Solubility and Crystal Size of Sirolimus in Different Organic Solvents

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The solubility of sirolimus drug in different organic solvents, viz., acetone, chloroform, methanol, ethanol, and dichloromethane, has been measured for the temperature range of (295 to 345) K by a gravimetric method. The relation between solubility and crystal size formation has also been studied. It was found that the higher the solubility of material, the larger the crystal size and vice versa. The stability of crystals has been measured, and it was found that smaller crystals have a moderate level of stability when compared with that of larger crystals. From the observations of the present study, it may be suggested that for cardiovascular applications of sirolimus drug one can use ethanol and methanol as a solvent for deriving crystals of less than 400 nm size.

Introduction

Nowadays, nanosize particles are used by many industries, such as cosmetics, dyes, electronics, pharmaceuiticals, etc.[1] Nanosize particles have also gained importance in the field of drug delivery.[2] An increasing number of newly developed drugs are poorly soluble in water, and poor solubility results in low bioavailability and/or erratic absorption of the drugs.[2] Sirolimus (an immunosuppressant drug) is a triene marcolide antibiotic isolated from *Streptomyces hygroscopicus* and is known to have poor solubility in water.[3,4] Due to the physical properties of sirolimus, such as water insolubility and log *P* (i.e., log of octanol−water partition coefficient), which is a measure of a drug's lipophilicity, of greater than 5, it is a challenging task to formulate it into either an intravenous or oral dosage form.[5] The solubility of sirolimus[6] is 2.6 $\mu g \cdot mL^{-1}$, which is far below the target solution concentration of 1 $mg \cdot mL^{-1}$. It is difficult to enhance the solubility of sirolimus by the generation of a salt form because of the lack of an ionizable group of sirolimus in the pH range[6] of 1 to 10.

Over the past ten years, the number of poorly soluble drugs has steadily increased. Estimates state that (40 to 60) % of the drugs in the pipelines have solubility problems.[7,8] Poor solubility in water correlates with poor bioavailability. If there is no way to improve drug solubility, then it will not be able to be absorbed from the gastrointestinal tract into the bloodstream and reach the site of action.[9] Tong[10] has pointed out that solubility is one of the most important physicochemical properties studied during pharmaceutical preformulation, hence accurate solubility data are essential to ensure the robustness of the finished product. Park et al.[11] studied the estradiol solubility in phosphate buffer saline and crystal habits in different organic solvents and found that estradiol crystal habits prepared from ethanol and methanol had larger crystal size.

Crystallizing solvents having various polarities are preferred since molecules in such solutions tend to form different types of hydrogen-bonded aggregates.[12] Some solvents favor crystallization of a particular form because they selectively adsorb to specific faces of the crystal, and some of the commonly used solvents are water, methanol, ethanol, propanol, iso-propanol, acetone, acetonitrile, ethyl acetate, hexane, etc.[13] Also, crystal habit in organic solvent plays an important role in affecting the crystal product physicochemical properties, such as solubility, dissolution rate, compressibility, and bulk density that have an effect on the product biological activity and production cost.[11] As sirolimus is used in local drug delivery on medical devices, such as drug eluting stents and balloons, it is important to understand the solubility and crystal habit of it for its biological activity.[14−16] On the basis of the available literature, it can be said that drug crystals of less that 400 nm are suitable for arterial penetration and tissue uptake for cardiovascular applications.[17−19]

Therefore, the study of the solubility of a drug and its crystal habit is necessary from pharmaceutical industry requirements. The solubility of sirolimus in organic solvents directly affects the size of crystal formation, yield, and cost of production. Hence, it is necessary to know the solubility of sirolimus in different organic solvents. In the present work, an attempt has been made to study the solubility of sirolimus in different organic solvents, viz., acetone, chloroform, methanol, ethanol, and dichloromethane from (295 to 345) K at atmospheric pressure using the gravimetric method.[20] The crystal size (*L*) of the drug in respective organic solvent has been derived under sonication in a setup similar to our previous work,[21] and we tried to establish a relationship between the solubility of the drug in organic solvent and the size of crystal formation in antisolvent media.

Materials and Methods

Materials. Sirolimus ((3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-hexadecahydro-9,27-dihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3*H*-pyrido [2,1-c] [1,4]-oxaazacyclohentriacontine-1,5,11,28,29-(4*H*,6*H*,31*H*)-pentone), $C_{51}H_{79}NO_{13}$, molecular weight 914.175, CAS RN 53123-88-9) was provided by Gayatri Medico Distributors, Bharuch, India, with a purity of 98 %. All organic solvents (HPLC grade) used as solubilizing agents, for dissolving the sirolimus, were supplied by Himedia Lab, Mumbai, India. For sonication experiments, biodegradable Tomadol 23-6.5 was used as a surfactant (provided by Air Products Asia Inc., Singapore), and Millipore water was used as water base (antisolvent media for crystal fall-out). All the chemicals and drug were used as supplied without further purification.

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Methods. To keep homogeneity in solubility and sonication experimental work, data generation and easy analysis of solubility and crystal size data, all the parameters, and quantities of materials, except temperature, have been kept constant. The quantities of materials used in solubility experiments were 10 mL of organic solvent and 8 mg of sirolimus drug. The quantities of materials used in sonication experiments were as follows: 12 mL of Millipore water, 8 mg of sirolimus drug, 1 mL of organic solvent, and 475 μL of surfactant. All the experiments were carried out at an ultrasound frequency of 20 kHz and for 30 min of sonication time and temperature in the range of (295 to 345) K, with an interval of 5 K. We used the gravimetric method[20] to study the solubility of sirolimus in different organic solvents. Sirolimus was dissolved in organic solvent and agitated at 318 K for 40 min and then filtered and dried under vacuum below 303 K. We used an approach similar to that of our previous work,[21] except a sonicator, for the solubility study. The experiments were performed in a glass-lined stainless steel (SS) beaker of 25 mL, having a facility of chilled water circulation in a jacket to control the temperature. The entire setup was comprised of a glass-lined SS beaker with jacket, circulating pump, and magnetic stirrer. For weighing of the drug, a Sartorius balance (uncertainty of $\pm$ 0.00001 g) was used. The temperature of the beaker was measured through thermocouple, and a data link was provided to a controller. All the experiments were carried out under amber light and room temperature of 293 K in view of the photosensitive nature of sirolimus drug. The thermostat was set to maintain the temperature in a range within 0.10 K from a stated set point. For every experiment, a certain amount of organic solvent was added into the crystallizer, which helped to stabilize the temperature at the stated range. An excess amount of sirolimus crystals was added into the crystallizer and sealed by rubber plugs. A time of 5 h was provided for stirring of solution and for establishment of equilibrium between solids and solution, and 2.5 h settling time was provided for suspension media. A 10 mL glass syringe was used for extraction of clear solution, filtered through a 0.2 μm PTFE filter, and instantaneously injected into a Petri dish. The said Petri dish was weighed before injecting suspension. After injecting the suspension in a Petri dish, the said dish was weighed and transferred to a dryer oven at 323 K. A time of 7 h was provided for drying; after drying, the Petri dish weight was measured, and multiple readings were taken to avoid errors. Each experiment was repeated four times with the aim to derive accurate results. The solubility was calculated based on the weight of dried solids and evaporated solvent. The saturated mole fraction solubility of solute (x^c) in solvent was calculated[22] as

$$x^c = \frac{m_1/M_1}{m_1/M_1 + m_2/M_2} \quad (1)$$

where m_1 and m_2 represent the mass of solute and solvent, respectively; and M_1 and M_2 represent the mole weight of the solute and the solvent, respectively. The uncertainty of mole fractions in the solubility values is established to be $\pm$ 2.0 %. The uncertainty of temperature in the experiments is observed to be $\pm$ 0.10 K.

Nanosize crystals of sirolimus in different organic solvents were obtained through sonication experiments following the procedure reported earlier.[21]

Crystal Size Measurement. The mean size of sirolimus crystals in nanosuspension was determined by photon correlation spectroscopy (PCS) on a Particle Size Analyzer (Master Sizer 2000 Ver 3.01, Malvern Instruments). It gives information about the mean size of the bulk population in terms of volume weighted mean and surface weighted mean, specific surface area, analysis of crystal population for 10 %, 50 %, and 90 % with its mean crystal diameter, and the polydispersity index. The size distribution was expressed by equivalent volume diameters at 10 % (d10 %), 50 % (d50 %), and 90 % (d90%) cumulative volume. In this study, we have taken mean average diameter into consideration. Each experimental value was gained by PCS results from three independent experiments, each performed in triplicate. The measurement temperature was chosen as 293 K.

Stability. Zeta potential measurements were performed on the same Malvern Zetasizer at 298 K. Zeta potential is relative to the double electric layer on the surface of colloidal particles, and a range of (−20 to −11) mV corresponds to the threshold of agglomeration in dispersions.[23] A higher value of zeta potential means higher stability with other conditions staying the same. As described by Müller et al.,[24] a zeta potential value of $\pm$ 30 mV is the minimum for physically stable nanosuspension solely stabilized by electrostatic repulsion, and the corresponding value is about $\pm$ 20 mV in the case of a combined electrostatic and steric stabilization. According to ASTM standards,[25] the zeta potential of particles in the range of (−30 to −60) mV can be considered as stable particles, and those with potential more than −61 mV are highly stable particles.

Scanning Electron Microscopy. For evaluation of nanosize particles of crystals formed, scanning electron microscopy (SEM) has been carried out on a ZEISS Ultra-60 field emission scanning electron microscope.

Results and Discussion

The experimental solubility data of sirolimus in organic solvents, viz., acetone, chloroform, methanol, ethanol, and dichloromethane, at different temperature were presented in Table 1 and Figure 1 for better visual observation. Absolute temperature is represented as T, and x^c and x^e are the calculated and experimental values of solubility, respectively. From Figure 1 it can be said that the solubility of sirolimus in organic solvents is temperature dependent. The results indicate that acetone has higher solubility and methanol has comparatively lower solubility at different temperatures.

Solid−liquid phase equilibrium theory states that solubility of a substance in pure organic solvent depends on the temperature and can be correlated by the semiempirical equations, suggested by Wang et al.[26] and Apelblat and Manzurola[27] deduced from solid−liquid phase equilibrium[28,29]

$$\ln(x^e) = a + \frac{b}{T/\mathrm{K}} + c\ln(T/\mathrm{K}) \quad (2)$$

where a, b, and c are empirical constants. With the help of the least-squares method, using the experimental values of T and x^e, values of parameters a, b, and c are estimated and are listed in Table 2 along with the root-mean-square deviation (rmsd), which is defined as follows[30]

$$\mathrm{rmsd} = \left[\frac{1}{N}\sum_{i=1}^{N}(x_i^e - x_i^c)^2\right]^{1/2} \quad (3)$$

where N is the number of experimental points.

Experimental results show that solubility of sirolimus increases with temperature in different organic solvents, but the rate of solubility is different in different organic solvents, which

Table 1. Mole Fraction Solubility of Sirolimus in Different Organic Solvents at Different Temperatures

T/K	10^3x^e	$100(x^c - x^e)/x^e$	T/K	10^3x^e	$100(x^c - x^e)/x^e$
		Acetone			
295	3.16	0.44	325	4.87	−0.52
300	3.21	0.75	330	5.11	−0.47
305	3.43	−0.07	335	5.45	1.69
310	3.88	−0.24	340	6.27	0.56
315	4.33	0.72	345	7.21	0.87
320	4.70	1.05			
		Chloroform			
295	2.72	1.32	325	3.86	0.78
300	2.89	0.57	330	4.13	0.68
305	3.11	−0.23	335	4.37	1.11
310	3.25	−0.46	340	4.58	0.52
315	3.46	1.88	345	4.95	0.93
320	3.69	0.24			
		Dichloromethane			
295	1.90	1.22	325	3.06	−1.75
300	2.01	−1.75	330	3.33	−0.06
305	2.20	−0.69	335	3.51	−0.48
310	2.46	0.21	340	3.97	0.78
315	2.71	0.37	345	4.33	0.92
320	2.79	−0.48			
		Ethanol			
295	1.37	−0.02	325	2.37	0.70
300	1.43	−0.74	330	2.59	−0.99
305	1.63	1.26	335	2.75	−0.63
310	1.84	0.33	340	2.91	0.58
315	1.97	0.68	345	3.17	−1.01
320	2.10	−0.11			
		Methanol			
295	0.72	1.57	325	1.63	0.32
300	0.88	0.33	330	1.71	−0.21
305	1.07	0.23	335	1.83	−0.11
310	1.23	−0.14	340	1.88	−0.29
315	1.43	−0.54	345	2.05	0.95
320	1.46	0.70			

reflects from the values of slope and correlation coefficient (R^2) in Figure 1. From Figure 1, it can be concluded that acetone has a higher solubility and methanol has a lower solubility.

Also, sonication experiments were carried out for the formation of nanosize crystals of sirolimus in these organic solvents to study the relation between the solubility and crystal size. The data of crystal sizes formed in respective organic solvents were

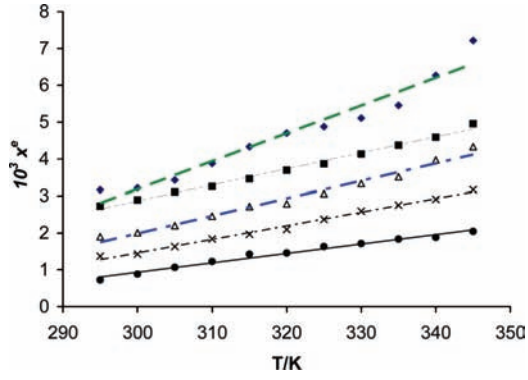

Figure 1. Experimental mole fraction solubility, x^e, of sirolimus in different organic solvents at different temperature ranges: ◆, acetone; ■, chloroform; Δ, dichloromethane; ×, ethanol; ●, methanol. Straight line equation for data fitting and R^2 values of experimental solubility: ◆, acetone, $y = 0.0755x - 19.48$, $R^2 = 0.9486$; ■, chloroform, $y = 0.0434x - 10.154$, $R^2 = 0.9921$; Δ, dichloromethane, $y = 0.0473x - 12.206$, $R^2 = 0.9788$; ×, ethanol, $y = 0.0366x - 9.5304$, $R^2 = 0.993$; ●, methanol, $y = 0.0256x - 6.7545$, $R^2 = 0.9818$.

Table 2. Parameters of Equation 2 for Sirolimus in Different Organic Solvents

solvent	a	b	c	10^5 rmsd
acetone	−1.44	1193.06	−2.12	4.17
chloroform	12.47	228.01	−3.22	3.42
dichloromethane	0.29	1640.16	−0.92	2.70
ethanol	23.24	401.70	−4.28	1.73
methanol	106.51	−5932.53	−22.32	0.83

Table 3. Crystal Size (nm) of Sirolimus in Different Organic Solvents at Different Temperatures

	organic solvents				
T/K	acetone	chloroform	dichloromethane	ethanol	methanol
295	1421	1352	1105	875	641
300	1328	1125	981	802	594
305	1188	987	902	765	531
310	1047	825	863	735	453
315	922	765	703	621	367
320	883	721	652	547	328
325	828	685	587	475	240
330	797	632	525	412	211
335	773	594	487	358	164
340	742	563	432	287	125
345	734	540	385	205	89

shown in Table 3 and Figure 2, and it can be observed that methanol gives small crystals as compared to other organic solvents. By comparing Figures 1 and 2, it can be concluded that the higher the solubility, the larger the crystal sizes formed; whereas the lower the solubility then the smaller the crystal sizes for sirolimus drug in organic solvents. The logarithmic plots of both solubility and nanosize crystals as a function of temperature (figures not shown) have good data fitting with minimum variation.

In our experiments, the zeta potential of nanosize crystals of sirolimus in nanosuspensions was found to be in the range of (-44.7 ± 1.75) mV to (-58.2 ± 0.75) mV for different organic solvents. From Tables 3 and 4, it can be observed that the value of zeta potential is lower for smaller crystals. A moderate level of stability of crystals is in ethanol and methanol, whereas higher stability of crystals is in acetone.

From SEM images (Figure 3), it can be observed that the crystal size and shape are solvent dependent. This is due to the

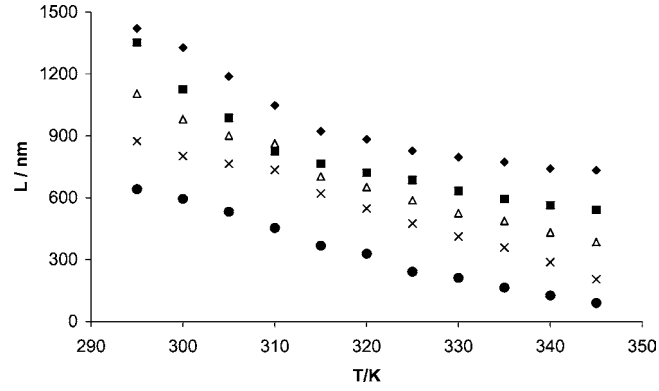

Figure 2. Sirolimus crystal size (nm) in different organic solvents at different temperature ranges: ◆, acetone; ■, chloroform; Δ, dichloromethane; ×, ethanol; ●, methanol.

Table 4. Effect of Organic Solvent on Zeta Potential (E) at 298 K

organic solvent	E/mV
acetone	-58.2 ± 0.75
chloroform	-52.3 ± 1.22
dichloromethane	-54.7 ± 1.37
ethanol	-47.2 ± 1.55
methanol	-44.7 ± 1.75

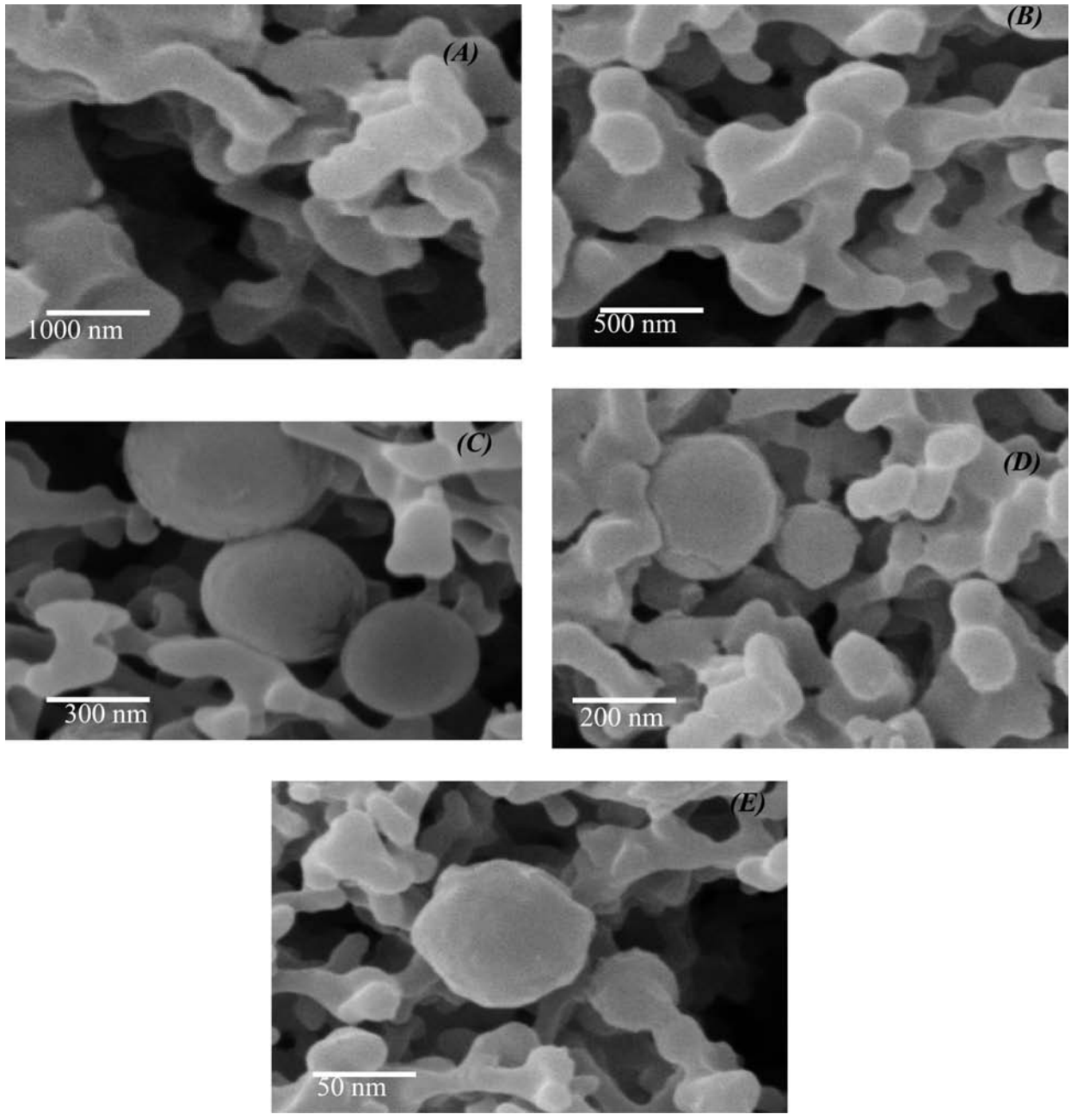

Figure 3. SEM images of crystals derived from: (A) acetone; (B) chloroform; (C) dichloromethane; (D) ethanol; (E) methanol.

nature of solvents; solvents favor the crystallization of a particular form because they selectively adsorb to specific faces of crystals, which is in line with the findings of Brittain.[13]

The experimental data of the present work indicate that the solubility of sirolimus drug varies in different organic solvents and also found that the solubility increases with temperature. These findings about the solubility of sirolimus are in contradiction to the findings of Simamora et al.[6] where they had found that it is impossible to enhance the solubility of sirolimus by generation of a salt form because of the lack of an ionizable group of sirolimus in the pH range of 1 to 10.

Suspension media of different organic solvents have different saturation solubility under sonication. A lower solubility of sirolimus drug in suspension media is responsible for smaller nuclei formation (in methanol), whereas a higher solubility of sirolimus drug in suspension media is responsible for larger nuclei formation.[31] During the sonication process, the organic solvent evaporates and the drug precipitates[32] in water media through phase transfer following the Ostwald ripening; i.e., nuclei formed in process grow over a period of time until the saturated solubility of suspensions supports it.[31] After this, size reduction of particles took place under the cavitation formed in the sonication process, and similar findings were reported by Radtke[33] in his study of nanoparticle formation through homogenization.

The growth of crystal in suspension exhibits Ostwald ripening, which is due to the differences in saturation solubility above the surface of crystals of different sizes and high variations in solubility with alterations in temperature during the process.[31]

The values of zeta potential indicate long-term physical stability of particles of sirolimus without Ostwald ripening (i.e., a typical tendency of larger particles to increase their size over a period of time, whereas the smaller particles dissolve in a highly disperse medium) due to the uniform size of nanoparticles derived in experiments.[31]

The crystal of sirolimus, derived in methanol, ethanol, and dichloromethane, having a size of lower than 400 nm in an experiment is well suited for medical applications, mainly in the cardiovascular area, because the smallest blood capillaries in the human body are in a range of (5 to 6) μm.[31] As pointed out by Muller and Keck[31] in their study, it can be said that with the help of nanoparticle formation a poorly soluble drug can also be made 100 % bioavailable and can be utilized in the medical treatment.

Figure 3(A) indicates that the crystals obtained in acetone are larger in size (> 700 nm), are cylindrical in nature, and do not have a uniformity in shape. Figure 3(B) indicates that the crystals obtained in chloroform are medium in size (> 500 nm), and the majority of the particles is cylindrical with some of the particles being spherical in nature and not having a uniformity in shape. Figure 3(C) indicates that the crystals obtained in dichloromethane are smaller in size (>300 nm), and the majority of the particles is spherical with some of the particles being cylindrical in nature and having uniformity in shape. Figure 3(D) indicates that the crystals obtained in ethanol are smaller in size (> 200 nm), and the majority of the particles is spherical with some of the particles being cylindrical in nature and having uniformity in shape. Figure 3(E) indicates that the crystals obtained in methanol are smaller in size (> 50 nm), and the majority of the particles is spherical with some of the particles being cylindrical in nature and having uniformity in shape.

Conclusions

From the present study, it can be concluded that for obtaining nanosize crystals of sirolimus drug one has to consider their solubility in organic solvent. The particle size of nanocrystals and solubility of drug are proportionately correlated to each other, i.e., the higher the solubility then the larger the crystal size and vice versa. Hence, for cardiovascular applications of sirolimus drug, where crystals of less that 400 nm size are suitable for arterial penetration and tissue uptake, one may use either ethanol or methanol for deriving nanocrystals in water.

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