Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Comparative Study of Different Solubility Enhancement Techniques and Various Excipients on Solubility and Dissolution Rate of Racecadotril

VRUSHALI TAMBE*, PRIYANKA BAGADE, S.K. SHRIVASTAVA

Progressive Education Society's Modern College of Pharmacy [for ladies] Moshi, Pune-412105, Maharashtra

ARTICLE DETAILS

Article history:
Received on 31 January 2014
Modified on 22 February 2014
Accepted on 24 February 2014

Keywords:
Racecadotril,
Solubility,
β-CD,
Solvent evaporation,
Fusion

ABSTRACT

The present investigation envisages effect of various excipients and methods on solubility of Racecadotril which is used as an antidiarrhoeal. Solubility of antidiarrhoeal is paramount important for rapid onset of action. Complexation of drug with Beta-cyclodextrin and Hydroxypropyl Beta-cyclodextrin complexes was done by various methods like physical mixing, kneading, lyophylisation, microwave-irradiation, solvent evaporation and fusion. Mannitol and PEG6000 were used for solid dispersion. To ascertain stability of complexes at various pH, the phase solubility studies were carried out at different pH. Complexes were evaluated by DSC, XRD, SEM and IR. A_L type profile was obtained with β-CD and HP-β –CD. The stability constants values are in the range of 100-1000 M^{-1}. β-CD showed greater solubility enhancement as compared to all excipients. Enhancement of dissolution rates with increasing quantity of β-CD in the complex was observed. Complex formation is confirmed using various analytical techniques which primarily may be due to hydrogen bond formation.

INTRODUCTION

Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown. Poorly water soluble drugs often require high dose in order to reach therapeutic plasma concentrations after oral administration [1]. Most of the drugs are weakly acidic and weakly basic with poor aqueous solubility. This creates the major problem in formulation development of new chemical entities. Various techniques used for the improvement of the solubility of poorly water-soluble drugs include micronization, chemical modification, pH adjustment, solid dispersion, complexation, co-solvency, micellar-solubilization, hydrotropy etc. [2].

Racecadotril (RAC), [+-]-N-[2-[[Acetylthio] methyl]-1-oxo-3-phenylpropyl] glycine phenylmethyl ester acetorphan [3] is an enkephalinase inhibitor which decreases water/electrolyte secretion in intestinal lumen. As it is used as antidiarrhoeal, it should have fast onset of action. But due to its low solubility, development of optimised dosage form is challenging. Thus there is need to increase its dissolution at site of absorption.

Hence study was undertaken with the aim of increasing solubility of RAC with the use of Beta-cyclodextrin (β-CD), Hydroxypropyl Beta-cyclodextrin (HP-β-CD), Mannitol and PEG6000 by using different methods and to compare performance of excipients and methods.

*Author for Correspondence:
Email: vrushali.tambe@rediffmail.com

MATERIALS AND METHODS

Materials: Racecadotril was a gift from Symed lab Hydrabad, India.β-CD and HP- βCD were obtained as gift from Emcure pharmaceutical Hinjewadi, Pune, Maharastra. PEG 6000, Mannitol were purchased from Loba chemi. Chemicals used for HPLC analysis were of HPLC grade.

Phase solubility study of RAC with β-CD:

Phase solubility studies were performed by the method reported by Higuchi and Connors [4,5]. Excess amounts of RAC was added to 20 ml of distilled water, phosphate buffer pH 7.4, pH 5 and 0.1 N HCl containing various concentrations of β-CD and HP-β-CD. The drug to excipient ratios were 1:1, 1:2, 1:4, 1:6, 1:12 and 1:18. The suspensions were vigorously shaken for 48 hrs on rotary shaker. After equilibrium was attained,

the samples were filtered through a whattman filter paper [#41]. RAC concentration was determined using UV/VIS double beam spectrophotometer at 233nm [Shimadzu -1800 model]. Phase solubility constants in different solvent systems and phase solubility relationship were studied. The stability constants in different pH conditions were determined using the formula:

$$K_{1:1} = \frac{\text{Slope}}{S_0 [1- \text{Slope}]}$$

Where, Slope is obtained by plotting the moles of cyclodextrins concentration Vs moles of Racecadotril, S_0 is the equilibrium solubility of the drug in the absence of the cyclodextrins.

Comparison of Performance of Various Carriers:

Inclusion complex by solvent –evaporation method: [6]

For the preparation of co-evaporated product, aqueous solutions of β-CD and HP-β-CD were added to a methanolic solution of RAC in 1:2 drug-carrier ratios. The resulting mixtures were triturated for 45min. Mixtures were dried at 45-50°C, sieved and stored in desiccators for the further evaluation and characterization.

Solid dispersion by solvent –evaporation: [6]

Aqueous solutions of Mannitol and PEG 6000 were added to methanolic solutions of RAC in 1:2, drug: carrier ratio. The resulting mixtures were triturated for 45min. and dried.

Evaluation of Complexes:

In-vitro dissolution studies: [7]

Dissolution studies were conducted using a USP II paddle method (50 rpm, 37°C, and 900 ml dissolution medium) with a TDT-06T dissolution tester (Electrolab Pvt. Ltd, India). The RAC and co-evaporated products containing 100 mg RAC were tested in 0.1 N HCl. 5ml aliquots were withdrawn from the dissolution medium at predetermined intervals and replaced with 5 ml of dissolution medium. The drug concentration was determined using UV-visible double beam Spectrophotometer at 233 nm.

Preparation and evaluation of complexes prepared by various methods:

Various complexes containing β-CD were prepared by Physical mixture, Kneading, Solvent evaporation, Microwave, Lyophilization and Fusion method.

Physical mixture [PM]: [8]

The physical mixture of RAC: β-CD was prepared by mixing RAC with β-CD in 1:1 ratio in mortar for about 45min with constant trituration and passed through 100 # mesh.

Kneading method [KN]: [9]

B-CD was dissolved in water. RAC was added and the mixture was kneaded for 45 min and kept in hot air oven at 45-50 °C for 1day. The formed dry mass was passed through sieve no 100#.

Solvent evaporation method [SE]: [6, 10]

Complex was prepared using the procedure mentioned above.

Microwave irradiation method [MW]: [11]

An aqueous solution of β-CD was prepared by varying volume of water (15ml, 25ml, 35ml, 45ml and 55ml) to study effect of water level on dissolution rate. This solution was added to a methanolic solution of RAC. The resulting mixtures were triturated for 45 minutes. Mixtures were kept in micro wave oven (Catalyst system) at 140 watt for 25- 45 min. Dried mass was sieved.

Lyophilization method [LYP]: [12-14]

RAC and β-CD in 1:1 ratio were dispersed in water. Resulting mixture was stirred for 24hr on magnetic stirrer. The formed solution was frozen for 3-4 days and lyophilized (Alpha- 1-2 LD plus) over a period of 24 hr using a freeze drier. The resulting dry mixture was sieved using # 100.

Fusion method [FU]: [15]

1gm of RAC was melted in petridish on oil bath and to this molten mass 1gm of β-CD was added. Complex was kept in oil bath for 15-20 min with continuous stirring with glass rod. It was cooled in ice bath with continuous stirring. The formed mass was sieved.

Evaluation of complexes:

In-vitro dissolution studies: Dissolution studies of complexes were carried out by same procedure as mentioned above. The complex equivalent to 100 mg of Racecadotril was taken for dissolution study.

Study of concentration of β-CD on dissolution rate of complexes prepared by solvent evaporation and fusion method

Preparation of complexes:

From results obtained by dissolution testing two methods viz. solvent evaporation and fusion

method were selected as appropriate methods for solubility enhancement of RAC. The complexes were prepared in 1:1, 1:1.5 and 1:2 RAC to β-CD molar ratios.

Evaluation of Complexes:

In-vitro dissolution studies:

Dissolution studies were conducted by weighing complex equivalent to 100 mg of RAC and applying same procedure as mentioned previously.

% Practical yield: [16]

Percentage practical yield of each method was calculated using following formula.

$$PY = \frac{\text{Practical mass (complex)}}{\text{Theoretical mass (drug + carrier)}} \times 100$$

Assay of Complexes containing RAC using UV-Visible Spectroscopy and HPLC: [17]

1. UV Visible Spectroscopy: An accurately weighed complex equivalent to 2.5 mg of RAC was mixed and sonicated with methanol for 5 min. Volume was made up to 25ml with distilled water. The filtered solution was diluted with distilled water to get drug concentration of 10µg/ml and was analysed at 233 nm.

2. HPLC: The complex equivalent of 2.5 mg of RAC was weighed and the volume was made up to 25ml with HPLC grade methanol. Solution was filtered through whattman filtered paper and diluted further with HPLC grade methanol. 40µg/ml dilution was injected in HPLC system (Cyberlab). C18 (Neosphere, 4.6×250 mm) column was used with methanol as mobile phase at flow rate of 1ml/min. Detection wavelength was 209 nm.

Drug-Excipient interaction study:

1. **TLC analysis** [18]
   TLC was used to confirm that during a complexation process drug has not undergone any chemical changes or degradation. Commercially available TLCplates were used for analysis. The solvent system consists of iso-propyl alcohol to Hexane (7:3 ratio). The developed chromatograms were observed in UV-chamber. The retention factor (R_F) of each sample was compared with standard.

2. **FTIR analysis** [19,20]
   The spectrum of RAC and complexes were recorded using an FTIR– spectrophotometer.

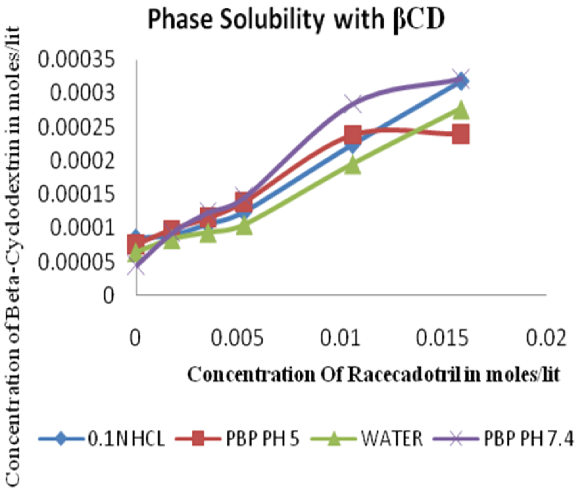
3. **Differential scanning calorimetric Analysis [DSC]** [19,20]
   The Drug and complexes were placed into in aluminum cup and sealed and heated 10°C/min at 50-350°C in nitrogen atmosphere (Shimadzu).

4. **Scanning Electron Microscopy [SEM]** [21]
   Surface morphology of RAC, β-CD and complex were determined by Scanning Electron Microscopy (Juol-Jsm 6360A apparatus).

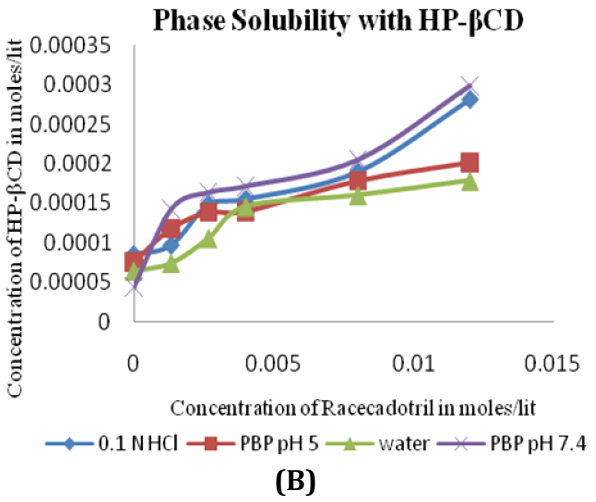
5. **Powder X- Ray diffraction studies- [PXRD] [19,20]**
   The powder X-ray diffraction patterns for RAC, β-CD and complexes were obtained using D8 advanced model of Brukar Axs Company and % Crystallinity was determined.

RESULTS AND DISCUSSION

In phase solubility analysis of RAC, A_L type of curves was obtained with cyclodextrins as shown in Fig1. Stability constants of complexes in various medium are given in Table 1.It suggests formation of inclusion complexes with 1:1 stoichiometry. Change in the pH of medium leads to change in stability constant of complex. All constants were in the range of ideal value i.e. 100 – 1000 M^{-1} indicating stability of complexes at wide pH ranges. RAC also exhibit pH dependent solubility.



(A)



(B)

Figure 1: Phase solubility studies of RAC [A]-withβ-CD, [B] - with HP-βCD

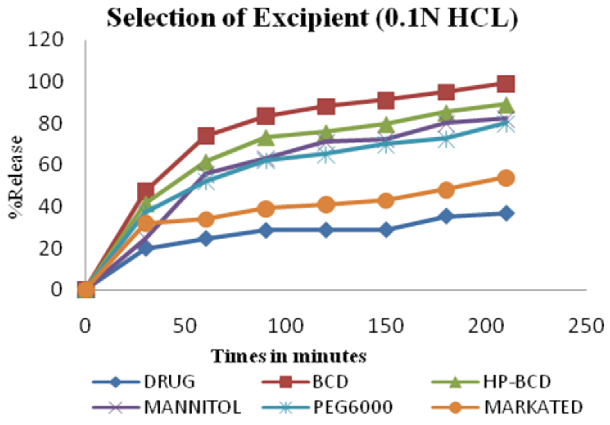


Figure 2: *In vitro* drug release profile for selection of carrier by solvent evaporation method in 0.1N HCL.

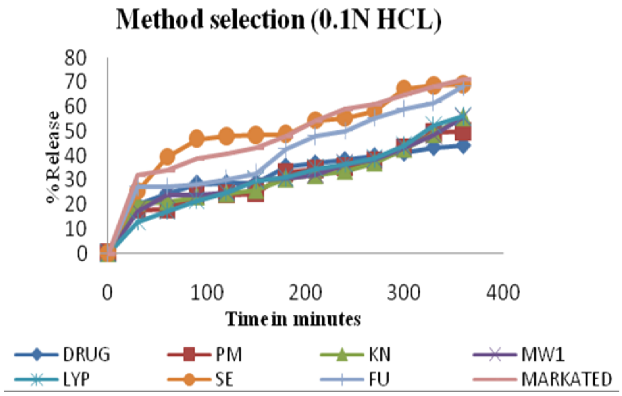


Figure 3: *In vitro* drug release profile for selecting appropriate method in 0.1N HCL

Table 1: Stability constants of complexes

Medium	B-CD	HP-β-CD
0.1N HCL	238.85M^{-1}	128.88M^{-1}
Phosphate buffer pH 5	134.26M^{-1}	120.72 M^{-1}
Water	204.14 M^{-1}	140.75M^{-1}
Phosphate buffer pH 7.4	368.74M^{-1}	415.60M^{-1}

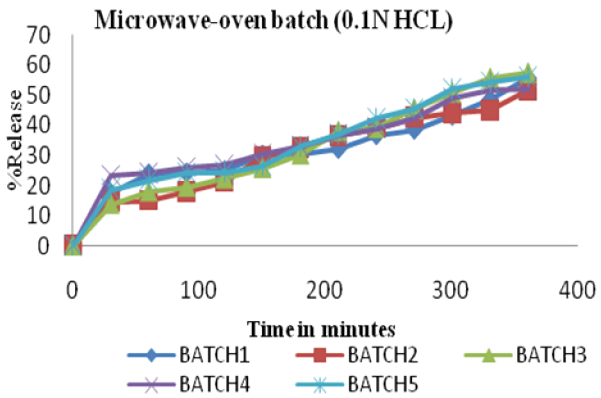


Figure 4: *In vitro* drug release profile for microwave irradiation batches in 0.1N HCL.

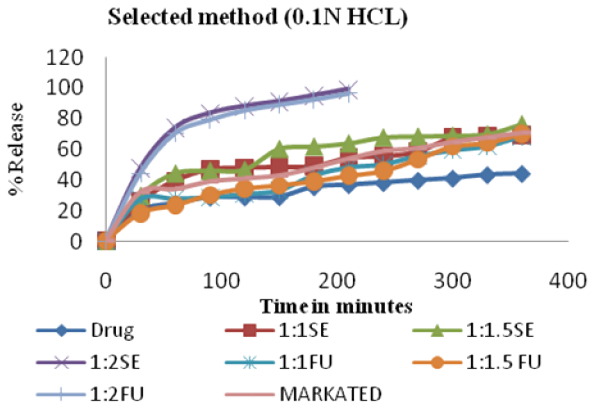


Figure 5: *In vitro* drug release profile for complexes prepared by solvent evaporation and fusion method in 0.1N HCL.

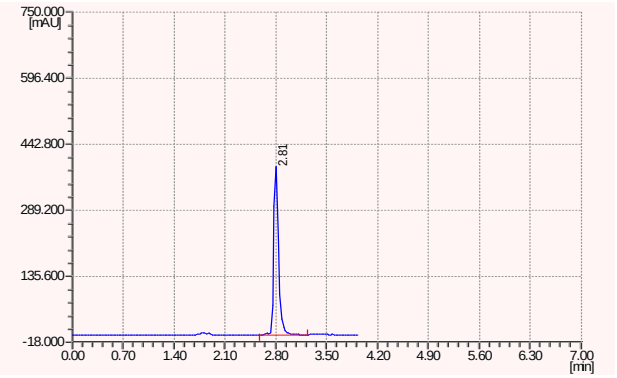
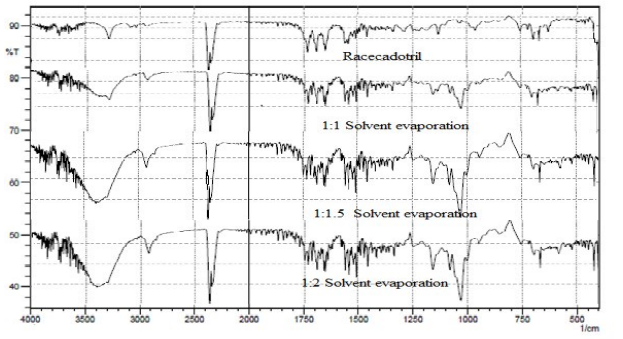
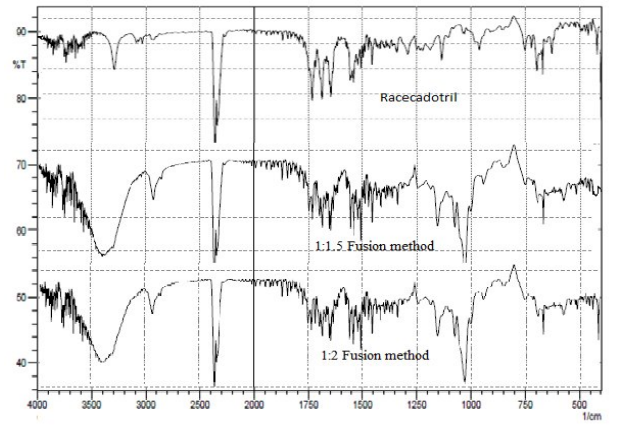


Figure 6: HPLC Chromatogram of RAC

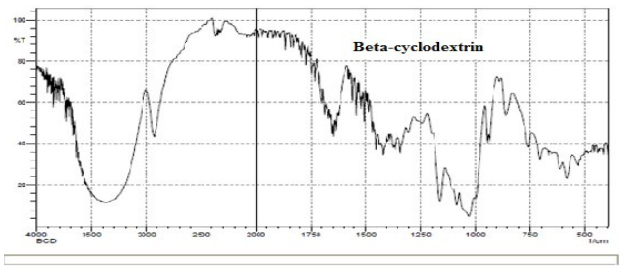
Figure 7: Photograph of TLC plates after spot development



(A)



(B)



(C)

Figure 8: FTIR spectra of RAC with complexes prepared by [A] - Solvent evaporation method [B] - Fusion method and [C] - β-CD

In vitro dissolution profiles of complexes prepared by solvent evaporation method using β-CD, HP-β-CD, Mannitol and PEG 6000 in 1:2 ratios of drug to excipient are shown in Fig 2. Complex prepared using β-CD had highest % drug release. Therefore the β-CD was selected as appropriate carrier for solubility enhancement of RAC.

Dissolution profiles of RAC to β-CD (1:1) complexes prepared by various methods [Physical mixing, Kneading, Microwave-irradiation, Lyophilization, Solvent evaporation and Fusion] arepresented in Fig 3. Dissolution rate was faster for complexes prepared by solvent evaporation and fusion method. This might be due to more intimate association of RAC with β-CD. It was concluded that the solvent evaporation and fusion method were better for solubility enhancement of RAC.

In Microwave irradiation method, results obtained by varying level of water for dissolution of β-CD on dissolution rate of RAC are shown in Fig 4.Dissolution studies of various batches prepared by varying water level have shown that there was no significant effect of water level on dissolution profiles.

Dissolution data of batches prepared with increasing concentration of β-CD by the Solvent evaporation and Fusion method is shown in Fig 5. There was a linear increase of RAC solubility as a function of cyclodextrin concentration. It was found that 1:2 Complex prepared by Solvent evaporation and Fusion method showed faster release as compared to 1:1 and 1:1.5 complexes.

Comparative data of % Practical yield, Assay and drying time analysis is shown in Table 2.

The drying time for complexes prepared by the Microwave-irradiation method was significantly lesser as compared to all other methods.

Chromatogram obtained for assay of formulations is shown in Fig 6. The $R_{F\ values}$ obtained by TLC analysis of RAC and the complexes are mentioned in Table no. 3 and the developed chromatograms are shown in Fig 7.The retention time of RAC was found to be 2.81 minutes.

The $R_{F\ values}$ RAC and complexes are in the same range and no additional spots were developed after development of chromatogram. This indicates that the drug remains intact during the complexation.

The FTIR spectra of RAC as shown in Fig 8 displayed peaks at 3300cm^{-1} [N-H stretch]. Only

N-H stretching peak was broadened which might be due to hydrogen bond formation between RAC amine [-NH-] and primary and secondary alcoholic [-OH] group of β-CD. This results into complex formation leading to solubility enhancement.

DSC thermograms of RAC, β-CD and complexes prepared by Solvent evaporation and complexation method are shown in Fig 9. RAC has shown one endothermic peak at83.64^{0}C indicating melting point of drug. In the DSC curve of pure β-CD, the peak corresponding to the evaporation of water appeared in the temperature range of 50-150^{0}C. The thermogram also displayed melting endotherm with sholder which indicated the presence of more than one crystal form. Decrease in edothermic peak intensity in thermogram of complexes indicates that RAC got converted from crystalline to amorphous form. RAC may have penetrated into the β-CD cavity replacing the water molecule leading to enhancement in solubility. 1:2 complex showed significant decrese in the intensity of endothermic peak.

The surface morphology as determined by scanning electron microscopy of the RAC, β-CD and complex is shown in Fig 10. RAC existed as needle shape crystals as observed in SEM analysis**,** whereas β-CD consists of irregular size crystals. In complex, original morphology of both component disappeared and tiny particles of regular size were present. The reduced particle size increased the surface area and close contact between the hydrophilic carriers and RAC might be responsible for solubility enhancement. There was also significant improvement in the flow property of RAC.

XRDs of RAC, β-CD and complexes prepared with increasing concentration of β-CD by Solvent evaporation method are presented in Fig 10. % Crystanallity data is presented in Table 4. RAC was found to be crystalline indicated by numerous sharp peaks in XRD. Comparison of XRDs of complexes in different ratio prepared by solvent evaporation and fusion method has shown decreased percent crystallinity with increase in β-CD concentration. 1:2 complex was more amorphous. % Crystanallity was found to decrease in order 1:1β-CD < (change sign) 1:1.5β-CD < 1:2β-CD (SE) < 1:2β-CD(FU) indicated that the complex gets converted in the amorphous form as the β-CD concentration increases. Fusion method resulted in more reduction in % crystanallity. .

CONCLUSION

The solubility of RAC was significantly improved by Solvent evaporation and Fusion method. Complexation with β-CD showed highest dissolution rate as compare to HP-β-CD, Mannitol and PEG 6000. Results obtained from DSC, PXRD confirmed the conversion of crystalline to amorphous form of RAC which results in solubility enhancement of drug. FTIR and TLC results showed no degradation of RAC after complexation Significant decrease in drying time was obtained by microwave-irradiation method.

ACKNOWLEDGEMENT

The author is grateful to Symed Labs, Hyderabad and Emcure, Pune for providing assistance in the form of drug and excipients.

REFERENCES

[1] Vimula VR. Solubility Enhancement Techniques Int J Pharm Sci Res. 2010: 5, 41-51.

[2] Gupta A. Bioavailability Enhancement of poorly water soluble drug: A Review Int J Pharm Life Sci. 2011: 2, 640 – 645.

[3] Singh N, Narayan. Racecadotril: a novel anti-diarrheal. MJAFI. 2008: 64, 361-362.

[4] Namelia N, Maria M. Phase solubility studies of the inclusion complexes of Repaglinide with β-Cyclodextrin and β-cyclodextrin derivatives. FARMACIA. 2010: 58,620- 28.

[5] Moriwaki C, Costa GL. Enhancement of solubility of Albendazole by complexation with β-Cyclodextrin. Brazilian J Chemo Eng. 2008: 25, 255-267.

[6] Senthilnathan B. Solubility and Dissolution Enhancement profile of Telmisartan using various techniques. Int J Pharm Tech Res. 2011: 3, 1738.

[7] Rao A, Shivaligam RM, Rao S. Formulation and evaluation of aceclofenac solid dispersions for dissolution test enhancement. Int J Pharm Sci Drug Res. 2010: 2, 146-150.

[8] Sachan KN, Pushakar S. Enhancement of solubility of acyclovir by solid dispersion and inclusion complexation method. World Appl Sci J. 2010: 7, 857-864.

[9] Akabari BV, Valaki BP, Vidyasagar G. Enhancement of solubility and dissolution rate of Rosuvastain calcium, Int J Pharm Bio Arch. 2011: 1, 511-520.

[10] Dhat A.P. Solubility Enhancement of Setrinidazole using Solid dispersion

technique. Int J Res. In Pharm. and bio medical Sci. 2011: 2, 1135.

[11] Moneghini M, Gingone. G. Influence of microwave technologies on physical chemical properties of solid dispersion with nimisulide. Power Tech. 2009: 195, 259-263.

[12] Bansal KA, Bachati A. Excipient used in lyophilization of small molecules. J. Excipients and Food Chem.2010: 1, 41-50.

[13] Haugh GM, Murphy MC. Novel freeze drying methods to produce a range of collagen glycosaminoglycan scaffolds with tailored mean pore sizes, Royal college of surgenose in Ireland publications. 2010:16, 887-893.

[14] Khattab SI, Bandarkar FS. Lyophilized glicalazide – polaxmer solid dispersions for the enhancement of in vitro dissolution and in vivo bioavability. Int J Pharm Sci .2011: 3, 122-127.

[15] Gupta GD, Kumar S. Solubility enhancement – Emine role in poor soluble drug. Res J Pharma Tech. 2009: 2,220-223.

[16] Patanker D, Bhise S. Solubility of antihypertensive agent by solid dispersion technique, Int J Pharm Life Sci. 2011: 2, 970-975.

[17] Youngi Z, Wen J. Preparation and physicochemical properties of complex of Niriginin with Hydroxypropyl-beta-cyclodextrin, mdpi . J Molecules. 2010: 15, 4402-4417.

[18] Srinivas KK, Sakhitvel PA. High performance thin layer chromatographic analysis of Racecadotril in the bulk of drug. J.P.C- modern TLC. 2009: 22, 277-281.

[19] Deshmukh DB, Gaikwad VH, Pawar SP. Dissolution Enhancement of poorly water soluble diacerin by solid dispersion technique. J Pharma Sci Res. 2010: 2, 234-739.

[20] Dehghan MH, Hanwate RM. Comparative dissolution study of Glipizide by solid dispersion technique, J Pharm Sci Tech. 2010: 2, 293-296.

[21] Sachan KN, Pushakar S. Enhancement of solubility of acyclovir by solid dispersion and inclusion complexation method, World Applied Sci J. 2010: 7, 857-864.