Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 6(6); 269-273

ISSN: 0976 822X

Research Article

Enhancement of Saturation Solubility and *In Vitro* Dissolution of Carvedilol Nanoparticles by High Pressure Homogenization Technique

Ashok Kumar J[1]*, Ramkanth S[2], Lakshmana Prabu S[3], Gopal V[4]

[1]*Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, UCSI University, Kuala Lumpur-56000, Malaysia*
[2]*Annamacharya College of Pharmacy, Rajampet - 518126, Andhra Pradesh, India*
[3]*Department of Pharmaceutical Technology, Bharathidasan Institute of Technology, Anna University, Tiruchirappalli – 620 024, Tamil Nadu, India*
[4]*Mother Theresa Postgraduate and Research Institute of Health Sciences, Puducherry-605006, India*

Available Online: 11th November, 2015

ABSTRACT
The objective of the work was to enhance the saturation solubility and *in vitro* dissolution of poorly soluble (BCS Class II) drug, Carvedilol by high pressure homogenization technique. Carvedilol nanoparticles were prepared by emulsion-diffusion followed by high pressure homogenization (HPH) technique with stabilizers. The prepared nanoparticle evaluated for its physicochemical properties, morphology, saturation solubility and *in vitro* dissolution study. The Carvedilol nanoparticles were prepared in a spherical shape and the size range of 120 nm to 300 nm. Carvedilol nanoparticles observed maximum saturation solubility of 410.75±3.58 µg / ml (82.15 %) in acetate buffer (pH 4.6) and its i*n vitro* dissolution release was 94.5 % in phosphate buffer (pH 6.8) after one hour. Prepared Carvedilol nanoparticles has three folder enhanced saturation solubility and *in vitro* dissolution rate than the pure drug, irrespective of medium. The obtained results suggested that HPH might be an efficacious technique for converting poorly soluble drug into nanoparticles and ultimately the effect of particle size reduction may enhancing the solubility and *in vitro* dissolution rate.

Key words: Carvedilol, Saturation solubility, *In vitro* dissolution, Carvedilol nanoparticles, High pressure homogenization.

INTRODUCTION

New drug discovery, the number of insoluble drugs candidates has increased in recent years, with almost 70% of new drug candidates showing poor water solubility[1]. For these drug candidates, poor aqueous solubility and dissolution in the gastro intestinal fluids is a limiting factor to the *in vivo* bioavailability after oral administration. Therefore, *in vitro* dissolution has been recognized as an important element in the development and thus increasing the dissolution rate of poorly soluble drugs and enhancing their bioavailability[2,3]. There are numerous approaches reported in literature to reach this goal i.e. salt formation, solubilization, particle-size reduction, and solid dispersion[4,5]. Carvedilol, chosen in the current study, is a nonselective βblocker / α-1 blocker for the treatment of high blood pressure and mild to severe congestive heart failure. Carvedilol is a poorly water soluble drug a BCS class-II compound, attaining sufficient bioavailability of the drug is rate limited solely to dissolution and peak blood plasma level reaches 1.5 to 2 H after oral administration[6]. But due to their high permeability researches focus on solubility enhancement for BCS class-II compounds. Therapeutic efficacy of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. Solid dispersion with porous silica and inclusion complexes with cyclodextrins were described technique for enhanced Carvedilol dissolution[7-9]. It is well known that a poorly water-soluble drug requires more time to dissolve in the gastrointestinal fluid than it takes to be absorbed[10]. Limited solubility or dissolution in the gastrointestinal tract results in insufficient and variable absorption[11]. The solubility of drug is often intrinsically related to drug particle size; as a particle become smaller, surface area to volume ratio increases. The large surface area allows greater interaction with solvent, which causes an increase in solubility. Drug particle size reduction leads to an increase in the surface area and consequently the rate of dissolution as described by the Noyes-Whitney equation[12]. Low aqueous solubility has been a challenge for both formulation development of new chemical entities and generic development. Although some approaches are available for enhancing the dissolution of poorly soluble drug but has certain draw backs. Conventional methods of particle size reduction, such as comminution and spray drying, rely upon mechanical stress to disaggregate the active compound. Micronization is another conventional technique for the particle size reduction, which increase the surface area but does not increase equilibrium solubility. The dissolution velocity of micronized drug powders is enhanced by their enlarge surface area. The same effect, but more pronounced, is valid for nanonised drugs powder. Particle size reduction by homogenization

**Author for Correspondence*

is thus permitting an efficient, reproducible and economic means of solubility and dissolution velocity enhancement[13]. However, nanonisation is a new solution to poor water soluble and poorly bioavailable drugs. Nanosuspension is commercially possible approach to solve the poorly solubility as well as poor bioavailability problem of drugs. Nanotechnology in pharmaceutics is a technique to modify physicochemical, micrometrics and biopharmaceutical properties of the poorly soluble drugs, thereby improving their solubility[14,15]. *In vitro* dissolution rate and bioavailability of poorly soluble drugs such as spironolactum[16], budesonide[17], omeperazole[18] and meloxicam[19] have been improved by reducing their particles by high pressure homogenization. This study set out to enhance the saturation solubility and *in vitro* dissolution for Carvedilol by reducing their particle size using emulsion-diffusion followed by HPH, without influence of carriers.

MATERIALS AND METHODS

Materials

Carvedilol was obtained as a gift sample from Dr Reddy's Laboratories, Hyderabad, India. Polyvinylpyrrolidone K-30 (PVP K-30) and Sodium Dodecyl Sulfate (SDS) were purchased from Loba chemicals, Mumbai, India. Concentrated Hydrochloric acid, sodium acetate, potassium dihydrogen phosphate and other ingredients used were of analytical or Pharmaceutical grade.

Methodology

Preparation of Carvedilol nanoparticles

The drug was completely dissolved in suitable solvent. The obtained drug solution was injected into the water containing stabilizers under sonication by ultrasonic cleaner for 15 min. The nanoparticle dispersion is formed in the aqueous medium containing stabilizers. The disperolution was kept in a homogenization (Biologics, 20 KHZ-3000, USA) at 80 % pulser for 15 Min. Then the dispersion was stirred under mechanical stirrer for 30 Min to evaporate solvent by diffusion. The prepared nanosuspension was oven-dried at 60ºC for 12 H[20,21].

Scanning electron microscopy (SEM)

Scanning electron microscopy was conducted to characterize the morphology of particles. The samples were mounted on aluminium stubs using double adhesive tape, coated with gold in HUS-5GB vacuum evaporator. The Carvedilol nanoparticle particles morphology were observed using scanning electron microscope (Hitachi 3000, Japan) at acceleration voltage of 10 kV at required magnification[22].

Physicochemical properties

The prepared Carvedilol nanoparticles were characterized for physicochemical properties such as appearance, color, odor, melting point, pH, loss on drying and partition-coefficient. Appearance, color and odor of Carvedilol nanoparticles were observed and recorded, as it is important to establish a test panel early in the stability program. Melting point is the physical property often used to identify or check the purity of compound, the temperature at which a solid melts and becomes a liquid is the melting point. The melting point of Carvedilol nanoparticles can be determined by using mel-temp apparatus (Campbell electronic, Mumbai, India) and introducing a tiny amount into a small capillary tube by, attaching this to the stem of a thermometer in a heating bath, heating the bath slowly, and observing the temperatures at which melting begins and is complete. Pure samples usually have sharp melting points, for example 149.5 - 150 ºC or 189 - 190 °C; impure samples of the same compounds melt at lower temperatures and over a wider range, for example 145 - 148 °C or 186 - 189 °C[23].

The pH value of 10% aqueous solution of Carvedilol nanoparticles were determine by using digital pH meter (Systronic, Delhi, India) as per USP 32 <791>. Loss on drying (LOD) Carvedilol nanoparticle were performed as per monograph in USP 32 <731> (Dry a sample at 105 °C for 3 H: it loses not more than 0.5 % of its weight)[24]. The partition-coefficient (log *P*) value of Carvedilol nanoparticles were performed with *n*-octanol-phosphate buffer pH 6.8 mixture. The two phases were mixed in equal quantities and presaturated with each other for at least 24 H before the experiment. An accurately weighed quantity of Carvedilol nanoparticle was dissolved in 10 MI saturated phases and shaken for 24 H in a sealed container at 37 °C. The two phases were separated by centrifugation at 1000 rpm for 5 Min to achieve complete partitioning and analyzed for drug contents in the two phases. The partition-coefficient was expressed as the concentration of drug in the n-octanol phase (% w/v) divided by the concentration in the aqueous phase[25].

Saturation solubility studies

Saturation solubility is a compound specific constant, only depending on the temperature and the properties of dissolution medium. However, below a size of approximately 1-2 µm, the saturation solubility is also a function of particle size. The quantitative estimation of the solubility was made by preparing saturated solution of pure drug and its nanoparticles in constant volume of distilled water, acidic buffer (pH 1.2), acetate buffer (pH 4.6) and phosphate buffers (pH 6.8 and 7.4). These mixtures were stirred in a mechanical shaker for 24 H in room temperature. Visual inspection was carefully made to ensure there were excess particles in the mixture, indicating saturation has been reached. Aliquots from each sample were filtered through 0.45 µm whatman filter paper (discarding approximately the one third of filtrate) and filtrate was diluted suitably to determine the saturation solubility of samples. Drug solubility was evaluated spectrophotometrically using UV visible spectrometer (UV 2310, Techcomp, Mumbai, India) at 242 nm using respective medium as a reference. Saturation solubility measurements were performed in triplicate.

In vitro dissolution studies

In vitro dissolution profile of pure drug and Carvedilol nanoparticles were investigated with USP type II (Paddle method) apparatus (Cambell electronic DR-6, Mumbai, India). The amount equivalents to 50 mg of samples were put into the vessel containing 900 ml of phosphate buffer (pH 6.8). The dissolution media were maintained at 37±0.5 °C with paddle rotation speed at 50 rpm. An aliquot of 5

ml samples were withdrawn at 15, 30, 45 and 60 Min, replaced with an equal volume of fresh medium to maintain sink condition. Samples were filtered through a 0.45 µm whatman filter paper and assayed for the drug content spectraphotomatically at 242 nm using the corresponding dissolution medium as reference. *In vitro* dissolution tests were performed in three vessels each (n=3) for pure drug and Carvedilol nanoparticles.

RESULTS AND DISCUSSION

Preparation of Carvedilol nanoparticles

Carvedilol nanoparticles were prepared by emulsion-diffusion followed by high pressure homogenization technique in the presence of stabilizers. In this study, acetone was selected as the solvent, it can solubilize the drug in large amount and improve the diffusion rate to the water. Water containing the combination of polyvinyl pyrrolidone (PVP) and sodium dodecyl sulphate (SDS) stabilizers needs to have the good affinity to the drug nanoparticle for better stability. This obtained dispersion was subjected to high pressure homogenization at room temperature. After homogenization, the drug nanosuspension was oven-dried to form the drug nanoparticles.

Scanning electron microscopy

Morphology of drug nanoparticles in the suspension followed by oven-drying is shown in Figure 1. The prepared Carvedilol nanoparticles by high pressure homogenization are spherical in shape and the particle size range of 120 to 300 nm. The particles are discrete and uniform in size and there is no sign of aggregation due to stirring has been observed, and the particle size does not depends on stirring time.

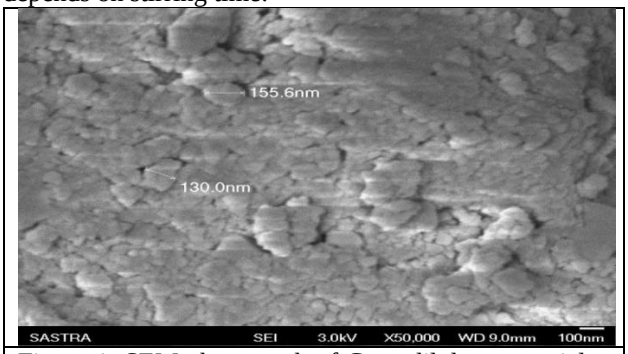

Figure 1: SEM photograph of Carvedilol nanoparticles (X 50,000)

Physicochemical properties

Physicochemical properties such as appearance, color, odor, pH, melting point, LOD and partition-coefficient reports of Carvedilol nanoparticles are illustrated in Table 1. The prepared Carvedilol nanoparticles was found to an odor less, white to half white crystalline powder and its pH was 7.7, which were complies with the certificate of analysis (COA) specification of Carvedilol Active Pharmaceutical Ingredient (API). The melting point of Carvedilol nanoparticle was found to be 114 ˚C, however complies the limit of 114-116 ˚C, it indicates that Carvedilol nanoparticle also have sharp melting point like pure drug. LOD value of Carvedilol nanoparticle was 0.42% and this value meet the USP specification limit of 0.5%. The partition-coefficient logarithmic (log *P*) value of Carvedilol nanoparticle was found to be 2.241 (Standard [log *P*] of Carvedilol is 2.79). The obtained logarithmic value of Carvedilol nanoparticle was much closer to the standard value of Carvedilol, which indicates that the Carvedilol nanoparticles possess sufficient lipophilicity.

Table 1: Physicochemical properties data for Carvedilol nanoparticles

Parameters	Results
Appearance	Solid crystalline powder
Color	White to half white color
Odor	Odor less
pH	7.7
Melting point	114 ˚C
LOD	0.42 %
Partition-coefficient (log *P*)	2.241

Table 2: Saturation solubility data for pure drug and Carvedilol nanoparticle

Solvents	Pure drug µg/ml (%)	Carvedilol nanoparticles µg/ml (%)
Distilled water	9.45 ± 0.52 (1.87 %)	22.30 ± 1.24 (4.46 %)
Acidic buffer (pH 1.2)	7.99 ± 0.46 (15.98 %)	188.15 ± 2.45 (37.63 %)
Acetate buffer (pH 4.6)	164.55 ± 2.64 (32.91 %)	410.75 ± 3.58 (82.15 %)
Phosphate buffer (pH 6.8)	157.6 ± 2.76 (31.52 %)	401.20 ± 3.86 (80.24 %)
Phosphate buffer (pH 7.4)	62.25 ± 1.32 (12.45 %)	152.65 ± 2.52 (30.53 %)

Saturation solubility studies

The saturation solubility study of pure drug and prepared nanoparticles in different physiological mediums were shown in Table 2. The results of solubility study indicated that Carvedilol nanoparticles possess a very high solubility in all physiological medium than pure drug. The saturation solubility of Carvedilol nanoparticles in distilled water, acidic buffer was found to be 22.30 ± 1.24 µg/ml (4.46%) and 188.15 ± 2.45 µg/ml (37.63%) respectively. Its saturation solubility increased on acetate buffer (pH 4.6) up to maximum of 410.75 ± 3.58 µg/ml (82.15%) and then decreased to 401.20 ± 3.86 µg/ml (80.24%) in phosphate buffer (pH 6.8). Pure drug solubility in distilled water, acidic acid (pH 1.2), acetate buffer (pH 4.6) and phosphate buffer (pH 6.8 and pH 7.2) was 1.87%, 15.98%, 32.91%, 31.52% and 12.45% respectively. Therefore Carvedilol nanoparticle solubility is pH dependent, it showed higher solubility in acetate buffer (pH 4.6) and less solubility at higher physiological pH levels. It has also been reported that, Carvedilol is practically insoluble in water and exhibits pH-dependent solubility. Its solubility is below 1 µg/ml at above pH 9.0, 23 µg/ml at pH 7, and about 100 µg/ml at pH 5 in room temperature. The solubility of Carvedilol in aqueous solutions with pH ranging from 1 to 4 is limited due to its protonation, resulting in "*in situ*" hydrochloride salt formation, which exhibits lower

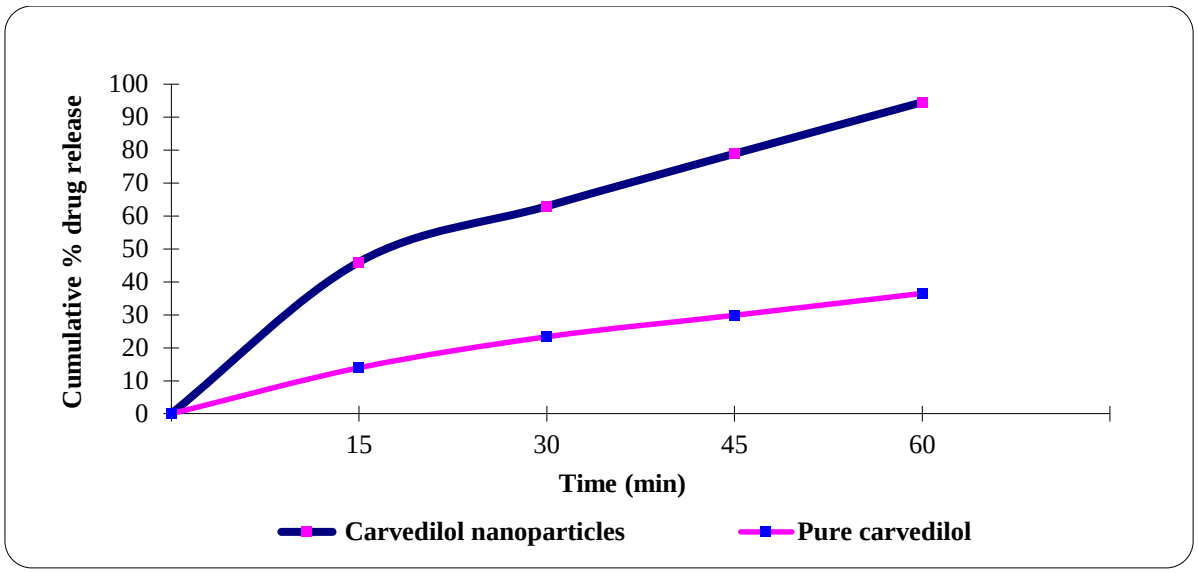

Figure 2: *In vitro* dissolution profile of pure drug and Carvedilol nanoparticles

solubility in media containing chlorine ions due to the common-ion effect[26].

Hence saturation solubility of Carvedilol nanoparticles was approximately three folders higher than its pure form in all pH range. Due to the reduction of the particle size from micron to nano scale may increase the surface area and also significantly increased the saturation solubility Carvedilol nanoparticles. An increased saturation solubility in the lumen of the gut increase the concentration gradient between lumen and blood, thus accelerating drug-diffusion, promoting the absorption.

In vitro dissolution studies

The selection of dissolution medium may be based on the solubility data and dosage range of the drug product. In this study phosphate buffer (pH 6.8) was used for *in vitro* dissolution medium, due to its second maximum solubility for drug and mimics the physiological pH of intestine. Some researchers have also reported that gastric residence times of solid dosage forms such as pellets or hard gelatin capsules are variable and can be extremely short (i.e., under 3 Min or even only 15 S) when taken into an empty stomach, preventing the drug from dissolving in acidic media[27]. It is acceptable that the dissolution media are not completely representative of gastrointestinal conditions. Yet it's produced in guidelines that a good method will be use a dissolution media, which is physiologically meaningful or closely mimics *in vivo* conditions[10].

Carvedilol nanoparticles were subjected to *in vitro* release studies in phosphate buffer (pH 6.8) and were compared with the release of pure drug are shown in Figure 2. From the result of *in vitro* release study, it was observed that the Carvedilol nanoparticles and pure drug gave the release of 46.03% and 13.94% respectively in 15 Min. Carvedilol nanoparticles displayed a dramatic increase in the rate and extent of *in vitro* dissolution release when compare to pure drug during the initial stage (first 15 Min). After 60 Min, the *in vitro* cumulative percent drug release was 94.50% and 36.50% for Carvedilol nanoparticles and pure drug respectively. The increase, *in vitro* release rate in the later stage can be attributed to the slight saturation of the drug in the dissolution medium. The initial fast release of Carvedilol nanoparticles can be due to less particle size, which lowers the surface tension around the nanoparticles. When the drug particles come in contact with medium, it lowers the surface tension and it results in to solvation of nanoparticles in the dissolution medium[28]. Here it was observed that the *in vitro* release of Carvedilol nanoparticles is three times higher than pure drug because of their particle size.

According to the Noyes-Whiteney equation, the increase in saturation solubility and decrease in particle size leads to an increased dissolution rate. An increase in saturation solubility is postulated by the particle size reduction due to an increased dissolution pressure explained by the Ostwaid-Freundlich equation[29]. When the particle reduced to nanometric range, dissolution velocity and dissolution pressure will increase which leads to the solubility due to the changes in the surface tension[30]. In addition nanoparticles have general adhesiveness to the gut wall after oral administration, which further enhance the bioavailability[13]. The present work was satisfactory preliminary study to enhancing saturation solubility and *in vitro* dissolution of Carvedilol, a poor water soluble drug by high pressure homogenization.

CONCLUSION

The saturation solubility and *in vitro* dissolution of BCS class II drug, Carvedilol was enhanced by emulsion diffusion followed by HPH technique. Reduced particle size and increased surface area may enhance the solubility and *in vitro* dissolution of Carvedilol. The acceleration of saturation solubility and *in vitro* dissolution release of Carvedilol nanoparticles is approximately three times more than that of pure drug. The bioavailability of Carvedilol is truly dissolution rate limited, so particle size reduction can significantly improve the performance of the drug. Nano metric sized drug particle of poorly water soluble drug (Carvedilol) has a dramatic effect on solubility, *in vitro* dissolution and consequently

bioavailability due to its particle size reduction by high pressure homogenization. Further detailed investigations are required to establish efficacious dosage form.

CONFLICT OF INTRESTS

Declared None

REFERENCES

1. Kawabata Y, Wada K, Nakatani M. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: basic approaches and practical applications. Int J Pharm 2011;420:1-10.
2. Hu J, Johnston KP, Williams RO. Nanoparticle engineering processes for enhancing the dissolution rates of poorly water soluble drugs. Drug Dev Ind Pharm 2004;30:33-245.
3. Costa P, Sousa Loba JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001;13:123-133.
4. Vemula VR, Lagishetty V, Lingala S. Solubility enhancement techniques. Int J Pharm Sci Rev and Research 2000;5(1):41-51.
5. Sharma D, Soni M, Kumar S, Gupta GD. Solubility enhancement-eminent role in poorly soluble drugs. Research J Pharm Tech 2010;2(2):220-224.
6. Tenero DM, Henderson LS, Baidoo CA. Pharmacokinetic properties of new controlled release formulation of Carvedilol. Am J Car 2006;98:5-16.
7. Odon P, Borut K, Franc V. Carvedilol dissolution improvement by preparation of solid dispersions with porous silica. Int J Pharm 2011;406:41-48.
8. Hirlekar R, Kadam V. Preparation and characterization of inclusion complexes of Carvedilol with methyl-cyclodextrin. J Incl Phenom Macrocycl Chem 2009;63,219-224.
9. Bhutani S, Hiremath SN, Swamy PV, Raju SA. Preparation and evaluation of inclusion complexes of Carvedilol. J Sci Ind Res 2007;66:830-834.
10. Horter D, Dressman JB. Influence of physicochemical properties on dissolution of drugs in the gastrointestnal tract. Adv Drug Deliv Rev 1997;25:3-14.
11. Martin A. Physical Pharmacy, 4th ed. Lea and Febiger, Philadelphia. 1993.
12. Muller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in drug therapy, Rational for development and what we can expect for the future. Adv Drug Deliv Rev 2001;47(1):3-19.
13. Keck CM, Mullur RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur J Pharm Biopharm 2006;62:3-16.
14. 14.Savjani KT, Gajjar AK, Savjani JK. Importance and enhancement technique. ISRN Pharm 2012;1-10. http://dx.doi.org/10.5402/195727.
15. Blagden N, De Matas M, Gavan PT, York, P. Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates. Adv Drug Deliv Reviews 2007;59(7):617-630.
16. Langguth P, Hanafy A, Frenzel. Nanosuspension formulation for low soluble drugs: Pharmacokinetic evaluation using spironolactone as model compound. Drug Dev Industrial Pharmacy 2005;31(3):319-329.
17. Jacobs C, Muller RH. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm Research 2002;19(2):189-194.
18. Moschwitzer J, Achleitner G, Pomper H, Muller RH. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. Eup J Pharm Biopharm 2004;58(3):615-619.
19. Amit JR, Madhabahi MP. Preparation and characterization of nanoparticles for the solubility and dissolution rate enhancement of meloxicam. Int J Pharm 2011;1(2):42-49.
20. Quintanar GD, Tamayo ED, Ganem QA, Allemann E, Doekler, E. Adaptation and optimization of the emulsification-diffusion technique to prepare lipidic nanospheres. Eur J Pharm Sci 2005;26:11-218.
21. Dolenc A, Julijana K, Baumgartner S, Planinsek, O. Advantages of celecoxib nanosuspension formulation and transformation into tablets. Int J Pharm 2009;376:204-212.
22. Shashikala P, Lavanya A, Bhagavanth Rao, H. Solid lipid nanoparticles for preparing controlled release drugs. Int J Pharm Sci 2012;4(1):69-72.
23. Shakeel F, Ahamed MA, Rhgigh, AM. Effect of surfactant on the crystal properties and dissolution behavior of aspirin. Asian J Research Chem 2009;2(2):202-206.
24. United State Pharmacopoeia 32/National formulary 27., US Pharmacopoeial convention. Inc. 2007.
25. Yuveraj Singh T, Chetan Singh C, Anshu Sharma. Development and evaluation of Carvedilol transdermal patches. Acta Pharm 2007;57:151–159.
26. Brook CS, Chen W, Spoors PG. Carvedilol phosphate salts and/or solvates thereof, corresponding compositions and/or methods of treatment US Patent 2007;7:268, 156.
27. Weitshies W, Kosch O, Monnikes H, Trahms L. Magnetic marker monitoring: an application of biomagnetic measurement instrumentation and principles for the determination of the gastrointestinal behavior of magnetically marked solid dosage forms. Adv Drug Deliv Rev 2005;57:1210–1222.
28. Basavaraj KN, Ganesh KD, Hiren MB, Veerandra KN, Manvi FV. Design and characterization of nanocrystals of lovastatin for solubility and dissolution enhancement. J Nanomed nanotech 2011;2(2):1-7.
29. Kesisoglou F, Panmai S, Wu Y. Nanosizing-oral formulation development and biopharmaceutical evaluation. Adv Drug Deli Rev 2007;59:631-644.
30. Chen Y, Liu J, Yang X, Xu H. Oleanolic acid nanosuspension: Preparation, *in vitro* characterization and enhanced hepatoprotective effect. J Pharm Pharmacol 2005;57:259-264.