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Research Article

FLUORESCENCE APPROACH TO MICELLAR SOLUBILIZATION OF AN AROMATIC COMPOUND: P-BENZOQUINONE (p-BQ)

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ABSTRACT

1,4-BQ or p-BQ are ubiquitous in nature and can be synthesized by diverse strategies. Quinones are the large class of compounds endowed with rich and fascinating chemistry. A large number of chemical derivatives with p-BQ as the basic subunit exhibit prominent pharmacological applications such as antibiotic, antimalarial, antitumor, antineoplastic, anticoagulant and herbicidal activity. Micellar solubilization of p-BQ in non-ionic and ionic surfactants monitored by fluorescence and absorption spectral techniques has been reported. The relatively week fluorescence of p-BQ was significantly enhanced in non-ionic micellar media formed by TX-100 surfactant. The influence of the surfactant, concentration and working experimental conditions on the fluorescence spectra of p-BQ is thoroughly evaluated and discussed. The solubilizing, action of the surfactants has been confirmed by the theoretically calculated spectral parameters like, empirical fluorescence coefficient, quantum yield, molar extinction coefficient and Stokes' shift. The author provides a unique format for the analytical and medicinal applications of p-BQ based on micellization of the surfactants which makes them promising drug carriers, employing fluorescence, absorption and light scattering measurements.

Keywords: *Micelles, p-BQ, Fluorescence, Solubilization*

INTRODUCTION

Transdermal drug delivery is the topical application of drugs to the skin in the treatment of skin diseases, wherein high concentrations of drugs can be localised at the site of action, thereby reducing the systematic drug levels and side effects [1-3]. Surfactants are frequently used as emulsifiers in formulations for dermal application [4]. It is generally recognizing that non-ionic surfactants possess the least toxicity and skin irritation potential [5] and therefore they have been widely investigated as skin penetration enhancers.

In biological systems, the effects of surfactants are complex, particularly their effect on cell membranes, which can lead to alteration is permeability [6].

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The effect of surfactants on membrane permeability describe an apparent concentration dependent biphasic action, such that an increase in membrane permeability occurs at low surfactant concentration, but this decreases at higher concentrations (CMC) of the surfactants. Above the CMC, the added surfactant exist as micelles in the solution and micelles are too large to penetrate the skin [7].

The utilization of micelles as drug carriers presents some advantages when compared to other alternatives such as soluble polymers and liposomes. Micellar systems can solubilize poorly soluble drugs and thus increase their bioavailability, stay in the blood long enough to provide gradual accumulation in the required area, their size permit them to accumulation area with leaky vasculature. Moreover, specific ligands can be attached to their outer surface to optimize the controlled release and specificity of pharmacological effect. Micelles can be

obtained in an easy and reproducible manner in large scale [8]. Therefore, the utilization of aqueous solutions for drug solubilization cab be advantageous for drug delivery purposes, with 2 possibilities of increasing water solubility of poorly soluble drug, improving bioavailability, reducing toxicity and other side effects, enhancing permeability across the physiological barriers, and substantial change a drug distribution [8].

p-benzoquinone(p-BQ) is widely used in medicine, herbicides, chemical reagents, dyes and tanning agents. For medical purposes, p-BQ is used in pharmaceutical industry for production of cortisone.

UV-VIS spectroscopy is an analytical spectroscopic technique used to measure the absorbance of certain molecules in UV-VIS region of the electromagnetic spectrum. Upon radiation with incident light, molecules absorb energy as their electrons are excited to higher energy levels and it is this absorbance that is measured with UV-VIS spectroscopy [9]. It is believed in this study increases the absorbance of the modified molecules which, by themselves, absorb highly in the UV-VIS range.

Fluorescence spectroscopy along with UV-VIS spectroscopy was utilized to examine the spectrometric characteristics of Ribonuclease A after exposure to p-BQ in addition to unmodified Ribonuclease A [10]. Information about excited states of radical ions can enable their use as powerful oxidising and reducing agents capable to driving chemical reaction. This article presents the first measurements of fluorescence from an excited state of the isooctane solution [11]. The low fluorescence quantum yield, $\phi_f = 0.003$ and the presence of a 0.5eV red shift of the emission band edge (593nm) from the absorption band edge (475nm) imply that the lowest energy transition in p-BQ, which is the source of weak fluorescence, is formally forbidden. This conclusion is supported by both semi empirical and an initio molecular orbital calculations.in addition, we determined the 63ns, with an excited state absorption spectrum peaking at 415nm [11].

MATERIALS AND METHODS

Materials:- The following surfactants were employed:

S.NO	Non-ionic surfactants	Anionic surfactants	Cationic surfactants	Manufacturer
1	TX-100 : Polyoxyethylene tert-octyl phenyl ether	SLS : Sodium Lauryl Sulphate	CPC : Cetyl Pyridinium Chloride	All the surfactants were either of sigma (USA) or BDH (UK) products.
2	Tween-80 : Polyoxyethylene Sorbitain monooleate	DBSS : Dodecyl Benzene Sodium Sulphonate	CTAB : Cetyl Trimethyl Ammonium Bromide	
	Tween-20 : Polyoxyethylene Sorbitain monolaurate	DSSS : Dioctyl Sodium Sulpho Succinate	MTAB : Myristyl Trimethylammonium Bromide.	

Method:-

The stock solution was prepared in double distilled water. All the experiments were performed around 23-25^{o}C and final concentration was kept at $5X10^{-5}$ M for fluorescence studies. For absorption studies, the concentration of p-BQ was kept at $4X10^{-4}$ M throughout the experiment.

All the fluorometric experiments was carried out with Perkin Elmer Fluorescence Spectrophotometer (Model No. 204 A) with a synchronizes strip chart recorder (Model No. 056). A xenon lamp was used as a light source. For recording the fluorescence

excitation and emission spectra, its slit width was kept at 10nm and a cell of 1cm path length was used.

The absorption measurements were made with Hewlett Packard (HP) 8452, and diode array spectrophotometer respectively. The light scattering studies were made with a Brice-galvanometer. Measurements are made at an angle of 90^{o} to the incident beam and the scattering intensity was measured in terms of galvanometer deflection.

The purity of the surfactants was checked by determining their CMC values the help of surface tension measurements, employing drop weight method. The values obtained coincide with reported values. The fluorescence quantum yield values were calculated relative to anthracene in methanol as standard. Fluorescence emission of anthracene is in the same range as that of p-BQ. Approximate corrections were made to compensate for different absorption of the compound and the standard. Each time the total intensity of fluorescence emission was measured for the standard and the sample from the area of the fluorescence spectrum recorded over the whole range of emission under identical conditions. Molar extinction coefficient data have been reported as its logarithm (log ε). The Stokes' shifts data have been calculated with change in its concentration.

RESULTS AND DISCUSSION

The aqueous solution of p-BQ showed maximum excitation peak at 290nm and emission peak at 330nm. All the non-ionic surfactants, on addition to p-BQ solution caused a continuous enhancement in its fluorescence emission intensity with increasing concentration. Among them TX-100 exerted the maximum effect with blue shift of 25nm. The changes in the fluorescence spectra of p-BQ on addition of TX-100 are given in Fig 1. For anionic surfactants added p-BQ solution, fluorescence intensity initially decreased and then increased at their higher concentration with a red shift of 25nm with DBSS and 5nm with DSSS. While with SLS emission intensity decreased with red shift of 5nm. The changes in the fluorescence intensity of p-BQ on adding DBSS are given in Fig. 2. For cationic surfactant added solution fluorescence intensity initially decreased slightly and then increased at their higher concentration with red shift of 5nm in emission peak position. While with CPC, decreasing effect on the fluorescence intensity of p-BQ accompanied by a small red shift of 5nm. Maximum effect of CTAB on p-BQ are given in Fig.3. the fluorescence intensity in presence and absence of surfactants are given Table 1.

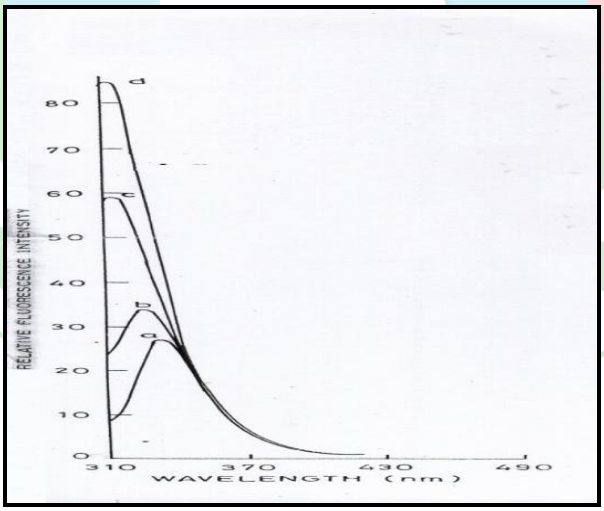

Fig 1. Influence of addition of TX-100 on fluorescence intensity of $5X10^{-5}$ M p-BQ solution.

(a) No surfactant , (b) 0.07% TX-100, (c) 0.1% TX-100, (d) 0.15% TX-100

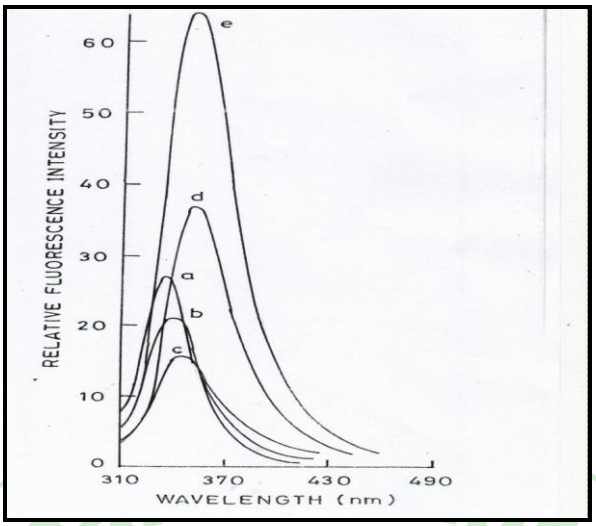

Fig 2. Influence of addition of DBSS on fluorescence intensity of $5X10^{-5}$ M p-BQ solution.

(a) No surfactant, (b) 0.1% DBSS, (c) 0.05% DBSS

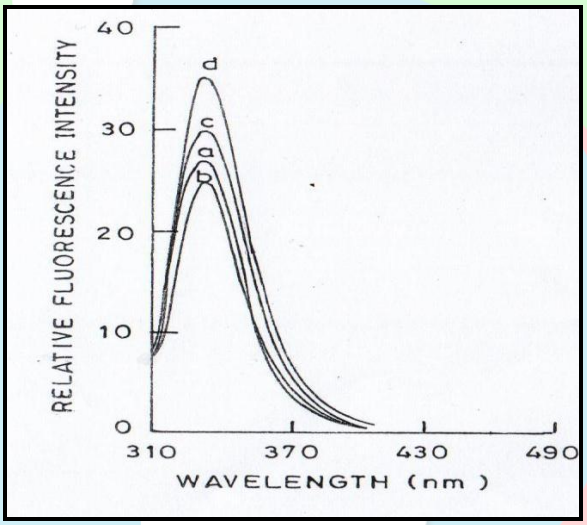

Fig 3. Influence of addition of CTAB on fluorescence intensity of $5X10^{-5}$ M p-BQ solution.

(a) No surfactant, (b) 0.07% CTAB, (c) 0.3% CTAB, (d) 0.7% CTAB

Table 1: Fluorescence intensity of p-BQ in absence and presence of surfactant

Name of surfactant	Fluorescence intensity in absence of surfactant	Concentration of surfactant used (%)	Maximum fluorescence intensity (nm)
TX-100	27	0.3	102
Tween-80	27	0.5	39
Tween-20	27	0.7	39
CPC	27	0.01	25
CTAB	27	0.7	35
MTAB	27	0.7	36
DBSS	27	0.5	64
DSSS	27	0.7	42
SLS	27	0.05	27

λ_{ex} = 290nm, λ_{em} = 330nm, P.M Gain = 3, Sensitivity Range = 1

The absorption spectra gave a peak at 290nm. All the non-ionic surfactants showed enhancement in absorbance with 5nm blue shift. For anionic surfactant added solutions absorbance increased without any shift. For cationic surfactants, with CTAB and MTAB, initially decreased and then increased with no shift in CTAB and small red shift with MTAB. While with CPC, continuously decreased and did not show any shift in λ_{max}. The changes in the absorption spectra with DBSS are shown in Fig.4.

The light scattering studies of p-BQ were made at an angle of 90^{o} to the incident light. During addition of each surfactant there occurred a sharp decrease in the galvanometer deflection with increase in surfactant concentration.

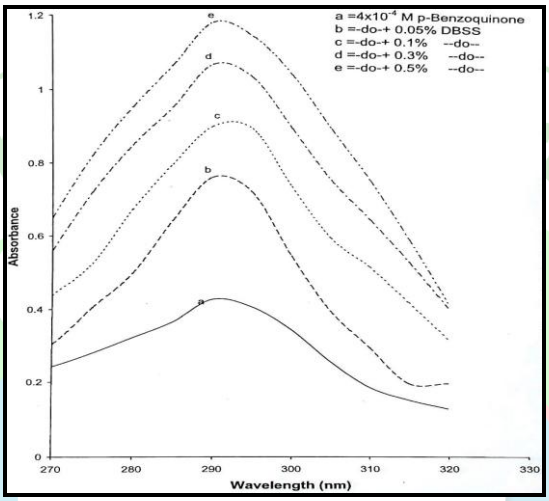

Fig 4. Influence of addition of DBSS on absorbance of 4X10^{-4} M p-BQ.

(a) No surfactant, (b) 0.05% DBSS, (c) 0.1% DBSS, (d) 0.3% DBSS, (e) 0.5% DBSS

The calculated fluorescence quantum yield (ϕ_f) of surfactant added p-BQ showed almost parallelism with changes in fluorescence intensity of the compound. In all surfactants non-ionic, anionic and cationic surfactants, micellar media the quantum yield values increased with the concentration. Highest (ϕ_f) values were obtained for TX-100 micellar media, which are given in Table-2. Molar extinction coefficient (log ε) values for all the non-ionic and anionic surfactants increased. While with cationic ϕ_f values gave different trend, with CTAB and MTAB, initially decreased and then increased at their higher concentrations. While with CPC it decreased. The empirical fluorescence coefficient values (K_f) for non-ionic surfactants increased gradually. With anionic surfactants showed different behaviour. The K_f values obtained with CTAB and MTAB both were similar, caused first decreased and then an increase in K_f values. K_f values obtained for CPC are in decreasing order.

The results indicate that the surfactant added system has a stronger emission intensity enhancement than the system in which there was no surfactant present. The maximum fluorescence enhancement was obtained by adding TX-100. On adding anionic and cationic surfactants gave red shift of 5nm in emission intensity. This may be due to a progressive increase in the presence of aggregated solute molecules with increasing solute concentration, rather than the enhancement in the local fields. Therefore, the emission of aggregate solute molecules is red shifted [12].

Table 2: Absorption maxima (λ_{max}), fluorescence maxima (λ_{em}), molar extinction coefficient (log ε) and quantum yield (ϕ_f) of p-BQ at different concentration of TX-100

S.No.	TX-100 in %	λ_{max} (nm)	log ε ($dm^3mol^{-1}cm^{-1}$)	λ_{em} (nm)	ϕ_f
1.	0.000	290	3.9344	330	0.1727
2.	0.07	290	3.9912	325	0.1767
3.	0.1	290	4.1271	315	0.1934
4.	0.15	290	4.1760	310	0.2088

The absorption spectroscopy of organic compounds is based on the transitions of n or π electrons to the π* excited state. This is because the absorption peaks for the transitions fall in an experimentally convenient region of the UV-VIS spectrum. The very weak absorption bands at the long wavelength side of the n- π*, singlet-singlet absorption band of p-BQ and its derivatives were examined [13]. On adding the surfactant, energy difference between the ground and excited states was slightly enhanced resulting in a small blue shift with water polarity due to n- π* transitions, thus this shift is because of the difference in the solution energy of the solute in the ground state and excited state in micellar media.

The result obtained can be explained based on solubilization by the micro heterogenous environment of micelles present in the surfactant solution at or marginally above CMC. The maximum enhancement in the fluorescence emission intensity of p-BQ was obtained with TX-100 micellar media, which has also been supported by absorbance, light scattering flux (σ_f) values and log ε values. This may be attributed to the increase in quantum efficiency of fluorescence. Furthermore, the quantum yields off fluorescence is higher in non-polar medium because of the lesser effect of other deactivation processes which compete fluorescence [14]. Also, that the rate of non-radiative processes is less in non-ionic micellar medium in comparison to those in aqueous medium. Another cause may be due to the absorption of the fluorophore at the micellar surface which decreases the collision of the fluorophore by water molecules.

The photoreduction of p-BQ is normal and reversed micellar system has been studied kinetically under high pressures up to 150 M Pa. Anionic sodium dodecyl sulphate (SDS) micelles accelerated the reaction, while cationic CTAB micelles restarted it. Pressure promoted the photoreaction in CTAB micellar system but not in SDS micelles. The location of p-BQ in the micellar system was studied using data for their Critical Micelle Concentrations. The mechanisms of the reactions are also discussed [15].

The vapour phase fluorescence spectra of p-BQ-h4 and d4 are reported and discussed in relation to the assignment of the low-lying singlet states. The low temperature, polarised single crystal electronic absorption spectra of p-BQ and several of its isotopic derivatives are reported. From the isotopes shifts and band polarisation of the various origins, a detailed vibrionic analysis is offered of the electronic absorption spectrum of p-BQ which indicates a near degeneracy of the 1 Au and 1 B 1g electronic stat

The molecule of p-BQ have been subsequently solubilized of incorporation into the interior non-polar core of the micelles. Sufficiently large values of molar extinction coefficient (log ε) may be due to π → π* transitions, as on increasing the concentration of non-ionic surfactant, n-π* transition decreased. The log ε values of the p-BQ molecules in different micellar media follow the same trend as their emission intensity except DBSS and SLS.

The values of empirical fluorescence coefficient K_f obtained may be attributed to the increased sensitivity of the fluorometric analysis of the solubilization of organic molecules by surfactants which offer a protective microenvironment, leading to enhanced fluorescence of the solubilizate by shielding the excited state from non-radiative decay that normally occurs in bulk aqueous solution. The Stokes' Shift values for p-BQ increased gradually. For very dilute solutions of p-BQ, the values of Stokes' Shift slightly decreased and then remain constant. The variation in Stokes' Shift indicate that the large energy changes occur in the excited state of the molecule on varying the concentration of the compound at room temperature. The theoretically calculated spectral parameters are illustrated in Table 3.

Table 3: Stokes' shift data of p-BQ at room temperature

S.No.	Concentration of compound	λ_{ex} (nm)	F.I.	λ_{em} (nm)	F.I.	P.M. Gain	Sensitivity Range	Stokes' shift (cm^{-1})
1.	$1X10^{-3}$ M	305	31	335	45	2	0.3	2936
2.	$5X10^{-4}$ M	300	30	335	42	2	0.3	3482
3.	$3X10^{-4}$ M	295	26	335	97	3	0.3	4047
4.	$1X10^{-4}$ M	290	16	335	55	3	0.3	4632
5.	$7X10^{-5}$ M	290	11	335	43	3	0.3	4632
6.	$1X10^{-5}$ M	290	3	330	23	3	0.3	4179

CONCLUSION

The present analysis and interpretation suggest that experimental results observed and the theoretically calculated spectral data are found to be in good agreement. This proves the validity of the investigation made.

Experimental solubilities of hydrocortisone drug in ethanol + water mixtures at 298.2 K are reported. The solubility of drug was increased with the addition of ethanol and reached the maximum values of the volume fractions of 80% of ethanol [17].

Surfactants have significance in pharmacy because of their ability to increase the solubility of sparingly soluble substance in water [18]. Numerous drug delivery and drug targeting systems have been studied to minimize drug degradation and loss, to prevent harmful side effects, and to increase drug bio-availability [19-23]. Micellar systems can solubilize poorly soluble drugs and thus increase their bio-availability, they can stay in the body (blood) long enough to provide gradual accumulation in the required area, and their size permit them to accumulate in areas with leaky vasculature [24].

The solubility of a poorly soluble compound as a function of the concentration of surfactant, usually what happens is that the solubility is very low until the surfactant concentration reaches the CMC. At surfactant concentrations above the CMC of surfactant, indicating that solubilization is related to micellization.

Non-ionic surfactants usually are better solubilizing agents than ionic surfactants for hydrophobic drugs, because of their lower CMC values. The increased solubilization of p-BQ in TX-100 micellar solution was a consequence of interaction of TX-100 head group. i.e. its monomeric form and of the molar fraction of surfactant in the micellar form that is higher for non-ionic surfactants due to the low CMC.

Therefore, non-ionic surfactants could be considered the best alternative for solubilization of p-BQ, as well as another basic drug. This class of surfactants provides a reasonable molar solubilization and drug delivery purposes.

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REFERENCES

1. *Chein YW (1992) Transdermal drug delivery and delivery systems. Novel drug delivery systems. New York: Marcel Dekker, 301-380.*
2. *Chein YW (1987) Transdermal controlled systemic medications. New York: Marcel Dekker Inc.*
3. *Vyas SP, Khar RK (2002) Transdermal drug delivery controlled drug delivery-concepts and advances. New Delhi, India: Vallabh Prakashan 411-447.*
4. *McNaught AD, Wilkinson A (1997) compendium of chemical Terminology, 2nd edition. The Gold Book.*
5. *Walter KA (1990) Surfactants and percutaneous absorption. In: Prediction of percutaneous penetration: methods, measurements, modelling, scott RC, Guy RH, Hadgraft J (Eds). IBC Technical Services Ltd. London, UK, 148-162.*
6. *Attwood D, Florence AT (1983) surfactant sysytems: Their chemistry, Pharmacy and Biology, Chapman and Hall ltd., London, UK.*
7. *Scheuplein RJ, Ross L (1970) Effects of surfactants and solvents on the permeability of epidermis. Journal of the society of Cosmetic Chemists 21: 853-873.*
8. *Torchilin VP. J control, Rel 2001; 77: 137-172.*
9. *Fluorescence and UV-VIS studies of quinone-induced protein modification. Skoog et. al., 2007.*
10. *Fluorescence and UV-VIS studies of quinone-induced protein modification, Kim et. al., 2012.*
11. *Andrew R. Cook. *Larry A. Curtiss. * and John R. Miller, J. Am. Chem. Soc., 1997, 119(24), pp 5729-5734.*
12. *Baldo, et. al., Chem. Phys. Lett. 347, 297 (2001).*
13. *Akira kuboyama, Bull. Chem. Soc. Japan, 35, 295-298 (1962).*
14. *Robinson R J and Dennis E A. Acc. Chem. Res, 1983,16; 257.*
15. *Katsuhiro Tamura, Masatoshi Abe and Masayoshi Tearl, J. Chem. Soc. Faraday Trans.1, 1989, 85, 1493-1500.*
16. *Thomas M. Dunn, Anthony H. Francis, March 1974, DOI: 10, 1016/ 0022-2852 (74) 90213*

17. *Hany S.M. Ali, Peter York, Nicholas Blagden, Shahla Soltanpour, William. E. Acree Jr. and Abolghasem Jouyban. J. Chem. Eng. Data, 2010, 55(1), pp. 578-582.*
18. *Mall. S., Buckston, G., Rawlins, D.A. Dissolution behaviour of sulphonamide into sodium dodecyl sulphate micelles: A thermodynamic approach. J. Pharm. Sci., 85(1); 75-78, 1996.*
19. *Allen, T.M., Hansen, C.B., Menenez, D.E.L. Pharmecokinetics of long circulating liposomes. Adv. Drug Deliv. Rev, 16: 267-284, 1995.*
20. *Canto, G.S., Dalmora, S.L., Oliveira, A.G.Piroxicam encapsulated in liposomes: characterization and in vivo evaluation to topical anti-inflamatory effect. Drug Dev. Ind. Pharm., 25: 1235-1239, 1999.*
21. *Gref, R., Minamitake, Y., Peracchia, M.T., Trubetskoy, V.S., Torchilin, V.P., Langer, R. Biodegradable long-circulating polymeric nanospheres, science, 263: 1600-1603, 1994.*
22. *Jones, M.C., Leroux, J.C. Polymeric micelles- a new generation of colloidal drug carriers. Eur J. Phar. Bio. Pharm, 48: 101-111, 1999.*
23. *Torchillin, V.P. Structure and design of polymeric surfactant- based drug delivery systems. J Control Rel, 73: 137-172, 2001.*