REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

Solubility Enhancement of Simvastatin Drug by Using Different Technology And B-Cyclodextrin (B-CD) And Chitosan (Chitosan) Polymers

Asha Subhashrao Chopde[1]*, Dr. Sunil Thakral[2]

[1]*Research Scholar, Faculty of Pharmaceutical Sciences, Madhav University Pindwara (sirohi), Rajasthan-307026 India
[2]Faculty of Pharmaceutical Sciences, Madhav University Pindwara (sirohi), Rajasthan-307026 India

***Corresponding Author:** Asha Subhashrao Chopde
*Research Scholar, Faculty of Pharmaceutical Sciences, Madhav University Pindwara (sirohi), Rajasthan-307026 India

Abstract
In the current study, inclusion complexes of simvastatin using β-cyclodextrin (β-CD) and chitosan (chitosan) through three different techniques: physical mixing, kneading, and spray drying. These complexes were formulated in drug-to-cyclodextrin molar ratios of 1:1 and 1:2. All formulations underwent in vitro evaluation. The resulting inclusion complexes appeared white, fine, and non-sticky in texture. Among the different methods, the kneading and spray drying approaches with chitosan (1:1 molar ratio) showed superior drug release compared to the pure drug. Likewise, the physical mixture of simvastatin with β-CD at a 1:2 molar ratio demonstrated improved dissolution. Kinetic analysis revealed that the drug release followed first-order kinetics rather than zero-order. In summary, the findings suggest that both β-CD and chitosan are promising carriers for enhancing the solubility and dissolution rate of simvastatin, making them suitable for formulating fast-release drug delivery systems.

INTRODUCTION

The key characteristic of a dosage form is its capacity to release the active ingredient at an appropriate rate and in a sufficient quantity to produce the desired pharmacological effect at its target site. When a drug is administered through an extra vascular route and has a systemic action, its effectiveness is closely linked to the quantity of the drug that reaches the bloodstream. Furthermore, if the drug's pharmacological effects are immediately related to its plasma concentration, the absorption rate becomes crucial; as it impacts both the peak concentration and the time it takes to reach that peak. In this context, bioavailability refers to both the amount of the active drug that enters the bloodstream and the speed at which it does so.

Methods Used For Increasing the Dissolution Rate of Poorly Soluble Drugs

As far as the definition of bioavailability is concerned, a drug with poor bioavailability is the one with – Poor aqueous solubility and/or slow dissolution rate in the biological fluids.
1. Poor stability of the dissolved drug at the physiologic pH.
2. Inadequate partition coefficient and thus poor permeation through the bio membrane.
3. Extensive pre systemic metabolism.

The three approaches in overcoming the bioavailability problems due to such causes are:
1. The Pharmaceutical Approach which involves modification of formulation, manufacturing, process or the physicochemical properties of drugs without changing the chemical structure.
2. The Pharmacokinetic Approach in which the pharmacokinetics of drugs is altered by modifying its chemical structure.
3. The Biological Approach where by the route of drug administration may be changed such as changing from oral to parenteral route.

The second approach of chemical structure modification has a number of draw backs like very expensive and time consuming, require repetition of studies and a time consuming for regulatory approval.
For a drug to exhibit pharmacological activity, it must have some level of solubility in water. Additionally, most drugs need to be lipophilic to effectively pass through biological membranes through passive diffusion. The limited solubility and slow dissolution rate of poorly water-soluble drugs in the aqueous gastrointestinal fluids often result in inadequate bioavailability. Poor dissolution rates and low oral bioavailability of such compounds pose significant challenges in the development of pharmaceutical formulations. The solubility of a drug in water plays a key role in determining its effectiveness, and it is also influenced by the specific formulation used[1-6].

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

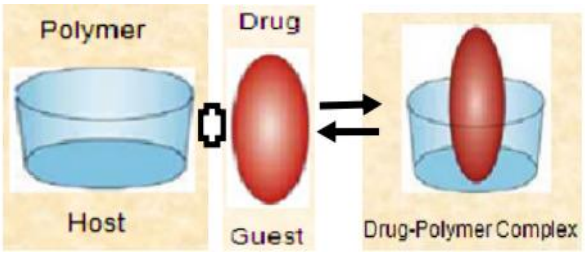


Fig. 1: Cyclodextrin-polymer Drug complex

A. Calibration curve for Simvastatin in distilled water
Procedure
Preparation of Standard Stock Solution.(SS-I and SS-II)
100 mg of Simvastatin was accurately weighed into 100 ml volumetric flask and dissolved in small quantity of methanol. The volume was made up to 100 ml with the methanol to get a concentration of (1000 ⌈g/ml.) SS-I. From this, 10 ml was withdrawn and diluted to 100ml to get a concentration of (100 ⌈g/ml) SS-II.

Calibration Curve in Distilled water
From the standard stock solution (SS-II), 0.5,1,1.5,2,2.5 and 3ml were withdrawn and volume was made up to 10 ml with Distilled water to give a concentration of 5, 10,15,20,25 and 30⌈g/ml. Absorbance of these solutions was measured against a blank at 245.5 nm for the absorbance values are summarized in Table 6. Calibration curve was plotted, drug concentrations versus absorbance was given in the Fig. 4.

B. Calibration curve for Simvastatin in 0.1N HCl
Preparation of Standard Stock Solution
100 mg of Simvastatin was accurately weighed into a 100 ml volumetric flask and dissolved in a small quantity of methanol. The volume was made up to 100 ml with the methanol to get a concentration of (1000 ⌈g/ml.) SS-I. From this, 10 ml was withdrawn and diluted to 100ml to get a concentration of (100 ⌈g/ml) SS-II.

Calibration Curve in Distilled water
From the standard stock solution (SS-II), 0.5,1,1.5,2,2.5 and 3ml were withdrawn and volume was made up to 10 ml with 0.1N HCl to give a concentration of 5, 10,15,20,25 and 30⌈g/ml. Absorbance of these solutions was measured against a blank at 245.5 nm and the absorbance values are summarized in Table 7. Calibration curve was plotted, drug concentrations versus absorbance was given in the Fig. 5.

C. Calibration curve for Simvastatin in 7.4 pH phosphate Buffer
Preparation of Standard Stock Solution
100 mg of Simvastatin was accurately weighed into a 100 ml volumetric flask and dissolved in a small quantity of methanol. The volume was made up to 100 ml with the methanol to get a concentration of (1000 ⌈g/ml.) SS-I. From this, 10 ml was withdrawn and diluted to 100ml to get a concentration of (100 ⌈g/ml) SS-II.

Calibration Curve in Distilled water
From the standard stock solution (SS-II), 0.5, 1, 1.5, 2, 2.5 and 3 ml were withdrawn and volume was made up to 10 ml with 7.4 pH Phosphate Buffer To give a concentration of 5, 10,15,20,25 and 30⌈g/ml. Absorbance of these solutions was measured against a blank of at 245.5 nm and the absorbance values are summarized in Table 8. Calibration curve was plotted, drug concentrations versus absorbance was given in Fig. 6.

Phase solubility studies
The most widely used approach to study inclusion complexation is the phase solubility method described by Higuchi and Connors, which examines the effect of a solubilizer, i.e., CD or ligand on the drug being solubilized, i.e., the substrate.

Procedure: 50mg Simvastatin was added to 15ml distilled water containing 0-10mM β-cyclodextrin and transferred to 25 ml stoppered conical flask. The mixture was shaken for 72hrs. Aliquots of 2 ml were withdrawn and filtered immediately using a 0.45µ nylon disc filter. The filtered samples were diluted suitably and assayed for Simvastatin by measuring absorbance at 245.5 nm against blank. The experiments were conducted in triplicate. The same procedure was followed in chitosan.
The apparent solubility constant (K_c) according to the hypothesis of 1:1 stoichiometric ratio of complexes was calculated from the phase-solubility diagram using the following equation.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

$$K_{a:b} = \frac{\text{slope}}{S_0\ (1\text{-slope})}$$

The slope is obtained from the initial straight line portion of the plot of Simvastatin against cyclodextrin concentration, and S_o is the equilibrium solubility of Simvastatin in water.

Compatibility Study with IR.

a) Fourier Transform Infrared Spectroscopy

Infrared spectroscopy is one of the most powerful analytical techniques that offer the possibility of chemical identification. The IR spectra of SV and their complexes were obtained by KBr pellet method by JASCO FT/IR-5300 spectrometer. A resolution of 4 cm^{-1} was used and 4 scans were co-added for each spectrum over a frequency range of 4000-400 cm^{-1}.All sample was analyzed in duplicate.

Preparation and characterization of Simvastatin-β-cyclodextrin and chitosan Cyclodextrins inclusion complexes

Complexes of Simvastatin with ®-cyclodextrin (®-CD) and chitosan (chitosan) were prepared by different methods using different molar concentrations of ®-CD and (chitosan). The molar concentration used and methods adopted are summarized in table-11.

Methods used in present work

a) **Physical mixture:** Simvastatin with ®-CD in different molar ratios (i.e. 1:1M, 1:2M) and with chitosan in ratio (i.e., 1:1M,1:2M) were mixed in a mortar for about one hour with constant trituration, passed through sieve No. 100 and stored in a desiccators over fused calcium chloride.
b) **Kneading method:** Simvastatin with ®-CD in different molar ratios (i.e., 1:1M, 1:2M) and with chitosan in ratios (i.e.1:1M) were taken. First cyclodextrin is added to the mortar, small quantity of 50% Methanol is added while triturating to get slurry like consistency. Then slowly drug is incorporated into the slurry and trituration is further continued for one hour. Slurry is then air dried at 25°C for 24 hours, pulverized and passed through sieve no. 100 and stored in desiccators over fused calcium chloride.
c) **Spray drying method:** The inclusion complex of SV-βCD was prepared by spray drying method. Thedrug and β-CD were dissolved in isopropyl alcohol (IPA) and distilled water separately. Both the solutions were mixed together on a magnetic stirrer for 30min.The resulting solution was fed to mini spray dryer (Labultima-222,Mumbai) and sprayed in the chamber from a nozzle with diameter 0.7mm under the atomization pressure of 1.5kg/cm2 with a feed rate of 3ml/min. The inlet temperature was kept at 80 ˚c and out let temperature 60 ˚c ± 2 ˚c. The vacuum in the system was 60mmwc and aspirator was 45%.The same procedure was adopted to prepare inclusion complex of SV-chitosan.The product thus obtained was collected, packed and doubly wrapped in a aluminum foil and stored in a decicator till further use.

Drug Evaluation Studies

a) Drug Content Estimation

Inclusion complexes prepared by physical mixture, kneading, and spray drying methods were assayed for Simvastatin content by dissolving a specific amount of the complexes in methanol and analyzing for the SV content spectrophotometrically at 245.5 nm on a spectrophotometer.

b) Aqueous solubility

An excess amount of sample was added to 5 ml of the distilled water in test tubes sealed with stoppers. The test tubes were vortex-mixed for 5 min and then sonicated for 30 min. They were kept in a constant temperature shaking bath maintained at 37 ± 0.5 ˚C until reaching equilibrium (48 h). A portion of solution was withdrawn and then filtered with a nylon disc filter (0.45 µm) and adequately diluted with methanol. The drug concentration was determined at 245.5 nm by UV-spectrophotometer (UV-1240, Shimadzu, Japan).

c) *In vitro* dissolution rate studies Simvastatin-β-CD and chitosan complexes

Dissolution studies of SV, its complex with β-CD and chitosan and its physical mixture was performed using USP dissolution apart Us type (USPXX IV) with 500-ml dissolution medium at 37˚C± .5˚C and 50 rpm for 3 hrs, 0.1 N HCl, distilled water and 7.4 pH Buffer containing 0.25% (w/v) of sodium lauryl sulfate were used as different dissolution media. At fixed time intervals, 5-ml aliquots were withdrawn, filtered, suitably diluted, and assayed for SV content by measuring the absorbance at 245.5 nm using a spectrophotometer. Equal volume of fresh medium at the same temperature was replaced into the dissolution medium after each sampling to maintain its constant volume throughout the study. Dissolution studies were perform in triplicate (n=3) and calculated mean values of cumulative drug release were used while plotting the release curves. The percent drug released at various time intervals was calculated and plotted against time.

Data Analysis (Curve fitting analysis)
To analyze the mechanism of the drug release rate kinetics of the dosage form, the data obtained were plotted as:
1) Cumulative percentage drug released Vs time (In-Vitro drug release plots)
2) Log cumulative percentage drug remaining Vs Time (First order plots)

Zero order release rate kinetics:
To study the zero–order release kinetics the release rate data are fitted to the following equation.
F= K.t
Where 'F' is the fraction of drug release, 'K' is the release rate constant and't' is the release time.
When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0.

Characterization of complexes
a) X-ray diffraction study:
X-ray diffraction study was done to study the powder characteristics of Simvastatin and its inclusion complexes with β-cyclodextrin and chitosancyclodextrin. X-ray diffract grams were obtained by Philips diffract meter (PW 1140) and Cu-Kα radiation diffractograms were run at a scanning speed of 2°/min and a chart speed of 2°/ 2cm/ 2θ.

b) Differential scanning calorimetry (DSC)
The DSC measurements were performed using a Perkin Elmer Pyris (Shelton, CT) and mettler equipped with an intercooler 2P cooling accessory. Samples of 4mg were placed in standard aluminum pans and sealed with a lid. Heating scans by 10°C/min were applied with a nitrogen purge of 20ml/min, over a temperature range of 30°C to 285°C. An empty aluminum pan was used as reference.

c) Scanning electron microscopy (SEM)
The morphology of samples was determined using scanning electron microscope (SEM)(HITACHI S-3000N, Japan), operated at an accelerating voltage of 20 kV (. lament current of 1.75 l beam current of 30 – 40 mA and probe current of 250 pA). Samples were
Prepared by mounting 0.5 mg of powder onto a 5mm silicon wafer a .fixed via graphite tape to an aluminum stub. The powder was then sputter-coated for 40 s at beam current of 38 – 42 mA with a 200 A ° layer of gold/palladium alloy.

d) Particle size analysis
The particle size analysis of was carried out by using laser channel beam instrument (C I S-50, Anskermid, Netherland.). The range of particles used for scanning was 1nm -150mm. The lens was used is A lens the particle were suspended in liquid paraffin to give a concentration of 10^{-9} particles /ml with a SNF value of 1. The sample prepared was placed in to the cuvetts made of polystyrene of 1cm path length. The particles were analyzed for their size (length×breadh ×volume) by using laser channel beam.

Stability study
The selected formulations were packed in amber-colored bottles, which were tightly plugged with cotton and capped. They were then stored at 25^0C / 60% RH and 40^0C / 75 % RH for 6 weeks and evaluated for their physical appearance, drug content and drug excipients compatibility at specified intervals of time.

RESULT AND DISCUSSION
Simvastatin-β-Cyclodextrin and chitosan inclusion complexes were prepared in order to increase the solubility, absorption and bioavailability of Simvastatin and same was evaluated. In the present work inclusion complexes were prepared by physical mixture, kneading and spray drying methods.

A) STANDARD CALIBRATION CURVE OF SIMVASTATIN IN DISTILLED WATER
Standard calibration curve of Simvastatin was drawn by plotting absorbance v/s concentration. The ⌊max of Simvastatin in distilled water was determined to be 245.4 nm. The absorbance values are tabulated in Table 6. Standard calibration curve of Simvastatin in the Beer's concentration range between 0-40 ⌈g/ml is shown in Fig.4.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

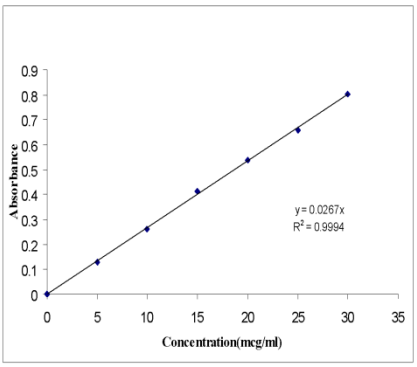


Fig. Standard calibration curve for Simvastatin in Distilled water at λ_{max} 245.4nm

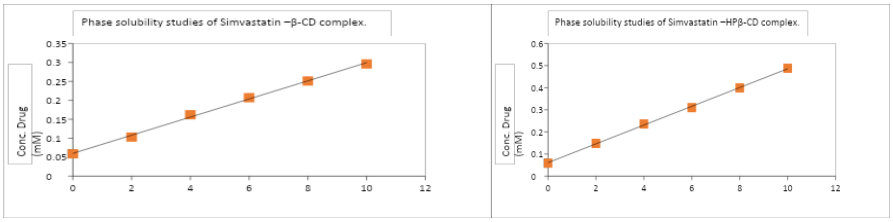


Figure No: Phase solubility of study of Simvastatin –chitosan complex.

Table No 11: Different formulations of Simvastatin with β-cyclodextrin and chitosan in molar ratio

Method	Drug to carrier	Drug to carrier ratio	Code
Physical Mixture	SV:β-CD	1:1	F_1
	SV:β-CD	1:2	F_2
	SV:chitosan	1:1	F_3
	SV:chitosan	1:2	F_4
Kneading method	SV:β-CD	1:1	F_5
	SV:β-CD	1:2	F_6
	SV:chitosan	1:1	F_7
Spray Drying	SV:β-CD	1:1	F_8
	SV:β-CD	1:2	F_9
	SV:Hβ-CD	1:1	F_{10}
Pure drug Simvastatin			F_0

Table No: *In vitro* drug dissolution profile of Simvastatin complex (F_2) in distilled water.

Ultraviolet visible spectroscopy

Determination of λ max

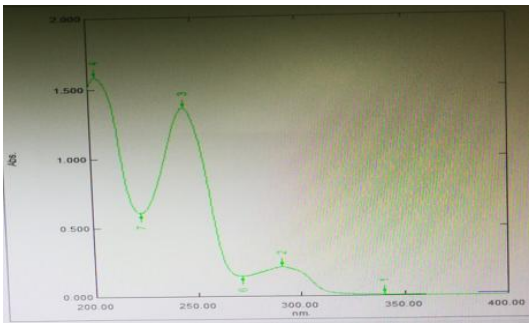


Fig. UV Spectrum of Efavirenz in methanol

An absorption maxima were found to be at 247 nm.Hence 247 nm was selected as λ max for further studies.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

FTIR spectroscopy

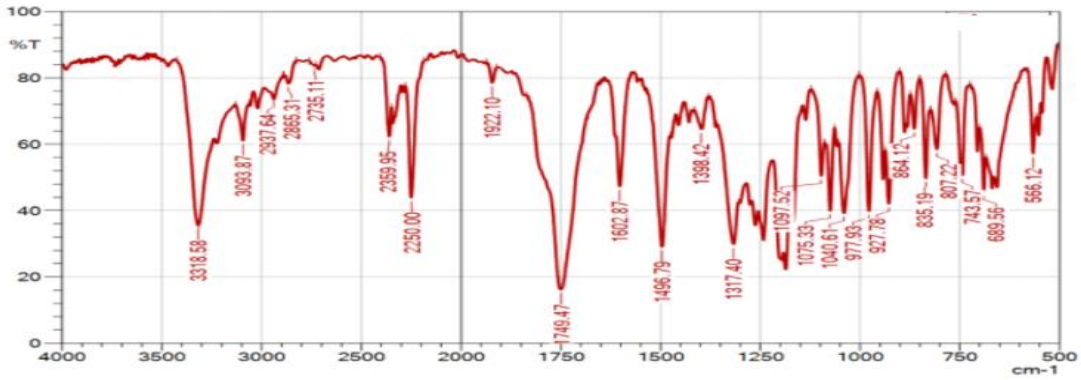


Fig. FTIR spectra of Efavirenz

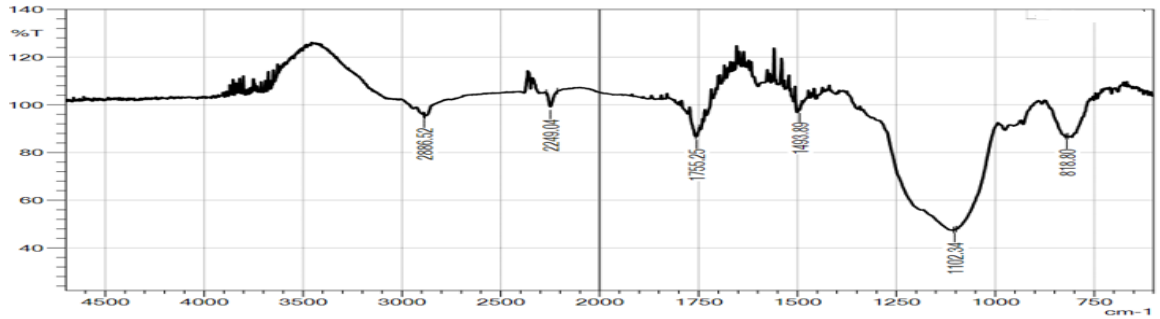
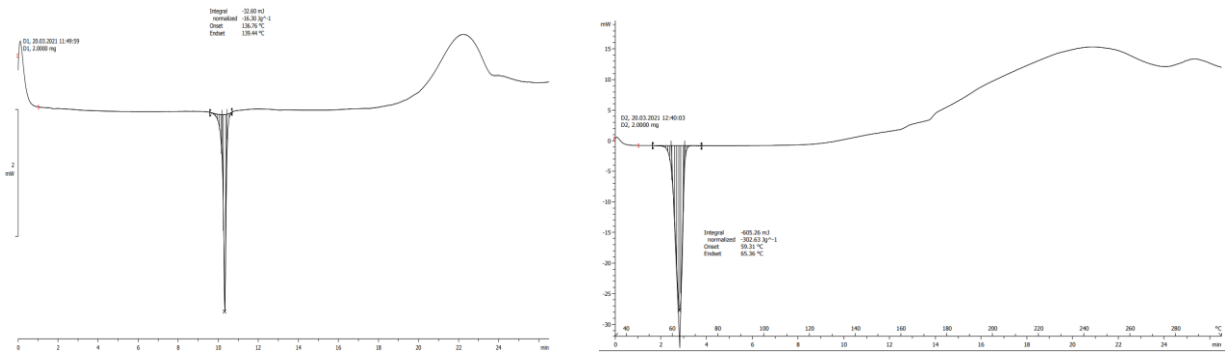


Fig. FTIR spectrum of complex formulation



a) **b)**

Fig. DSC thermographs (a) EFZ, (b) Complex

Table No.: *In vitro* drug release for (F_0 to F_{10}) formulations in 7.4 pH buffer.

Time in min	Formulations in 7.4 pH buffer.										
	F_0	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}
30	14.20	37.25	44.54	40.52	42.38	45.08	48.04	76.06	74.78	78.55	88.25
60	18.32	52.35	52.35	50.54	55.46	66.06	74.02	88.42	89.45	92.40	94.30
90	26.62	58.52	69.45	65.43	60.51	78.52	89.32	96.38	96.30	98.52	98.25
120	35.42	68.30	78.50	74.82	78.62	89.92	96.02	99.56	99.50	99.65	99.40
150	40.62	88.70	86.35	88.32	88.30	96.32	99.02	99.62	99.72	99.80	99.80
180	48.58	98.52	99.85	99.32	98.50	99.82	99.38	99.62	99.80	99.80	99.80

SUMMARY AND CONCLUSION

Simvastatin

In the present work inclusion complexes of simvastatin were prepared with cyclodextrin and chitosan by physical mixture, kneading and spray drying methods. The complexes were prepared in different molar ratios of drug and cyclodextrin namely 1:1 and 1:2. Prepared complexes were evaluated for *in vitro* drug. All the prepared inclusion complexes were white and fine without any stickiness. The drug content of the inclusion complexes was quite uniform (table-12). The percent drug content of the complexes was found to be in the range of 12% to 36%.

Cyclodextrins like ®-CD and chitosan can be used to prepare inclusion complexes of SV with improved solubility of the drug. Phase solubility studies of drugs with ®-CD illustrate the solubility enhancement capability of ®-CD, the stability constant (Kc) of SV:®-CD complex was found to be 410.25M^{-1}. The physical mixture with ®-CD (1:2M) was found to be higher than the pure drug and other prepared complexes. By comparing the first order and zero order kinetics, it was concluded that it followed the first order kinetics. Hence from the above results it can be concluded that ®-CD and chitosan can be used to formulate fast releasing formulations of drug.

REFERENCES

1. Zaheer A., Solubility enhancement of poorly water soluble drugs. IJPT. March-2011; 3(1): 807-823.
2. Kamalakkannan V., et al. Solubility enhancement of poorly soluble drugs by solid dispersion technique. JPR. 2010; 3(9):2314-2321.
3. Rasheed A., Ashok Kumar C. K., SravanthiV.Cyclodextrins as Drug Carrier Molecule, Sci Pharm. 2008; 76: 567–598.
4. Loftsson T., Brewster M.E. Pharmaceutical applications of Cyclodextrins. Drug solubilization and stabilization. J Pharm Sci. 1996; 85(10): 1017-25.
5. Loftsson T., Effects of Cyclodextrins on chemical stability of drugs in aqueous solutions. Drug Stabil. 1995; 1: 22-33.
6. Loftsson T., Olafsdottir B.J., FridriksdottirH., Jonsdottir S. Cyclodextrin complexation of NSAIDS: physicochemical characteristics. EurJ Pharm Sci. 1993; 1(2): 95-101.
7. Sharma D., Soni M., Solubility Enhancement Eminent Role in Poorly Soluble Drugs. Research J. Pharm. and Tech. April.-June. 2009; 2(2):807-823.
8. Amidon, Lennernas, Shah C., Pharm. Res. 1995; 12: 413-420.
9. Rita L., Current trends in β-cd based drug delivery system ijrap. 2011; 2(5): 1520-1526.
10. Szejtli J. Highly soluble cyclodextrin derivatives: chemistry, properties and trends in development. Adv Drug Deliv Rev.1999; 36(1): 17-28.
11. Szejtli J. in: Cyclodextrins and Their Industrial Uses, Ed. D. Duchtne, Editions de Santk, Paris, 1987; 173
12. Szetjli J. Introduction and general overview of cyclodextrin chemistry. Chem Rev. 1998; 98:1743-53.
13. Villiers., Eastburn S.D., Tao B.Y. Applications of modified Cyclodextrins. Biotechnol Adv. 1994; 12(2): 325-39.
14. Dass C.R., Jessup W. ApolipoprotiensA-I. Cyclodextrins and liposomes as potential drugs for the reversal of atherosclerosis. J Pharm Pharmacol. 2000; 52: 731-61.
15. Fujiwara T., Miyazawa T., Kobayeshi N. S. Soc. Chem. Lett. 1990; 739-742.
16. Higuchi T. & Connors K. A. (1965). Phase solubility techniques. In C. N.Reilley (Ed.), Advances in analytical chemistry and instrumentation Wiley-Interscience, New York. Journal of Psychiatry & Neuroscience. 2008; 4: 117-212
17. Huang C.Y. Determination of Binding Stoichiometry by the Continuous Variation Method: The Job Plot. Methods in Enzymology. 1982; 87: 509-525.
18. Job P. Ann. Chim. 1928; 9: 113-203.
19. Uekama K., Hirayama F., Irie T. Cyclodextrin drug carrier systems. Chem Rev. 1998; 98. (5): 2045-2076.
20. Thompson D.O. Cyclodextrins-enabling excipients: their present and future use in pharmaceuticals. Crit Rev Ther Drug Carrier Syst. 1997; 14: 1-104.
21. Brewster M.E., Verreck G., Chun I., Rosenblatt J., Mensch J., Van Dijck A., Noppe M., Arien A., Bruining M., Pewters J. The use of polymer-based electrospunnanofibers containing amorphous drug dispersions in the delivery of poorly water-soluble pharmaceuticals. Pharmazie. 2004; 59(5): 387-391.
22. Singh H., Tiwari A., Jain S. Preparation and In vitro- In vivo characterization of elastic liposomes encapsulating cyclodextrin –colchicine complexes for topical delivery of colchicines. The Pharmaceutical Society of Japan. 2010; 130(3):397-407.
23. Hedges R.A. Industrial applications of Cyclodextrins. Chem Rev. 1998; 98(5): 2035-44.
24. Prasad N., Strauss D. Reichart G. Cyclodextrins inclusion for food, cosmetics and pharmaceuticals. European Patent. 1,084,625 (1999).
25. Ekberg B., Anderson L., Mosbach, K. The synthesis of an active derivative of cyclomalto hexose for the hydrolysis of esters and the formation of amide bonds. Carbohydr Res. 1989; 192: 111- 7.