, PVA-naproxen, and

tryptophan in water $5x10^{-5}$ M, naproxen in water and naproxen in cyclohexane $5x10^{-5}$ M are di-exponential. Naproxen in cyclohexane $5x10^{-4}$ M is mono-exponential.

Table 2. Fluorescence time decays for PVA, PVA and tryptophan, PVA and naproxen, tryptophan in water and cyclohexane and naproxen in water and cyclohexane

Sample	τ_1		τ_2		τ_3		$<\tau>$
	(ns)	B_1	(ns)	B_2	(ns)	B_3	(ns)
PVA	0.72	0.017	0.170	0.242	3.62	0.002	0.693
PVA NAP $5x10^{-4}$	24.45	0.001	11.140	0.045			11.759
PVA NAP $5x10^{-5}$	3.88	0.384	11.070	0.041			5.559
H_2O NAP $5x10^{-4}$	5.81	0.048	9.067	0.074			8.111
H_2O NAP $5x10^{-5}$	9.55	0.036	5.540	0.013			8.855
CX NAP $5x10^{-4}$	9.08	0.054					9.080
CX NAP $5x10^{-5}$	8.05	0.038	2.270	0.012			7.577
PVA TRP $5x10^{-4}$	3.70	0.039	9.820	0.244			9.472
PVA TRP $5x10^{-5}$	2.59	0.019	5.340	0.033			4.740
H_2O TRP $5x10^{-4}$	2.64	0.051	5.290	0.004	0.50	0.009	2.930
H_2O TRP $5x10^{-5}$	2.93	0.037	1.160	0.005			2.840
CX TRP $5x10^{-4}$	2.15	0.009	0.340	0.055	7.38	0.028	6.428
CX TRP $5x10^{-5}$	0.29	0.127	2.870	0.009	7.98	0.011	5.220

Conclusions

This study provides an understanding of the physical and chemical properties of the PVA gel and its ability to hold the entrapped drug in the gel matrix as well as solvent effect on naproxen and tryptophan photochemical properties. Our current efforts in this study on using PVA with its spectral characteristics to prove abovementioned capability is mainly motivated by the promising application of such polymeric materials in medicine.

In particular, absorption and emission spectra depend on the solvent polarity; therefore the following considerations may be made:

a. Emission spectra of naproxen in PVA and water are comparable due to similar polarity (slight blue shift), emission spectra of naproxen in PVA versus cyclohexane demonstrate red shift due to decrease in solvent polarity.

Tryptophan typically has an emission peak ranging from 300 to 350 nm and maximum absorbance of 280 nm depending on the polarity of the environment. We can observe a blue shift ($n \rightarrow \pi^*$) between PVA and water and small difference between water and cyclohexane.

- In absorption spectra we can see a slight maxima shifts depending rather on concentration, very weekly on solvent polarity and media used.
- Immobilization of naproxen and tryptophan in PVA leads to extension of fluorescence time decays due to locked oscillatory states.
- Homogeneity of peak shapes is directed to the presence of naproxen and tryptophan and their concentrations. Maxima shifts depend on solvent environment.

Due to naproxen's low solubility, its oral administration results in gastrointestinal irritation, therefore alternative ways of administration are of great importance.

Although we demonstrated, under our experimental conditions, that PVA is a suitable material for the immobilization of naproxen achieved without use of chemical or reinforcing cross-linking agents and offers good performance owing to the closer structure and amount of immobilized drug, we are aware of the limitation for therapeutic applications.

Nevertheless a deeper knowledge of physical and chemical properties of this polymeric material can be of great interest, in order to demonstrate its utility in specific drug delivery applications.

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