Unusual Solubilization Capacity of Hydrophobic Drug Olanzapine in Polysorbate Micelles for Improved Sustained Drug Release

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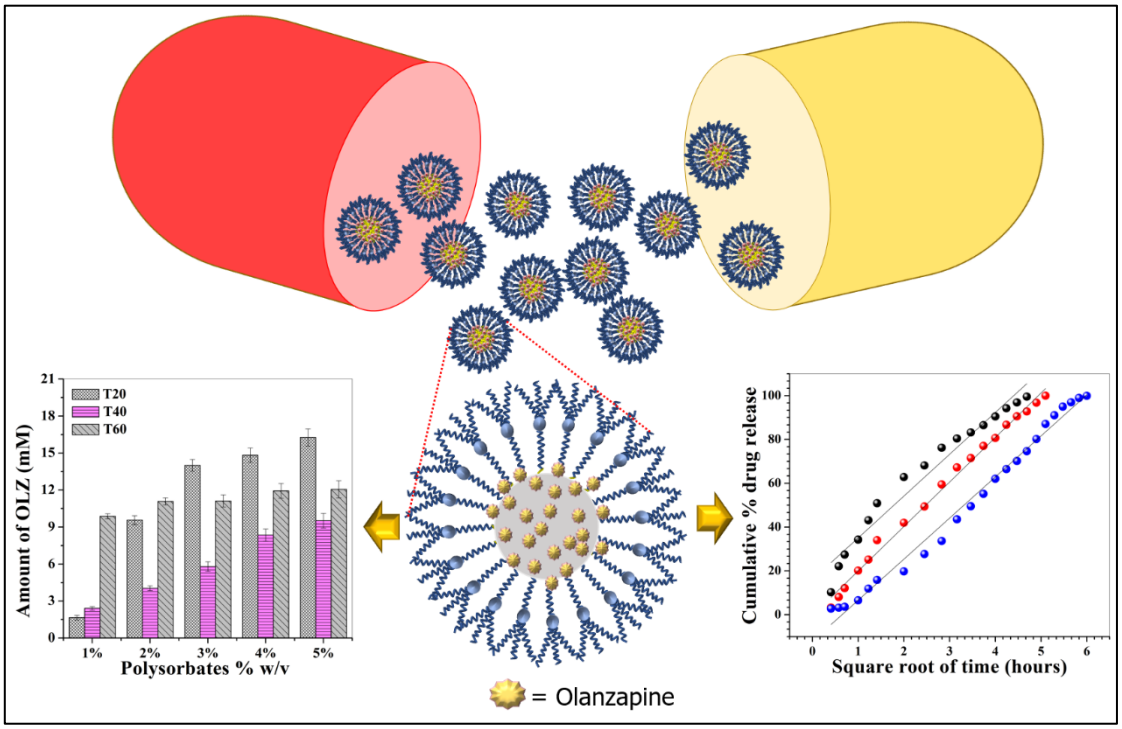
Graphical Abstract:



Highlights:

1) Polysorbate T20 and Polysorbate T20-Pluronic P84 mixed micelles accommodates a higher amount of hydrophobic drug Olanzapine.
2) Isothermal titration calorimetry confirmed the higher binding capacity of Olanzapine with Polysorbate T20.
3) SANS measurements suggested that the solubilization capacity of Polysorbates is also dependent upon aggregation number.
4) Both pure Polysorbates and Polysorbate-Pluronic P84 micelles showed the sustained release behavior.
5) This research output has a wider scope in colloidal chemistry as well as inpharmaceutical industry and manufacturing.

Abstract:

Polysorbates and Pluronic polymers are straightforward to use to improve the performance of hydrophobic molecules, however, colloidal systems of these polymers are not fully understood and loading of drug molecules in these Polysorbate micelles rely on a plethora of factors. Thus, it is a laborious task to select the optimal Polysorbate as a drug delivery vehicle. To pave the way for use of Polysorbates, three Polysorbates with different hydrophobicity were selected for oral delivery of the hydrophobic drug Olanzapine (OLZ). At higher concentration, Polysorbate T20 with low hydrophobicity accommodated a higher amount of OLZ than other Polysorbates T40 and T60 with higher hydrophobicity. The effect of mixed micelles of Pluronic P84 and Polysorbate (T20, T40, T60) on solubilization of OLZ was also studied at different concentration ratios and the higher OLZ solubilization was found to be in T20:P84 mixed micelles at 3:2 %w/v concentration ratio. Stronger interactions between OLZ and T20 were noticed with isothermal titration calorimetry (ITC), resulting in the higher OLZ solubilization in these micelles. Dynamic light scattering (DLS) and small angle neutron scattering (SANS) measurements revealed that mixed micelles of Polysorbates are greater in sizes than pure polysorbate micelles and the size decreased after loading of OLZ. Furthermore, SANS measurements suggested that decrease in the aggregation number after OLZ loading promoted the loading capacity of the Polysorbate micelle. Polysorbates micelles exhibited the sustained release behavior in biological relevant media, examined with *in vitro* dialysis release method. Therefore, it is believed that the finding of this work could be useful in the oral delivery formulations in which Polysorbates and Pluronics are primarily used.

Keywords: Olanzapine; Hydrophobic Drug; Loading Capacity; Polysorbates; Oral Delivery; Small angle neutron scattering

1.0 Introduction

Oral drug delivery is an advantageous method of drug administration due to high patient compliance, its non-invasive nature, and the fact that there is no need for sterile procedures.[1] Nevertheless, poorly water-soluble drugs encounter several difficulties for their absorption through the intestinal epithelium, which leads to decreased the bioavailability of drug.[2] Olanzapine is one of those poorly water-soluble Active Pharmaceutical Ingredient (API), which belongs to Class II of the Biopharmaceutical Classification System (BCS).[3] OLZ is a thienobenzodiazepine, which belongs to second generation antipsychotic drugs.[4] Schizophrenia is a severe and chronic mental disorder with approximately 20 million people worldwide suffering with this disease.[5] OLZ is a common medication prescribed for this disease; however, common side effects include sexual dysfunction, anticholinergic syndrome, tachycardia, orthostatic hypotension, central nervous system depression and hyperthermia.[6-8] Dose reduction is possible by improving the water solubility of OLZ, as that would lead to rapid dissolution in the gastrointestinal (GI) fluids and hence faster permeation through the GI membrane to reach systemic circulation.[9] Micelles are colloidal particles, which have been recently emerged as biocompatible and versatile systems for oral drug delivery.[10-11] Amphiphilic polymers are extensively exploited for hydrophobic drug delivery purposes as micelles formed by these polymers have the capability to encapsulate hydrophobic drugs within their hydrophobic micellar core.[12] Pluronics are triblock co-polymers made up of PEO−PPO−PEO structure (PEO: poly(ethylene oxide); PPO: poly(propylene oxide)) gained huge interest to use as solubilizer for hydrophobic drugs as they are non-toxic, non-ionic, US-FDA approved and biocompatible.[13,14] Moreover, Pluronics are modified with other additives for better entrapment of hydrophobic drugs for instance Pluronic grafted gelatin hydrogels. [15-17]

Another important class of surfactants are Polysorbates that are biocompatible, non-ionic and extensively used as a solubilizer in the pharmaceutical industry. These are approved by the United States Food and Drug Administration for their use in oral, injectable as well as topical products.[18-21] Wang and colleagues recently compared the stability of the natural drug Curcumin in different Polysorbates T20, T60 and T80. The higher stability was found to be in the case of T40 as compared to T20 and T80.[22] Due to the biotherapeutic delivery applications of Polysorbates, Nayem *et al.* evaluated the micellar morphologies of Polysorbate T20 and T80 using small angle neutron scattering (SANS) measurements.[23]

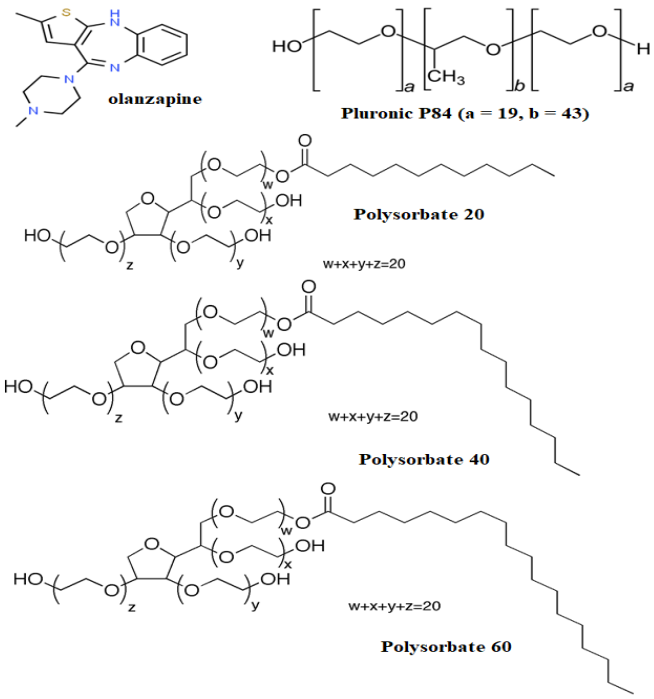
This is the first report which corroborates the systematic screening of the loading and solubilization capacity of OLZ drug in Polysorbates *viz.* T20, T40and T60 micelles with different hydrophobic chains. Moreover, the effect of triblock copolymer Pluronic P84 on the solubilization

capacity of different Polysorbates was studied. The reason behind choosing Pluronic P84 is that from our previous published work, it showed outstanding solubilization capacity for oxcarbazepine, clozapine and lamoterigine.[13,24,25] Complete thermodynamics of OLZ solubilization, locus of solubilization and binding capacity were investigated using UV-Visible spectroscopy and isothermal titration calorimetry (ITC). Furthermore, micellar characteristics in the absence and presence of OLZ drug was studied using dynamic light scattering (DLS) and SANS techniques. OLZ drug release behavior andkinetics of drug release were studied using different four kinetic models. As Polysorbates and Pluronics are extensively used to enhance and improve the performance of poorly water-soluble drugs, this report would be beneficial for formulation chemist as well as Pharmaceutical Scientists to design the formulations.

2. Materials and Methods

2.1 Materials

Pluronic P84, Polysorbate T20, Polysorbate T40, Polysorbate T60, and Olanzapine (OLZ) were procured from the Sigma Aldrich, and the molecular structures of the Pluronic P84, Polysorbates and OLZ have been shown in **Scheme 1.**

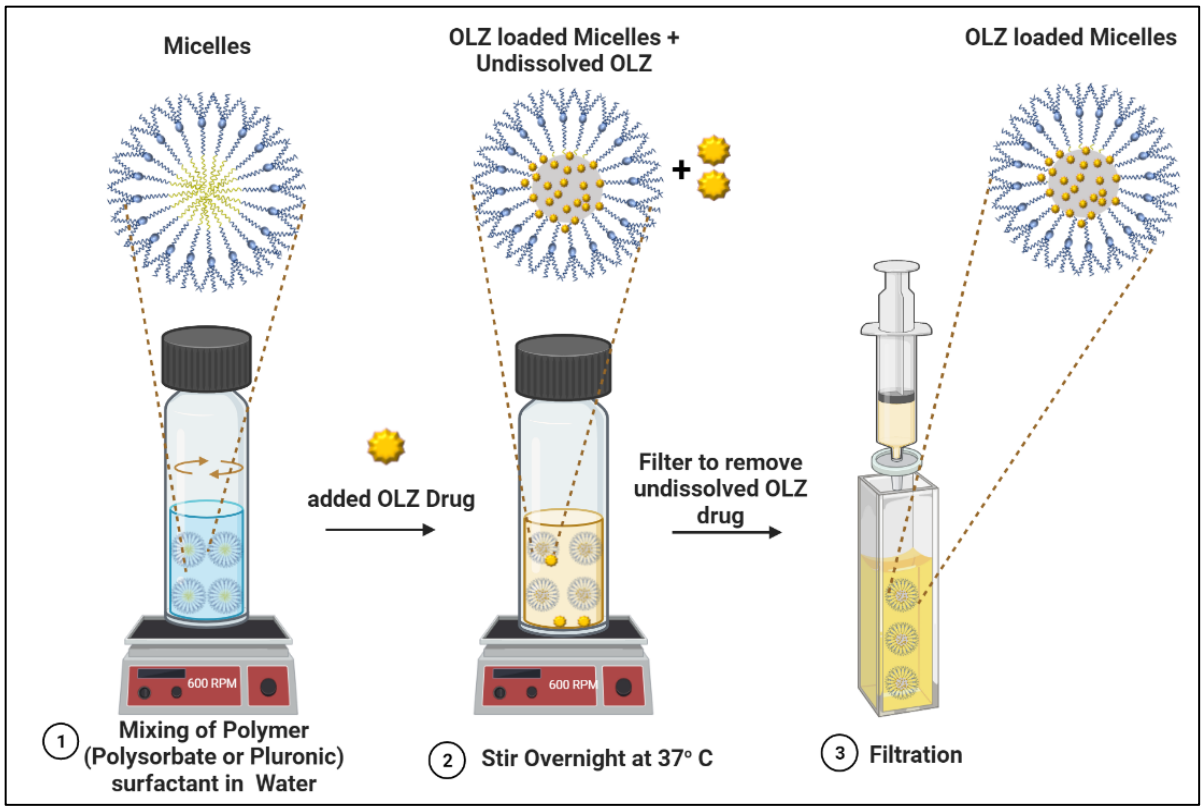


Scheme1: Chemical structures of materials used in the present study.

2.2 Methods

2.2.1 Solubilization of OLZ and thermodynamics calculations of solubilization:

OLZ solubilization in different Polysorbates and Polysorbate-Pluronic P84 mixtures have been performed by our previous reported method at 37°C.[26] Briefly, different Polysorbates (T20, T40, T60) and Pluronic P84 were weighed and dissolved in double distilled water and then extra amount of OLZ was added to the Polysorbates and their mixture with Pluronic P84. These solutions were kept on stirring at 37±0.1°C for 12 hours. After 12 hours, millipore filters (0.20µm) were used to remove the unloaded drug from the samples. The encapsulation process of OLZ in Polysorbate and their mixed micelles have been depicted in **Scheme 2.** The OLZ solubilized (encapsulated) in the micellar medium was estimated using Shimadzu (UV 1800) UV-Visible double beam spectrophotometer, OLZ showed maximum absorbance at 251 nm (λ_{max}), and the molar absorption coefficient was 14.823 $Lmol^{-1}$ cm^{-1}.



Scheme 2: Demonstration of the encapsulation of OLZ in the Polysorbate (T20 T40 and T60) and their mixed micelles with Pluronic P84.

Loading efficiency is the percentage of solubilized OLZ from the OLZ fed (18 mM) in polysorbates and mixed micelles as shown in equation below:

$$Drug\ loading\ efficiency = \frac{drug\ in\ micelles}{drug\ fed\ initially} \times 100 \quad \textbf{(1)}$$

The partition coefficient, P was also determined, where P is the ratio of drug solubilized in the

micelle to that in the water at a particular concentration of micelles:

$$P = \frac{S_{total} - S_w}{S_w} \textbf{(2)}$$

where S_{total} and S_w are the solubilities of OLZ in total and in water, respectively.

The thermodynamics involved in the process of dissolution of OLZ in micellar system was studied using partition coefficient to calculate the standard Gibbs free energy of solubilization using,

$\Delta G^S = -RTlnP$ **(3)**

where R is the universal gas constant and T is the absolute temperature. The standard Enthalpy of solubilization (ΔH^S) has been calculated using following equation derived from Gibbs Helmholtz relation[27]:

$$\Delta H^S = -RT^2 \frac{\partial\, ln(S_{total}/C)}{\partial T} \textbf{(4)}$$

Where C is the concentration of polymer used for the solubilization and value of temperature (T) used is 25°C.

Further, entropy of solubilization (ΔS) is determined using following equation:

$\Delta G^S = \Delta H^S - T\Delta S^S$ **(5)a**

2.2.2 ITC measurements

The enthalpy changes delineating the interactions and binding parameters pertaining to OLZ and Polysorbate T20 and T60 was determined by employing MicroCal ITC200 micro calorimeter at 37 ± 0.1 °C. The titrations were performed by adding 2 µL aliquots of stock solutions of polysorbate in 240 µL of 15 mM aqueous OLZ solutions. Blank experiments were performed by titrating polysorbate stock solution in water. The stock solution of OLZ have been prepared in Dimethyl sulfoxide (DMSO) and then diluted into the water for ITC measurements. The concentration of DMSO kept constant in both solutions to compensate the effect of DMSO. T20 binding affinities were obtained using 5% solution of T20, while 1% in case of T60 (due to high viscosity of the 5% solution which interrupted the working of the instrument).

2.2.3 DLS measurements

The hydrodynamic diameter (D_h) of Pluronic and polysorbate micelles was measured using a Malvern Zetasizer Nanoseries Nano-ZS instrument equipped with He-Ne laser (λ = 632.8 nm) at 37 ± 0.1 °C. Prior to experiment all the solutions were filtered using millipore 0.45 µm filter to avoid the interference of impurities.

2.2.4 SANS measurements

The SANS measurements were carried out on indigenously built SANS instrument operating at Dhruva reactor, Bhabha Atomic Research Centre (BARC), Mumbai, India.[28] All the sample measurements were carried out at 37°C. SANS data were fitted using a spherical core-shell structure

of micelles interacting via hard sphere repulsion. The radius of core and its polydispersity, hard sphere radius and volume fraction of micelles have been used as a fitting parameter. The polydispersity is fitted using log-normal distribution and aggregation number calculated from the core volume divided by the hydrophobic volume of the molecule.

2.2.5 *In vitro* drug release and Kinetics:

2 ml of OLZ micellar was trapped in dialysis bag (MW cut-off (MWCO)12-14 kDa) and submerged into 100 mL of a PBS solution (pH = 7.4) maintained at 37 ± 0.2 °C. Samples were taken periodically, filtered and assayed using UV-visible spectrophotometer.

3.0 Results and Discussion

3.1. Solubilization of OLZ

The solubility of OLZ is 0.0836 mM in purified water (experimentally determined), categorizing it as poorly solubilized antipsychotic agent. The solubilization of OLZ was enhanced by inclusion into polymeric pure and mixed micelles of Polysorbate T20, T40, T60 and Pluronic P84. The maximum solubilization at low Polysorbate (1-2% w/v) concentrations was in T60 micelles as depicted in **Figure 1 (a)**. The higher solubilization of OLZ at this concentration may be attributed to the higher hydrophobicity of T60 (C-18 stearate chain) than the other screened Polysorbates namely the T40 (C-16 palmitate chain) and T20 (C-12 laurate chain). The hydrophobic interaction between the longer hydrophobic T60 and OLZ plays an important role in solubilization of the OLZ drug.[22] On the other hand, at higher concentrations (3-5% w/v), the highest solubility of OLZ was observed in T20 micelles and this was further confirmed visually. More specifically, as can be seen in **Figure 1 (b)**, there is change of the cloudy solution of OLZ into clear yellow at or above 3% w/v T20 micellar solution without any filtration. However, these results are quite surprising and contrary to some previous studies in which surfactants with higher hydrophobicity showed highest solubility.[22,29] These Polysorbates belong to the polyethoxylated sorbitans, possessing similar head group but different hydrophobic chain. The structural architecture of T20 having strain hydrocarbon chain while T60 with bent chain, could be responsible for their different physicochemical properties.[30] Solubilization results can also be explained based on the Hydrophilic Lipophilic Balance (HLB) values, these are 16.7, 15.6, and 14.9 for T20, T40, T60 respectively. It is well reported in literature that the higher the HLB, better is the solubilization capacity for hydrophobic solutes.[31] But this was applicable to higher concentration of Polysorbates only and could not explain the OLZ solubilization behavior at lower Polysorbate concentration. Henceforth, hydrophobicity and hydrophobic interactions are better methods to define the solubilization capacity of the Polysorbates rather than HLB. The lower solubilization of OLZ at higher concentration of T60 was attributed to hydrophobic

interactions between T60 molecules that are more favorable to form micelles than the interactions between T60 and OLZ molecules. To further investigate the effect of Pluronic P84 on OLZ solubilization, mixtures of Polysorbates with Pluronic P84 were studied. These Pluronic micelles consist of PEO units forming a corona region and PPO units forming the hydrophobic core, both of which can solubilize the drug.[32] OLZ was dissolved in T20/T40/T60 and P84 mixed micelles at different ratio of 1:4, 2:3, 3:2, 4:1 %w/v. Solubility of OLZ in pure Pluronic P84 at 5 %w/v was found to be 5.980. Mixing of Pluronic P84 with Polysorbates enhanced the OLZ solubilization and found to be maximum in P84-T20 mixed micelles followed by T40 and was least in T60. At 1:4 % w/v concentration of T20:P84, solubilization was found to be higher *i.e.* 14.64 mM and further increasing concentration of Pluronic P84, not any greater increase in OLZ solubilization was observed. At 3:2 % w/v, the best solubilization was observed in the case of a T20:P84 mixed system which was slightly more soluble than pure T20 (5 %w/v). However, the addition of lower Pluronic concentration (1 %w/v) to Polysorbates (4 %w/v), a significant decrease in solubilization of OLZ drug was evidenced in T40 and T60 but with a slight decrease in T20. These results confirmed that at higher concentration of T20, presence of other surface-active molecules could not

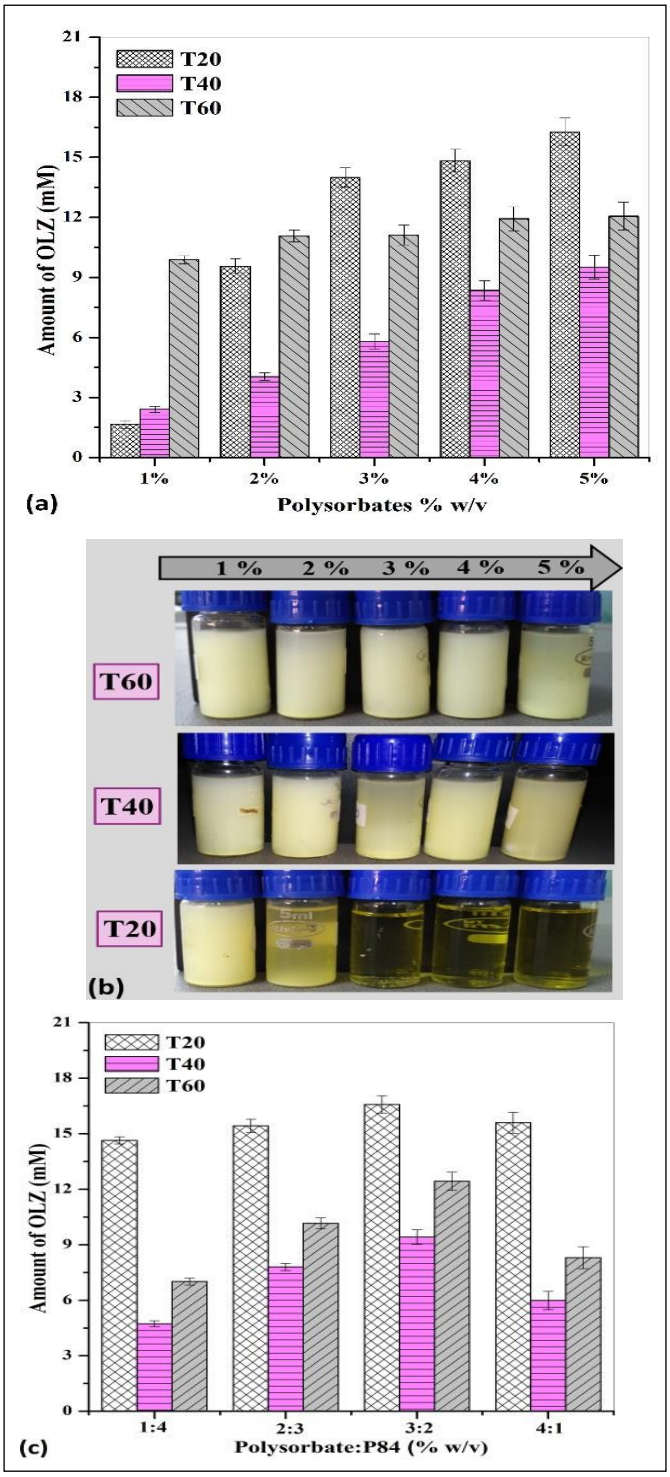


Figure 1:(a) Amount of OLZ solubilized (mM/5mL) and **(b)** Visual results of OLZ solubilization in Polysorbate (T60, T40 and T20) micelles at various concentrations (% w/v) at 37°C (Photographs were taken prior filtration), **(c)** OLZ solubilization in Polysorbate (T60, T40 and T20): P84(% w/v) mixed

perturb the solubilization capacity at the extent which was observed in T40 and T60. Therefore, for

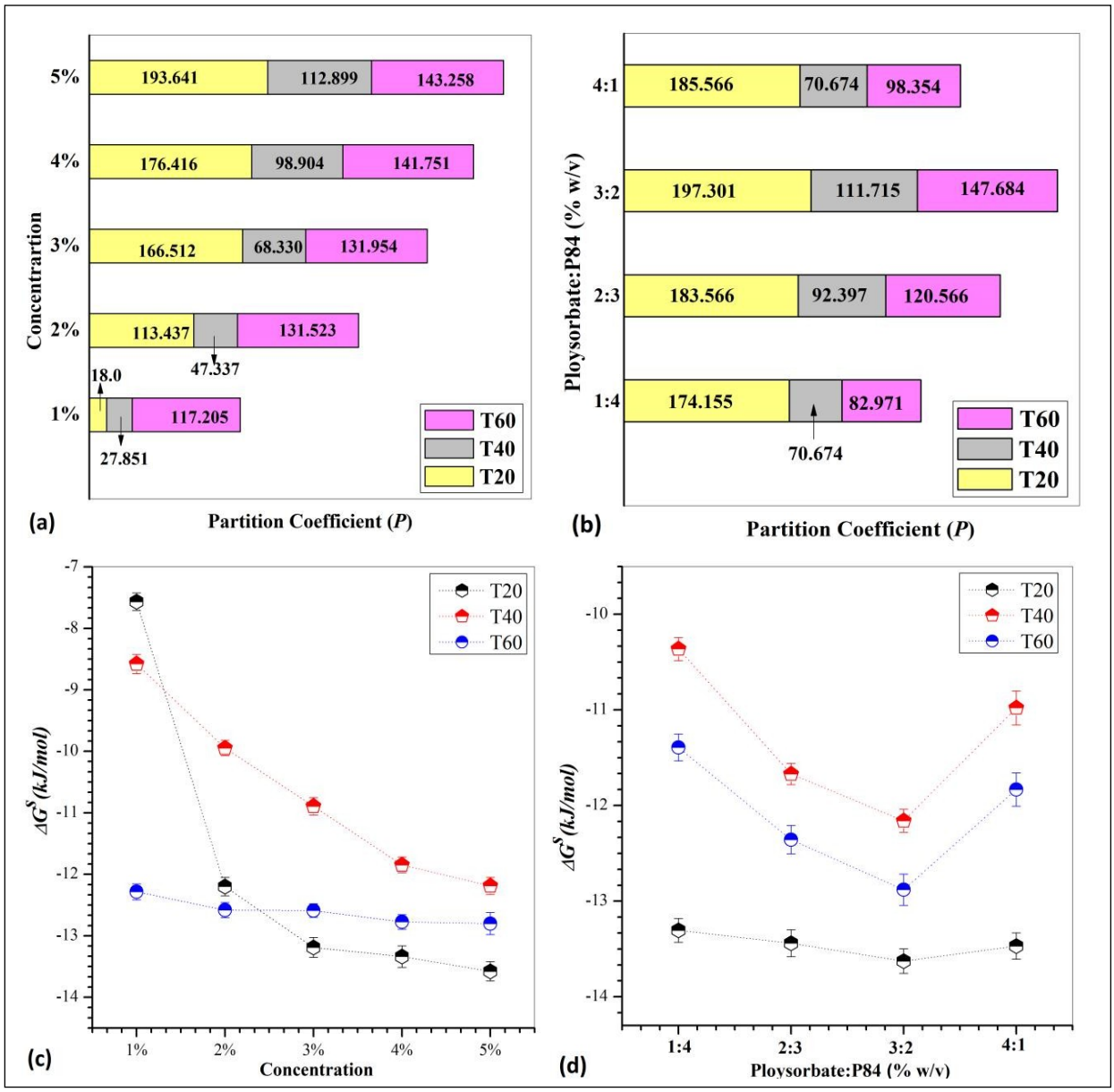


Figure 2: Partition coefficient (*P*) and Gibbs free energy of solubilization (ΔG^s) values for different Polysorbates (**a** and **c**) and Polysorbates-Pluronic mixed micelles (**b** and **d**).

OLZ drug delivery, T20 was found to be best Polysorbate amongst all exploredPolysorbates and was further confirmed from thermodynamics of OLZ solubilization. **Figure 2 (a)** elucidates that among all the Polysorbates, the value of Partition coefficient (*P*) was highest for T20 at 5% w/v corresponding to 193.64 while the lowest value was attributedto 1% w/v of T20. T20 showed a steep increase in Partition coefficient while T40 demonstrateda slow and gradual OLZ increase. On the contrary, in the case of T60, the maximum partitioning was observed at 2% w/v following the slight increase in the Partition coefficient upon further increasing concentration. Furthermore, in a Polysorbates: Pluronic P84 mixed system, T20:P84 have the highest partition coefficient, hence

showing excellent partitioning of OLZ in these micelles **(Figure 2 (b))**.[33] The trend for ΔG^s, ΔH^s, ΔS^s and drug loading efficiency (%) were found to be similar with solubilization and partition coefficient (P) trend. The drug loading efficiency was also found to be higher in case of Polysorbate T20 and Polysorbate T20-P84 mixed micelles as shown in **Figure S1** of the Supplementary information. In the case of Polysorbate 20 and T20-Pluronic mixed system, more negative values

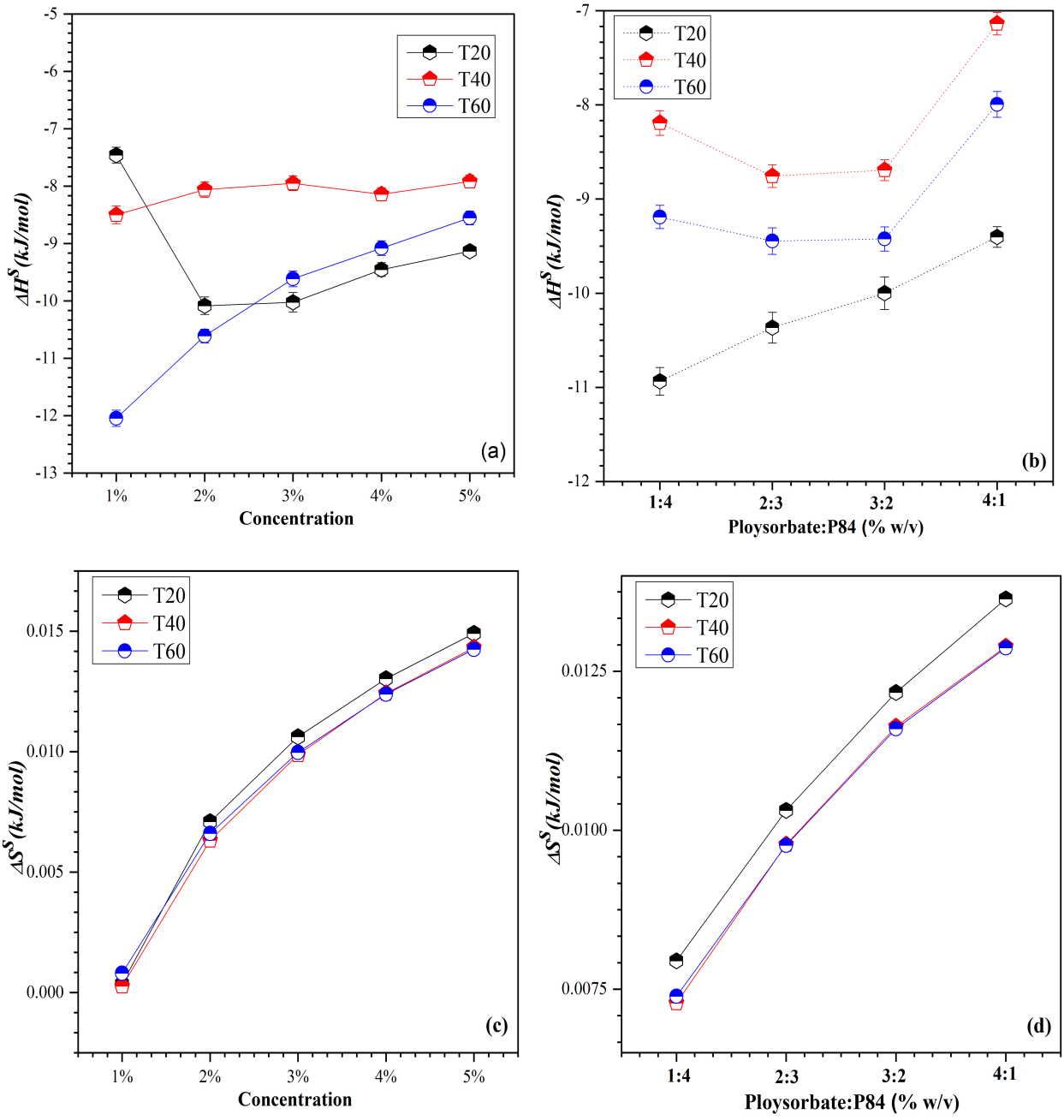


Figure 3: Enthalpy (ΔH^s) and entropy (ΔS^s) values for different Polysorbates (a and c) and Polysorbates-Pluronic mixed micelles (b and d).

of ΔG^s conferred the more favorable solubilization of OLZ in these micelles **(Figure 2 (c) and (d))**. The negative valuesfor ΔH^s **(Figure 3 (a) and (b))** show that the OLZ solubilization phenomenon

in Polysorbates and Polysorbate-Pluronic mixed system is energetically favorable since the inclusion of drug molecules in micellar cage is exothermic process.[34] For the solubilization to be spontaneous, the entropy should be negative but results of this study revealed that overall ΔS^s was positive as shown in Figure 3(c) and (d). This can be explained by the fact that the breakage of hydrogen bonding between water molecules in bulk water supersede the loss or decrease in entropy resulting from the restriction of OLZ molecules in the Polysorbates and Polysorbate-Pluronic mixed micelles.[35]

3.2. Isothermal Titration Calorimetry (ITC) measurements

ITC has been employed to investigate the binding of OLZ with micelles of Polysorbates T20and T60 at 310.15K in water. Heats of dilution of the titrants were essentially negligible as compared to the surfactant-drug reaction heats, and they were subtracted from the isotherms prior integration. Various thermodynamics as well as binding parameters obtained from the studies have been summarized in **Table S1** in the Supplementary information. It has been observed that the interaction enthalpy of OLZ with T20 and T60 was negative, indicating exothermic process, with higher values in the case of T60.[36,37] Surfactants with longer hydrophobic tails have a more negative enthalpy contribution to interaction energy.[38] Further, the stoichiometry of binding (n), the molar ratio of drug to surfactant, was calculated from the concentration of both solutions in syringe and cell at the point of saturation. The binding constant was found to be 11.10 and 0.002 for T20-OLZ and T60-OLZ respectively, and thus evidenced the stronger binding of T20 with OLZ compared to T60 and OLZ. Also, the positive entropy changes in T20 and negative entropy for T60 interpreted that in the case of T20 there might be structural rearrangement of the excluded water molecules in the bulk water leading to increase in entropy thus, contributing to high binding affinity with the drug molecule. The structural rearrangement of the solvent molecules also requires energy, which explains the low enthalpy of binding for T20 micelles with drug molecules

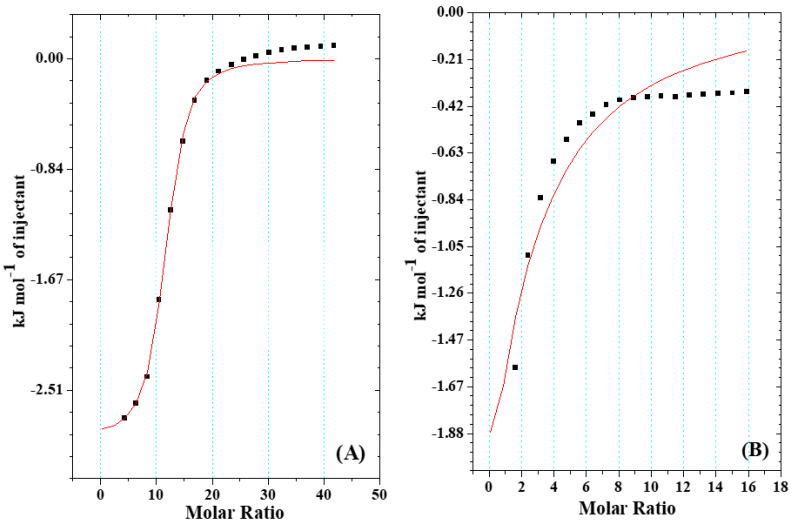


Figure 4: Isothermal Titration Calorimetric isotherms for binding of **(A)** 5% T20 + 15 mM OLZ and **(B)** 1%T60 + 15 mM OLZ at 37°C.

due to favorable entropy contribution to binding by release of water molecules associated with burial of significant hydrophobic surface upon binding or slight conformational changes. Thus, considering the decrease in entropy and low stoichiometry, it can be assumed that T60 undergo self-aggregation or more organized solvent rather than the drug-surfactant interactions. It is important to mention that the discrepancies in Van't Hoff (enthalpy of solubilization) and calorimetric enthalpy, owing to the effect of change in aggregation number and shape which has not been thoroughly taken into consideration in the data treatment by the Van't Hoff analysis, while in calorimetric measurements, the influence of the above-mentioned factors has been considered. [39] Hence, the OLZ solubilization phenomenon might involve the partition in the hydrophobic corona-core interfaces of the micelles to a greater extent, although the interactions are mainly exothermic in nature.[40]

3.3. Dynamic Light Scattering

To quantify the size of the micelles with and without the loaded drug, DLS experiments were performed to determine the hydrodynamic diameter (D_h) of the Polysorbate micelles of T20, T40 and T60 at 5 %w/v. The diameter refers to how a micelle diffuses within a fluid, which inthe present case, decrease in size in all the cases viz. T20 (10.10 to 8.72 nm), T40 (11.60 to 8.81 nm), and T60 (11.52 to 9.88nm) was observed **(Figure 5 (a)-(c))**. D_h of P84 was measured to be 15.77 nm which however (**Figure 5 (d))**, showed increase in the value to 18.67 nm on insertion of drug OLZ. The slight increase in the micellar size after drug encapsulationwas normally observed in the case of Pluronics.[26,29,32] On the contrary, in mixed micelles of T20:P84, a decrease in hydrodynamic diameter was observed as shown in **Figure 5 (e) and (f)**. The decrease in size in case of Polysorbate T20 and T20-P84 mixed micelles attributed to the effectof dehydration of water layer from the surface of the micelles and another reason is that micellar core size decreases because of Van der Waals interactions of drug with Polysorbates. In line with these results, Chen *et al.,* also demonstrated a decrease in the micellar size after the encapsulation of norcantharidin drug in poly(ethylene glycol)– poly(caprolactone) polymeric micelles.[41]

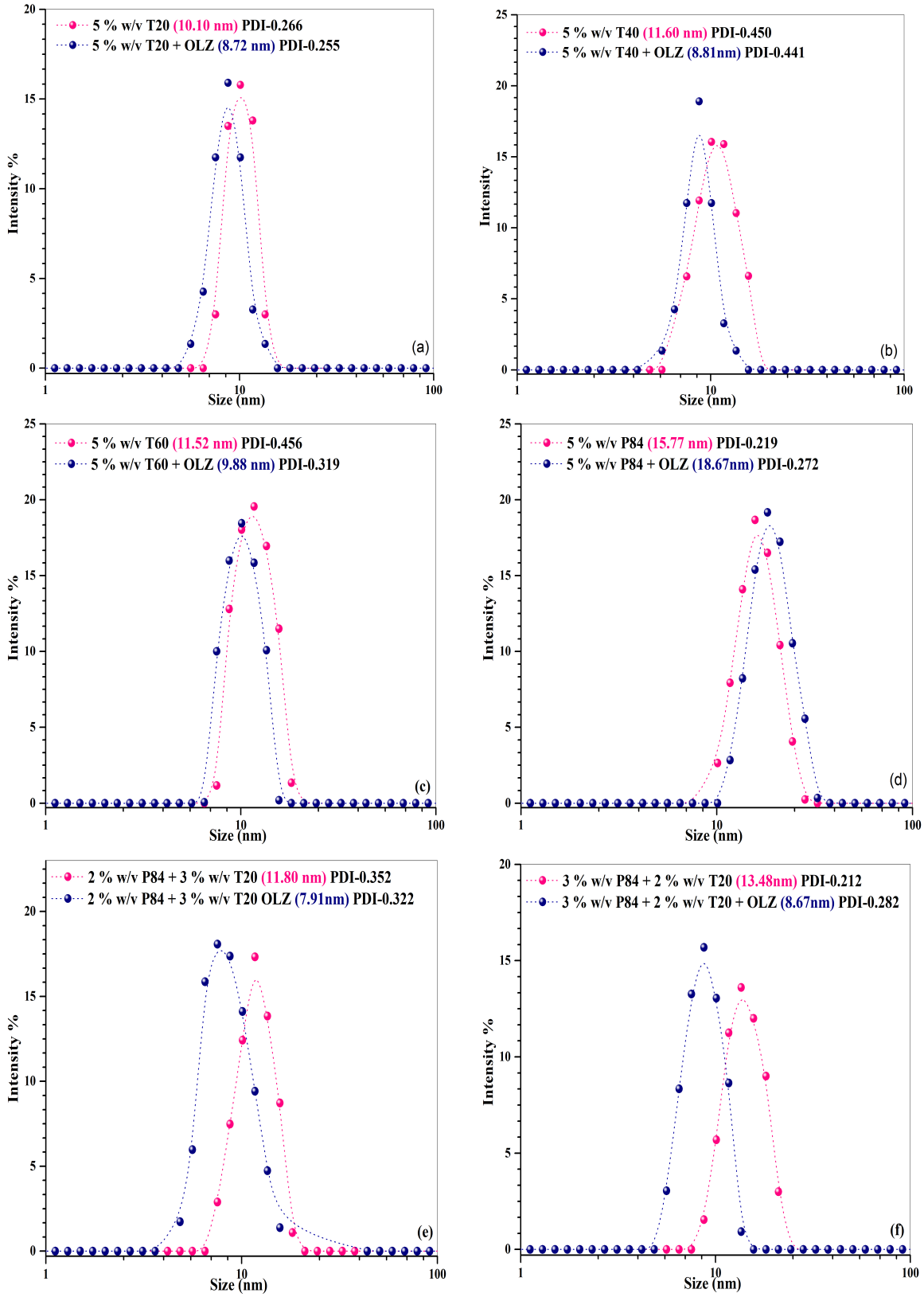


Figure 5: Hydrodynamic diameter (D_h) distribution plots for empty and OLZ encapsulated Polysorbates20 and Polysorbate 20-P84 mixed micelles.

3.4. SANS Measurement

Micellar parameters for PolysorbatesT20, T40 and T60 at 5 %w/v with and without OLZ were scrutinized using SANS measurements, with the scattering curves shown in **Figure 6**. Comparing the core radius (R_C) and hard sphere radius (R_{HS}) value depicted in **Figure 7 (a) and (b)** respectively, it was observed that R_C and R_{HS} of Polysorbate T20 were lower than T40 and T60. Due to the smaller hydrophobic chain, R_c for Polysorbate T40 and T60 is almost same, thus, whole hydrophobic chain of Polysorbate T60 did not contribute to form core despite having bigger hydrophobic chain than Polysorbate T40.On the contrary, R_{HS} value in case of T60 is higher than T40, so this means some part of hydrophobic region of polysorbate contribute to the formation of corona region of the micelles.Pluronic P84 has longer PPO forming core units and PEO forming Corona units so both R_C and R_{HS} values are higher than all investigated Polysorbates.[24] The mixing of P84 with polysorbates led to the increase in the RC and RHS values, confirmed the formation of mixed micelles. There is intercalation (possibly hydrophobic interactions) between PPO forming core region of Pluronic P84 with Hydrophobic chains of the Polysorbates and PEO-PEO interaction between PEO units of Polysorbates and Pluronic P84.[25] Aggregation number (Nagg) could better explain the solubilization capacity of OLZ in T20 rather than other two Polysorbates shown in **Figure 7 (c)**. Nagg of T20 is lower before and after the encapsulation of OLZ than T40 and T60 revealedthat lower amount of T20 monomers was needed to solubilize the drug. Upon the encapsulationof OLZ, a decrease in Nagg for pure as well as mixed system showed that presence of OLZ favors the micellization process, hence a smaller number of polymer monomers are

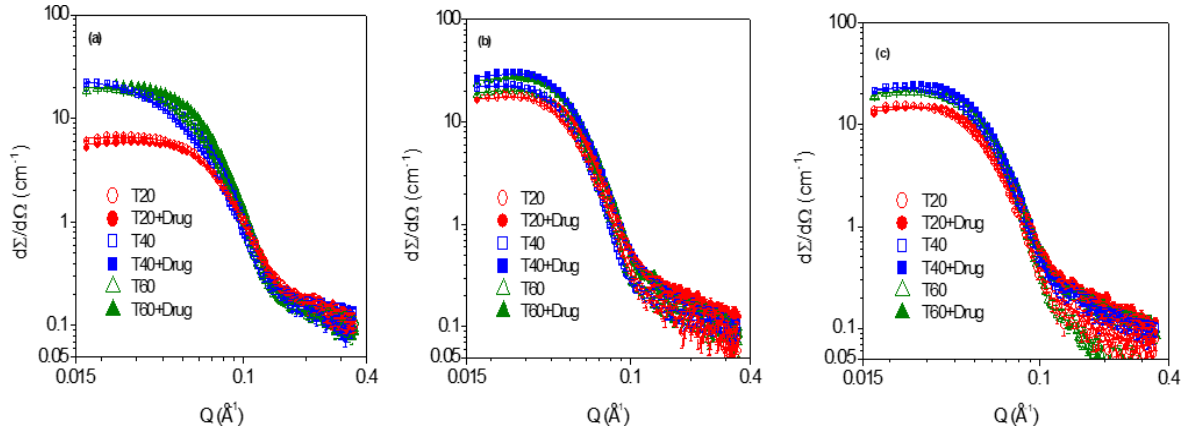


Figure 6: SANS scattering pattern for unloaded and OLZ loaded Polysorbates T20, T40, T60.

required to form the micelles.[42] Similar SANS results were reported by Sharma *et al.,* they observed that incorporation of drugs naproxen or indomethacin in the Pluronic F127 micelles led to the decrease in aggregation number (Nagg). Authors concluded that the decrease in Nagg results in the

formation of new micelles and thus increase the drug load.[43] Other parameter values of SANS have been provided in **TableS2** in Supplementary information**.**

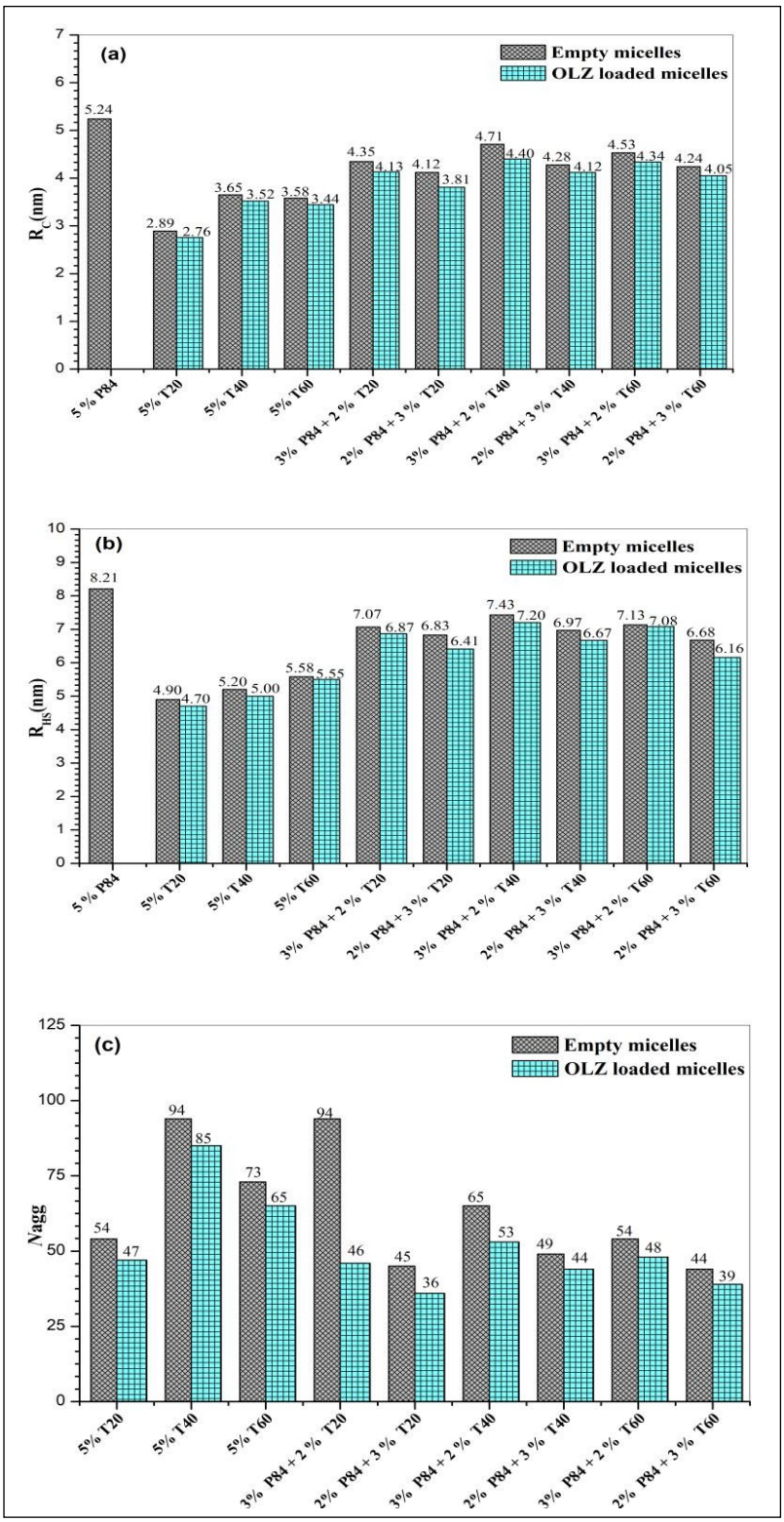


Figure 7: Micellar parameters for Pluronic P84, Polysorbates (T20, T40, T60) and Pluronic-Polysorbate mixed micelles: (a) core radius (RC), (b) hard sphere radius (RHS) and (c)

3.5. *In vitro* drug release:

The micellar systems with best solubilization capacity, namely, 4% T20, 5% T20 and 3:2 T20:P84 were chosen to study of OLZ release. It was found that all screened formulations showed sustained release behavior (**Figure 8**). The release pattern of OLZ in Polysorbates and Polysorbates mixed micelles follows the order 4% T20 >5% T20 >3:2 T20:P84; 4% T20,5% T20 and 3:2 T20:P84 showed full OLZ drug release after 22 hours, 26 hours and 36 hours respectively depicted in **Figure 8 (a)**. Slower release in case of 3:2 (% w/v) T20:P84 can be explained based on the micelles size, as it is well documented in the literature that the larger the size of a drug carrier, the slower the drug release.[44] Furthermore, drug release data were fitted to the following mentioned different kinetic models to shed more light on the exact kinetics followed by OLZ release as described below:

Zero order release kinetics: The pharmaceutical dosage forms following zero order release profile release the same amount of drug by unit of time and is given by Eq. (6)

$$Q^t = Q^0 + K^0 t \quad \textbf{(6)}$$

Qt is the cumulative drug release at time "t", Q^0 is the initial amount of drug and K^0 is the zero-order release constant. The formulations which follow the first order release kinetics implies that the drug release rate is independent of the concentration of the dissolved drug.[45]

First order release kinetics: Gibaldi and Feldman in 1967 explained the application of this model in drug dissolution studies.[46] The release of a drug which follows first order kinetics can be expressed by the Eq. (7):

$$\log Q_t = \mathrm{Log}Q_0 - K_t 2.303 \quad \textbf{(7)}$$

where Q_t is the cumulative amount of drug release at time "t", Q_0 is the initial amount of drug and K_t is the first order release constant.

Higuchi model kinetics: It is a mathematical model described drug release from matrix devices, proposed by Higuchi in 1961. Drug release is dictated by diffusion in the Higuchi equation[47,48] and expressed by the Eq. (8).

$$Q_t = K_H . t^{1/2} \quad \textbf{(8)}$$

where Q_t the cumulative amount of drug release at time "t" and K_H is the Higuchi release constant.

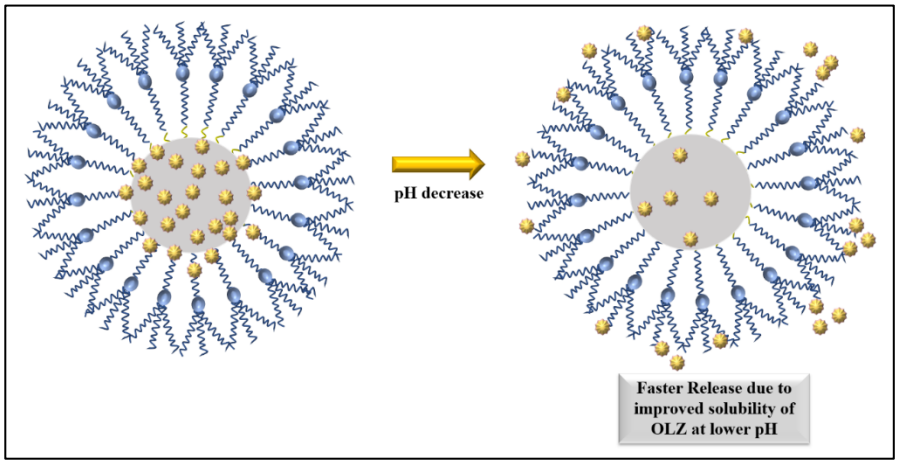
Korsmeyer-Peppas (KP) Model: In 1983 Korsmeyer *et al* derived a simple relationship which

described drug release from a polymeric system. The release of the drug which follows Korsmeyer-Peppas model can be expressed by the Eq. (9)

$M_t/ M_\infty = Ktn$ **(9)**

Where M_t/ M_∞ is fraction of drug released at time "t", n is the release exponent, K is a kinetic constant characteristic of the drug/polymer system.[49]

The most suitable kinetic model was selected based on the best goodness of fit (R^2). The formulation prepared at 4% T20 (**Figure 8 (b)**) followed first order kinetics release, conferring that OLZ release is independent of the concentration of OLZ entrapped in micelles.[50] The formulation prepared at 5% T20 followed the Higuchi model (**Figure 8 (c)**) and revealed that the OLZ release is diffusion controlled at this concentration. In case of the 3:2 (% w/v) T20:P84 formulation, the release followed the Korsmeyer-Peppas model with N value more than 0.45 implying that this formulation followed the Non-Fickian diffusion mechanism as shown in **Figure 8 (d)**. Non-Fickian diffusion mechanism signifies the functional interactions between OLZ and Polysorbate 20 and Pluronic 84 mixed micellar system that is responsible for this anomalous behavior.[51] On the other hand**, Figure 9 (a)** depicted the OLZ release at lower pH in simulated gastric fluid (pH=1.2) and release kinetics is depicted in **Figure 9 (b-e)**. It has been observed that OLZ drug released at faster rate at lower pH as compared to the release at higher pH which was slow. As OLZ is a weakly basic drug, the solubility of OLZ drug increases with decreasing pH, so, lower pH of the solution increased the solubility of OLZ and fast release of OLZ was observed from Pluronic micelles (**Schematic 3**). The results were corroborated with the results observed for ibuprofen release at different pH in which drug released faster due to its improved solubilization above pH 4.5. This was because ibuprofen is a weaky acidic drug and it dissolved fast at higher pH.[52,53] OLZ loaded T20 formulations at 4% and 5% w/v concentrations were stable for 2 months at room temperature (RT), while formulations with T40 and T60 showed OLZ precipitation after 2 weeks of the storage at RT.



Scheme 3: Mechanism showing OLZ release at lower pH

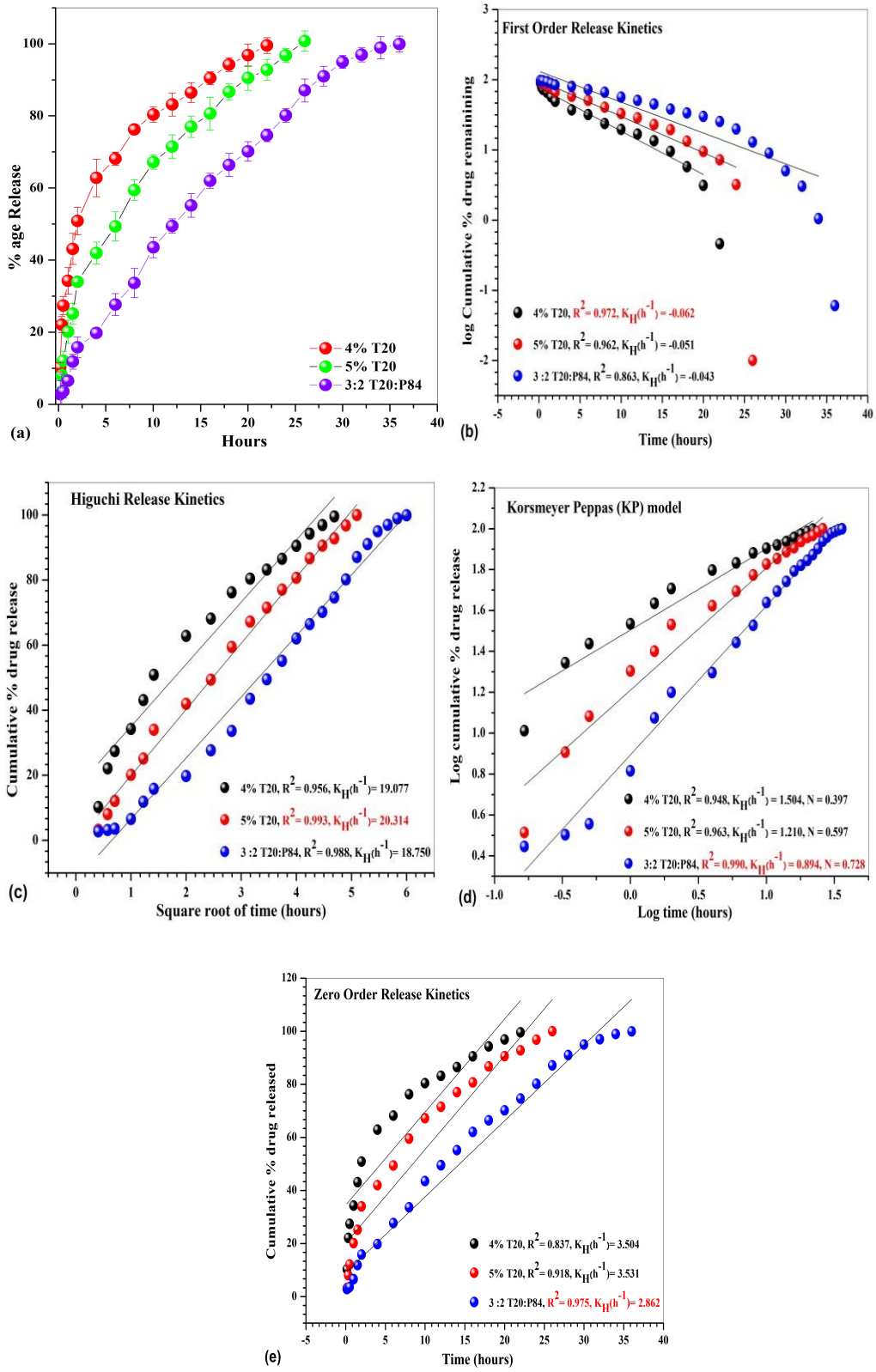


Figure 8: (a) *In vitro* OLZ release in phosphate buffer saline (pH = 7.4) and several kinetics models **(b)** to **(e)** for Polysorbate T20and Polysorbate T20-Pluronic P84

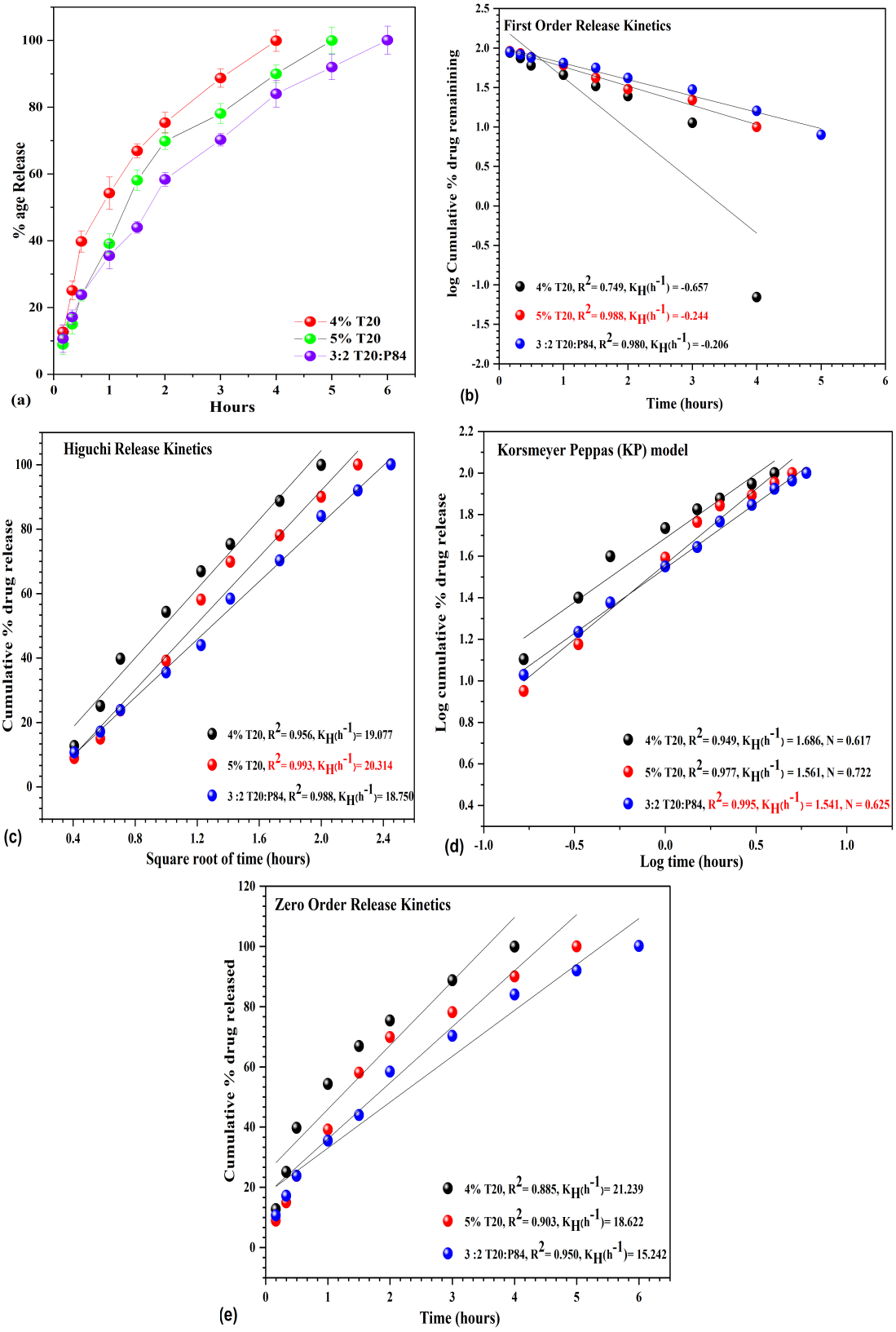


Figure 9: (a) *In vitro* OLZ release in simulated gastric fluid (pH=1.2) and several kinetics models **(b)** to **(e)** for Polysorbate T20and Polysorbate T20-Pluronic P84 mixed micellar formulations.

4.0 Conclusions:

All screened Polysorbates have similar hydrophilic head groups and only vary in the hydrophobic part. Therefore, a higher solubility was expected in the case of Polysorbate T60, as it contains a longer hydrophobic chain as compared to Polysorbates T20 and T40. However, these results are only valid at lower concentration but at higher concentration, this was not the case. At higher concentration, T20 demonstrated higher OLZ solubilization capacity and partition coefficient. Moreover, ΔG^s, ΔH^s were also favorable at this concentration range. The different concentration ratios of mixed micelles of Polysorbates T20, T40, T60 and Pluronic P84 were studied to look over their effect on the solubilization of OLZ. It was seen that T20:P84 mixed micelles at 3:2 % w/v concentration were found to have the higher OLZ solubilization as compared to the other mixed micellar systems studied. ITC measurements showed higher binding ability of T20 with OLZ than T60. DLS and SANS measurements evidenced an increase in the size of mixed micelles than pure Polysorbate micelles, however, after loading of OLZ drug in these micelles, a decrease in the size was observed. Moreover, SANS results revealed that Nagg is playing an important role in the OLZ solubilization, the higher solubilization capacity of Polysorbate T20 can be corelated to its lower Nagg number before and after OLZ loading. So, T20 can form micelles with a lesser number of monomers as compared to the other Polysorbates, upon increasing concentration of Polysorbates, higher number of micelles are available in case of T20 to solubilize OLZ than other Polysorbates. Polysorbate T20 and T20:P84 mixed micellar formulation exhibited the sustained release behavior of OLZ and by varying the concentration of Polysorbate T20 and mixing of Pluronic P84, drug release including the kinetics can also be controlled. The overall results indicated that this study will be advantageous in designing oral formulations for OLZ and other hydrophobic drugs. In the future work, these prepared formulations will be tested on 2D and 3D brain cell lines to investigate their anti-schizophrenic activities. This piece of work would be useful to design number of micellar formulations that can be used for the targeted drug delivery to brain as well as other organs.

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