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Solubilization of Tamoxifen Citrate by Cosolvency And Micellisation: A Comparative Evaluation

Saikat Ghosh[1]*, Tanushree Roy[2]

1 Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, West Bengal, India

2 Department of Pharmacology, Gupta College of Technological Sciences, Asansol-713301, West Bengal, India

ABSTRACT

Tamoxifen Citrate (TC), an antiestrogenic drug, is used in both pre and post menopausal breast cancer treatment in women. One of the major causes of its poor bioavailability is attributed to its poor aqueous solubility. In this study an effort has been made to improve the aqueous solubility of the hydrophobic drug using methods like cosolvency and micellisation. Cosolvents (ethanol, polyethylene glycol-400), and surfactants [polyoxyethylene sorbitan monooleate (Tween-80), Poloxamer-407 and Poloxamer-188] were tested for their solubilizing potential. Solubility enhancement approaches showed variable degrees of increase in solubility of TC. Solubility studies with different concentrations of Ethanol and PEG-400 at 37°C showed that higher efficiency of increasing solubility by cosolvency was achieved with ethanol (6.10 fold) than that with PEG 400 (5.62 fold) while in case of varying concentrations of surfactants (tween 80, Poloxamer 188 and Poloxamer 407). Poloxamer 407 exhibited maximum potential in solubilizing TC in water (7.1 fold). However, among the solubilizing techniques and solubilizing agents used, micellization with polysorbate 80 as surfactant was found to be the most effective. Data suggests that using simple techniques, improvement of solubility of TC can be attained which may help in improving its poor bioavailability.

Keywords: Tamoxifen citrate, bioavailability, cosolvent, surfactant, micellisation, Poloxamer.

*Corresponding Author Email: saikatghosh58@yahoo.com

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INTRODUCTION

Improvement in inherent solubility of sparingly soluble drugs offers an interesting challenge to the formulation scientists, since this is directly related to the bioavailability of the drug. A well experimented phenomenon relates solubility of drugs to be directly proportional to its rate of dissolution and thereby influencing its bioavailability to affected cell while reducing the dose of the drug to achieve the desired effects and hence minimizing the potential side effects[1]. As compared to other methods namely solid dispersion, complexation, micronisation etc., simple, cost effective methods such as cosolvency and micellar solubilizations may used for enhancing solubility of sparingly soluble drugs [2].

The antiestrogenic drug Tamoxifen citrate (TC) is the first choice treatment of breast cancer in both pre- and post-menopausal women. These effects may be related to its ability to compete with the ligand estrogen for binding sites in target tissues such as breast[3,4]. Chemically, TC is the isomer of a triphenylethylene derivative being (Z) 2-[4-(1, 2-diphenyl-1-butenyl) phenoxy]-N,N-dimethylethanamine-2-hydorxy-1,2,3-propanetricarboxylate(1:1). This agent belongs to Class II drugs of Biopharmaceutics Classification system (BCS) indicating that its bioavailability is limited by its poor dissolution rate in GI[3-5]. In fact, its solubility and dissolution rate are major factors in its bioavailability. Studies have reported that following a single oral dose of 20 mg tamoxifen, a very low average peak plasma concentration of 40 μg mL^{-1} (range 35- 45 ng/mL) is reached in 4-7 hours after dosing ascertaining poor bioavailability of the drug[6-8]. Since TC is poorly soluble in water (equilibrium solubility in water at 37 °C being 0.5 mg/mL), it is therefore important to improve its solubility to ameliorate its bioavailability[9, 10].

Cosolvents are generally organic compounds that are substantially miscible with water and enhance the solubility of sparingly soluble chemicals[10-12]. Cosolvents generally have hydrogen bond donor groups and /or hydrogen bond acceptor groups usually less than 3 carbons, which interact strongly with water and help to ensure mutual miscibility or at least aqueous solubility. They decrease the ability of the aqueous system to squeeze out nonpolar solutes, thereby increasing the solubility. Polarity of cosolvent can be characterized by some of the solvent properties, such as surface tension (γ), solubility parameters (δ), dielectric constant (ε) and logarithm of the alcohol water partition coefficient (log Ko/w)[13].

Ethanol is one of the most commonly used liquid cosolvent. Ethanol is an excellent solubilizing agent. It is one of the most efficient cosolvent for nonpolar solutes that can be used parenterally. Unlike most other cosolvents, ethanol reduces viscosity. Its effectiveness as a solubilizing agent at low concentrations and its low viscosity make ethanol ideal for use in combination with water.

The concentration of ethanol in parenteral products is limited to about 10% in 1ml injection. However it can be used in higher concentration (normally not more than 25%) for oral products. Although alcohol concentrations in excess of 50% can be irritating, there is no legal limit to its concentration in topical products [7, 11, 12].

Polyethylene glycols are nontoxic and nonirritating. They are used in a large variety of pharmaceutical preparations and can be tolerated in very high concentrations in topical, oral and parenteral dosage forms. Thus we have selected these two cosolvents- ethanol which reduces viscosity and polyethylene which increases viscosity[11-13].

Surfactants are usually amphiphilic organic compounds that possess both hydrophobic groups (tail) and hydrophilic groups (head). A peculiar phenomena associated with the surfactants is their ability to form micelles above a certain surfactant concentration known as the critical micelle concentration (CMC). A micelle is defined as an aggregate of surfactant molecules dispersed in a liquid colloid. A typical micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions in contact with aqueous layer, sequestering the hydrophobic single-tail regions in the inner micelle core. Formation of micelles is important step in increasing the aqueous solubility of poorly water soluble drugs using surfactants [14, 15].

Polysorbate 80 is high molecular weight hydrophilic polymer widely used as surfactant. It (commercially also known as Tween 80) is a nonionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid, and is often used in foods. The hydrophilic groups in this compound are polyethers also known as polyoxyethylene groups which are polymers of ethylene oxide. In the nomenclature of polysorbates, the numeric designation following polysorbate refers to the lipophilic group, in this case the oleic acid [9, 10, 14, 15].

Poloxamer 407 is a hydrophilic non-ionic surfactant of the more general class of copolymers known as poloxamers. Poloxamer 407 is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol. Poloxamer 407 in formulations lead to enhance solubilization of poorly water-soluble drugs and prolong release profile for many galenic applications (e.g., oral, rectal, topical, ophthalmic, nasal and injectable preparations) but do not clearly show any relevant advantages when used alone[15, 16].

Poloxamer 188 is also a nonionic copolymer surfactant with a triblock structure, comprised of two hydrophilic segments, the poly(oxyethylene) (PEO), and a central hydrophobic segment, the poly(oxypropylene) (PPO), taken together by ether bonds. Combination with other excipients like Poloxamer 188 or mucoadhesive polymers promotes Poloxamer 407 action by optimizing

sol–gel transition temperature or increasing bioadhesive properties. Here we chose the above surfactants as representatives of only non-toxic type of surfactants mostly rich in OH-group as do the selected cosolvents [15, 17, 18].

Although there have been enormous research works using different techniques of solubilization, yet a comparative profiling is very scarce. In this study, we evaluated the efficiency of solubilization approaches such as micellar solubilization, and cosolvency on the aqueous solubility of TC with a few selective pharmaceutically acceptable cosolvents namely (ethanol and PEG 400) and surfactants (tween 80 and poloxamers) in a comparative approach.

MATERIALS AND METHODS

Materials:

Tamoxifen citrate was obtained as a gift sample by Khandelwal Laboratories Pvt. Ltd. Mumbai, India, Poloxamer 407 and Poloxamer 188 were obtained from Sigma-Aldrich, Bangalore, India. Tween-80 was purchased from S. D. fine- chem Pvt limited, Mumbai, India. Absolute ethanol was purchased from Merck Ltd., Mumbai, India. Polyethylene glycol-400 was obtained from SRL Pvt. Ltd., Mumbai, India.

Critical micelle concentration (CMC) determination:

Initially critical micelle concentrations of the surfactants (tween 80, poloxamer 407 and poloxamer 188) were assessed by varying the concentration of the solubilizing agent for a particular concentration of the drug (5 mg). We adopted the method of electrical conductivity for CMC determination. In case of each of the surfactants, the conductivity was plotted against increasing concentration of surfactants (tween 80, poloxamer 188, poloxomer 407) present in water [19, 20]. An abrupt change of slope marked the CMC.

Phase solubility study:

In both the approaches (cosolvency and micellization) of solubilization, solubility of TC was determined by placing an excess amount of TC (10 mg) in water (5 mL). in different test tubes containing increasing concentrations (Table I) of various cosolvents and surfactants so that the total volume in each case remained to 5 mL. Three sets of sample vials were prepared for each particular solubilizing agent. The test tubes were shaken mechanically in a shaking water bath at 37 °C for 48 hr. At equilibrium (after 2 days, as preliminary studies showed that this period of time was sufficient to ensure saturation at 37 °C), aliquots were removed, centrifuged for 10 min at 10000 rpm. After proper dilution with water the samples were analyzed spectrophotometrically at 235 nm using Shimadzu UV/Vis spectrophotometer (Japan) taking appropriate blank solution. Concentrations of drug in the different solutions were determined

from the respective calibration curves prepared with TC in the respective compositions of cosolvent/surfactant solution. The cosolvents used were ethanol (EtOH) and poly (ethylene glycol) 400 (PEG 400). The surfactants were polyethylene sorbitan monooleate (Tween 80), poloxamer 407 and poloxamer 188.

Statistical Calculations:

Data were assessed by one-way ANOVA analysis followed by Tukey comparisons post test using Graphpad Instat software (Graphpad Software Inc., CA, USA). P value < 0.05 has been considered as statistical significance.

RESULTS AND DISCUSSION

Improvement of solubilization of TC by various solubilizing agents namely ethanol, PEG-400, tween 80, poloxamer 407 and poloxomer 188 was assessed by varying the concentration of the solubilizing agents.

Cosolvency:

When the Cosolvency potential of ethanol was compared to PEG 400 with increasing concentration range (from 1% v/v to 15% v/v), ethanol was found to be more efficacious than PEG 400 in enhancement of solubility of TC. Interestingly when solubility of TC was improved by using them, it was found that initially up to concentration (10% v/v) PEG- 400 enhanced the solubility of TC more than ethanol. But in higher concentration (above 12% v/v) ethanol improved solubility of TC more than PEG 400 (Figure 1a &1b, Table 2, 3).

Cosolvents are widely used in pharmaceutical industry for solubilization purpose. They work by reducing hydrogen bond density of aqueous system and create a less polar environment in bulk [2, 9, 10]. This results in more solubilization of sparingly soluble or less soluble drug molecules. Cosolvents generally possess nonpolar regions which do not interact strongly with water and they decrease the capability of water molecules to squeeze out nonpolar solutes from the aqueous system [9-12]. Figure 1b shows linearity in semi logarithmic plot of TC solubility versus cosolvent volume fractions while the solubility versus volume fraction of the cosolvents (Figure 1a) shows non linearty. The findings (from Figure 1a&1b, Tables 2, 3) suggest exponential increase in TC solubility with the increasing concentration of the cosolvent, ethanol and PEG400. Both ethanol and PEG-400 obey First order solubilization kinetic. Solubility study of TC with different concentrations of the cosolvents, ethanol and PEG-400, at 37 °C showed that efficiency of ethanol (15% v/v) as cosolvent was higher (6.10-fold) than that of 15% PEG-400 (5.62-fold) compared to the inherent solubility of TC in water.

A relationship between the total drug solubility (D_{tot}) and cosolvent concentration (C) in a drug-

cosolvent-solvent mixture has been described by using the equation**,**

$$\log D_{tot} = \log D_u + \sigma C$$

where D_u and σ are drug solubility in water and cosolvent solubilization power, respectively [21, 22]. The value of σ is inversely correlated with the polarities of both the solute and the cosolvent. The more nonpolar the solvent and the solute, the larger is the σ value [9-13]. For a single nonpolar solute, cosolvent solubilization power '*σ*' depends only on cosolvent polarity[14]. Table 3 indicates that solubility enhancement of TC follows the cosolvent order as: EtOH (σ: 0.036) > PEG-400 (σ: 0.025). The less polar is the cosolvent, the more effective it is at disrupting hydrogen bonding interactions in water molecules[9, 11]. In the present study more efficient improvement of solubility of TC by ethanol may be because ethanol is the less polar solvent than PEG-400 [9, 11].

However, the primary disadvantages of cosolvency include the potential for biological effects and the potential for drugs that have been solubilized using cosolvents to precipitate upon dilution with aqueous fluids. The biological effects of a cosolvent that may limit or eliminate its use in drug formulations include their general toxicity, target organ toxicity, tissue irritation, or tonicity with respect to biological membranes. In addition, precipitation of drug upon dilution with aqueous media or during injection or application to mucous membranes must always be considered in deciding if a cosolvent can be used as a vehicle for poorly water soluble drugs. Other considerations include the viscosity, tonicity, and taste, as well as the effect of cosolvents on the solubility and stability of formulation components other than the drug. Cosolvent based parenteral formulations suffer from several disadvantages such as pain and tissue damage at the site of injection and precipitation of the drug on dilution in several cases. Furthermore, parenteral administration of the organic solvent can also cause haemolysis[11, 12, 23]. Often, non-aqueous solvents can effectively dissolve the drugs with inadequate aqueous solubility. However, the toxicity of the non-aqueous solvents is a major concern. Additionally, the solubility enhancement generally is significantly reduced in the mixture of non-aqueous solvent and water, compared to non-aqueous solvent alone.

Micellization:

In case of CMC determination, a linear relationship was observed between the absorbance and the concentration of the surfactant was obtained beyond a certain point of surfactant concentration in a concentration dependent manner. Below this point no remarkable improvement in absorbance of the drug surfactant solution and thereby the concentration of the dissolved drug was seen. For tween 80, poloxomer 407 and poloxomer 188 they were found to be 0.41 mM/L, 0.020 mM/L, and 0.12 mM/L respectively. So, these were the CMC values of

surfactants used in this experiment. Maximum CMC value (0.41 mM/L) was obtained for Tween 80 (Table 1), which was about 3.5 times and 20 times more than those of poloxamer 188 and 407 respectively. Data suggest that poloxamer 407 is maximally capable solubilizing agent (among these three agents studied) to solubilize compounds by forming micelles at a much lower concentration than the other surfactants used. This hypothesis was verified further by other experimentations.

When the surfactants were compared for drug total solubility (which was also concentration dependent and enhanced with the increasing concentration), interestingly it was found poloxomer 407 showed more value than poloxomer 188 and tween 80 (table 1, figure 2). Although tween 80 has more CMC value (Table 1) as reported earlier, poloxomer 407 enhanced the solubility of TC more than Tween 80 proving our earlier hypothesis. Further, tween 80 induced improvement of solubility was much less than that enhanced by poloxomer 407 and poloxamer 188 (figure 2, Table 3).

Table 1: Solubility profile of Tamoxifen citrate with concentration of the tested surfactants.

Surfactant	CMC	Concentration of surfactant	Solubility of drug
Poloxamer 407	0.020 mM/L	0.082	2.1647±0.023
		0.205	2.7138±0.048
		0.409	3.5183±0.011
		0.818	4.7526±0.022
Poloxamer 188	0.12 mM/L	0.121	1.3951±0.012
		0.303	1.8639±0.021
		0.602	2.2767±0.031
		1.031	2.8397±0.017
Tween 80	0.41 mM/L	0.821	1.8278±0.027
		2.032	2.2851±0.017
		4.011	3.1936±0.015
		8.031	4.6656±0.030

The solubility of drug (mMoles/ litre) is expressed as Mean ±SD (n=3). P-values were found to be $p<0.05$, for all the surfactants at each concentration and hence considered to be significant.

Table 2: Solubility profile of Tamoxifen citrate with varying volume-fractions of cosolvents.

Volume fraction (v/v)	Ethanol Solubility of drug	Log Solubility	PEG 400 Solubility of drug	Log Solubility
1	1.6982±0.01	0.2299	2.1812±0.017	0.3387
5	2.4200±0.014	0.3838	2.9581±0.029	0.471
10	3.5402±0.023	0.549	3.6304±0.014	0.5599
15	5.4142±0.031	0.7335	4.9877±0.022	0.6979

The solubility of drug (mMoles/ litre) is expressed as Mean ±SD (n=3). In both cases volume fraction was varied from 1-15%. P-values were found to be $p<0.05$, for all both the co-solvents at

each concentration and hence considered to be significant.

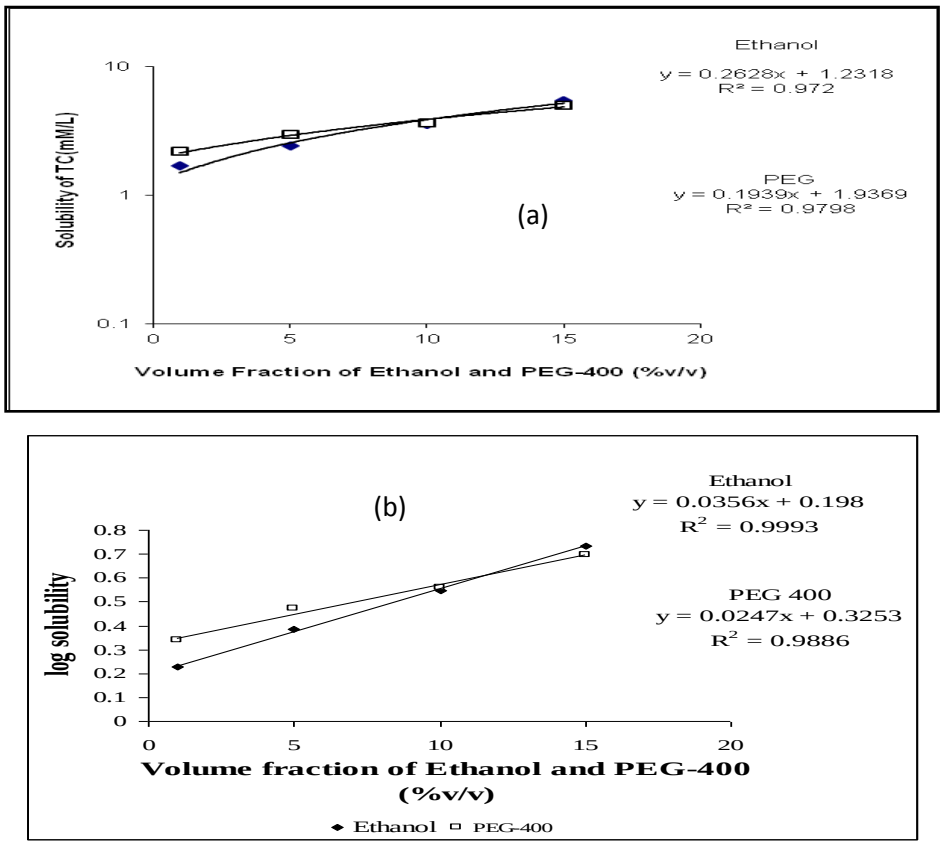

Figure 1: (a) Solubility of Tamoxifen citrate plotted against Volume fraction of co-solvents. (b) Log Solubility of Tamoxifen citrate plotted against Volume fraction of co-solvents.

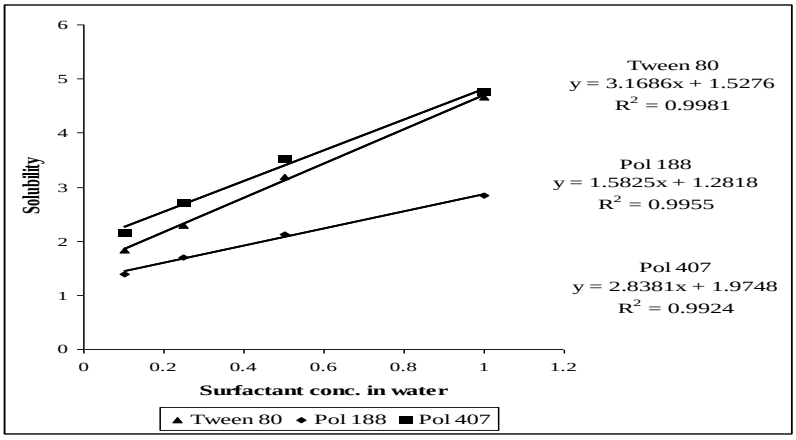

Figure 2: Solubility of Tamoxifen citrate plotted against Surfactant concentration for the tested surfactants.

Micelle formation is one of the important mechanisms to solubilise solutes. Incorporation of solute molecules to the micelles depends on the degree of nonpolarity of solutes and their micellar partitioning performances[9, 14, 15]. The more nonpolar the solute, the more likely it is to be incorporated near the non-polar core or center of micelles[14, 15].

Researchers have described a relationship of micellar surfactant concentration and solubility of solute (drug) [19, 20]. Total drug solubility (D_{tot}) depends on inherent solubility (D_u) and concentration of micellar surfactant (S) (i.e., the total surfactant concentration minus the critical micellar concentration) and is presented by

$$D_{tot} = D_u + \kappa D_u S,$$

where κ is micellar partition coefficient. Product of κ and D_u reflects number of surfactant molecules required to solubilise one solute molecule [19, 20, 24]. Table 1, Figure 2 shows the effects of Poloxamer-407, Poloxamer-188 and Tween 80 on solubility profiles of TC, respectively. These show that TC solubility was enhanced in the following sequences: Poloxamer-407 (κ: 2.28) > Poloxamer-188 (κ: 0.97) >Tween-80 (κ: 0.25). Poloxamer-407 was found to improve the solubility of TC maximally among the surfactants tested. Due to higher micellar partitioning, more nonpolar TC molecules were incorporated in the Poloxamer-407 micelles and the water solubility of TC was increased 7.1-fold as compared to 5.8 fold increase due to Polaxamer-188.

The choice of CMC is never unambiguous, since the change in slope occurs over a more or less narrow range of concentrations, whose magnitude depends on the physical properties being measured and sometimes on the nature of the data and on the way they are plotted. Different variables, mainly temperature and pressure can affect the CMC value of surfactants [19, 20].

However, in general, a disadvantage of micellar solubilization is the large quantity of surfactant required to provide the several orders of magnitude increases in solubility often desired. In addition, surfactant toxicity at high concentrations, particularly haemolysis, is a well-documented problem [23]. The rapid reversibility of solubilization is another difficulty in the use of micellar solution as an approach for parenteral or oral route.

Comparative evaluation:

The solubility of TC and its correlation with the varying concentrations of solubilizing agents is depicted in Table 3. In case of cosolvents, TC was solubilized more with ethanol than PEG 400 after reaching a certain concentration of cosolvent only. In case of surfactants poloxomer 407 showed better result in terms of solubility than poloxomer 188 and tween 80. However, on comparative evaluation as evidenced from table 3, solubility of TC when compared to its inherent solubility was found to have increased 7.1-fold in the presence of Poloxomer 407 as compared to 15% ethanol (6.10-fold), Poloxamer-188 (5.8 fold) 15% PEG-400 (5.62 fold) and tween 80 (5.54 fold) . Further looking into the disadvantages associated with the usage of each class of agents, Poloxamer 407 was found to be the most suitable solubilizer for TC among all the tested solubilizers.

Table 3: Comparison of solubilization parameters of Tamoxifen citrate for all tested solubilizing agents.

Solubilizing agents	Concentration Range	Concentrations tested	Relation of the solubility of Drug to solubilizing agent	Dependence of D_{tot} on D_u ([C]:Solubilizing agent concentration)
Ethanol	1-15% (v/v)	1%, 5%, 10%, 15% (v/v)	y = 0.2628x + 1.2318, R^2 = 0.9724	$10^{0.036[C]}$
PEG-400	1-15% (v/v)	1%, 5%, 10%, 15% (v/v)	y = 0.1939x + 1.9369, R^2 = 0.9798	$10^{0.025[C]}$
Tween- 80	0.8- 8 mM/L	0.8, 2, 4, 8 (mM/L)	y = 0.3842x + 1.5276, R^2 = 0.9981	0 .25[C]
Poloxamer- 407	0.08- 0.8 mM/L	0.08, 0.2, 0.4, 0.8 (mM/L)	y = 3.4712x + 1.9735, R^2 = 0.9924	2.28[C]
Poloxamer- 188	0.1-1 mM/L	0.1, 0.3, 0.6, 1 (mM/L)	y = 1.3069x + 1.3906, R^2 = 0.9955	0.97[C]

'C' is expressed in % in case of cosolvents (ethanol and PEG-400) and mM in all other cases (surfactants) Dtot is total drug solubility in a mixed solvent and cosolvent concentration (C); Du is drug solubility in water.

CONCLUSIONS

In this study, the effect of the solubilization approaches- micellar solubilization and cosolvency on the aqueous solubility of TC was observed on a comparative basis. From the above results, it can be concluded that solubility of poorly water soluble drug Tamoxifen Citrate can be better improved by micellar solubilization using Poloxomer 407. However care must be taken to optimize the amount of the surfactant required to solubilize a particular concentration of Tamoxifen Citrate so as to provide better solubility and bioavailability affording lesser toxicity and side effects thereby ascertaining the safety and efficacy of such formulations. Thus, further studies need to be done to correlate all these above factors to help formulate a therapeutically efficient formulation of Tamoxifen citrate

REFERENCES

1. Au JLS, Jang SH, Zheng J, Chen CT, Song S, Hu L, Wientjes MG. Determinants of drug delivery and transport in solid tumors. J Controlled Rel. 2001; 74:31-46.
2. Martin A. Solubility and Distribution Phenomena. In, Sinko PJ (Eds.), Martin's Physical Pharmacy and Pharmaceutical sciences 6th ed., Philadelphia; Lipincott Williams & Wilkens; 2011: 182-96.
3. Sarmah JK, Bhattacharjee SK and Mahanta R. Preparation of cross-linked guar gum nanospheres containing tamoxifen citrate by single step emulsion in situ polymer cross-

linking method, J. Incl. Phenom. Macrocycl Chem 2009; 65(3): 329–34.

4. Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. Adv Drug Del Rev 2004; 56(11): 1649-59.
5. Buckley MMT and Goa KL. Tamoxifen: a reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic use, Drugs 1989; 37: 451-90.
6. Lien EA, Solheim E, and Lea OA, Distribution of 4-hydroxydesmethyl tamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. Cancer Res1989; 49: 2175-83.
7. Government of India. Ministry of health and family welfare. Indian pharmacopoeia; Vol.-III. The Controller of Publications, New Delhi; 2007: 1153-56.
8. Monteagudo E, Gándola Y, González L, Bregni C and Carlucci AM. Development, characterization and in-vitro evaluation of Tamoxifen microemulsions. J Drug Del. 2012, article I D 236713, 11 pages doi:10.1155/2012/236713.
9. Yalkowsky SH and He Y. Handbook of Aqueous Solubility Data. Florida: CRC press, Boca Raton; 2003.
10. Strickley RG. Solubilizing excipients in oral and injectable formulations. Pharm Res. 2004; 21(2):201-30.
11. Rubino JT. Cosolvents and Cosolvency, In, Swarbrick J and Boylan JC (Eds.), Encyclopedia of Pharmaceutical Technology, Third Edition, Informa Healthcare, New York; 2006: 806-19.
12. Rytting E, Lentz KA, Chen XQ, Qian F, Venkatesh S. Aqueous and cosolvent solubility data for drug-like organic compounds. AAPS J 2005; 7(1): E78–E105.
13. Rytting E, Lentz KA, Chen XQ, Qian F, Venkatesh S. A quantitative structure-property relationship for predicting drug solubility in PEG 400/water cosolvent systems. Pharm Res 2004; 21: 237–44.
14. Florence AT and Attwood D, Physicochemical Principles of Pharmacy, Macmillan Press, London; 1989.
15. Rangel-Yagui CO, Pessoa Junior A, Tavares LC. Micellar solubilization of drugs, J Pharm Pharmaceut Sci 2005; 8(2):147-63.
16. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A Review of Poloxamer 407 Pharmaceutical and Pharmacological Characteristics. Pharm. Res 2006; 23(12): 2709-28.
17. Giffard M, Delfosse V, Sciara G, Mayer C, Cambillau C, and Hajji M E. Surfactant Poloxamer 188 as a New Crystallizing Agent for Urate Oxidase. Crystal growth design

2009; 9: 4199-4206.

18. Remon JP. Absorption enhancers, In: Swarbrick J and Boylan JC (Eds.), Encyclopedia of Pharmaceutical Technology. 3rd ed., New York: Informa Healthcare; 2006: 13-18.
19. Domínguez A, Fernández A, González N, and Iglesias E. Determination of Critical Micelle Concentration of Some Surfactants by Three techniques. J Chem Edu 1997; 74 (10): 1227-31.
20. Mokrushina L, Churyusova T, Savchuk K, Morozova YU and Smirnova N. Critical micelle concentration and phase behavior of aqueous mixtures of dodecylsulfates and sodium ethoxydodecylsulfate. Fluid Phase Equilibria 2002; (194-97): 1077–87.
21. Millard JF, Alvarez-Nunez FA, Yalkowsky SH. Solubilization by cosolvents. Establishment useful constants for the log-linear model. Int J Pharm 2002; 245: 153-66.
22. Jouyban A. Review of the cosolvency models for predicting solubility of drugs in water-cosolvent mixtures. J Pharm Pharmaceut Sci 2008; 11 (1): 32-58.
23. Golightly LK, Smolinkse SS, Bennett ML, Sunderland III EW, Rumack BH. Pharmaceutical excipients adverse effects associated with inactive ingredients in drug products (Part I). Med. Toxicol1988; 3: 128-65.
24. Ran Y, Zhao L, Xu Q, Yalkowsky SH. Solubilization of Cyclosporin A. AAPS Pharm. Sci. Tech 2001; 2:23-26.