INCLUSION COMPLEX SYSTEM; A NOVEL TECHNIQUE TO IMPROVE THE SOLUBILITY AND BIOAVAILABILITY OF POORLY SOLUBLE DRUGS: A REVIEW

Patil J.S.*, Kadam D.V., Marapur S.C., Kamalapur M.V.
Department of Pharmaceutics, B.L.D.E.A's, College of Pharmacy, BLDE university campus,
Bijapur-586 103, Karnataka (India)
***Email:** pharmajspatil@gmail.com

ABSTRACT

Orally administered drugs completely absorb only when they show fair solubility in gastric medium and such drugs shows good bioavailability. The solubility and dissolution properties of drugs play an important role in the process of formulation development. Problem of solubility is a major challenge for formulation scientist which can be solved by different technological approaches during the pharmaceutical product development work. Solid dispersion, solvent deposition, micronization are some vital approaches routinely employed to enhance the solubility of poorly water soluble drugs. Each approach suffers with some limitations and advantages. Among all, complexation technique has been employed more precisely to improve the aqueous solubility, dissolution rate, and bioavailability of poorly water soluble drugs. Cyclodextrins, the unique cyclic carbohydrates are successfully utilized as the potential complexing agents which form inclusion complex with insoluble drugs. A comprehensive literature survey was made collect the rightful utilization of cyclodextrins as complexing agents and permeation enhancers. Various techniques have been investigated to explain the methods for preparation of inclusion complexes. In the present review, an attempt was made to discuss various complexation techniques and tried to briefly highlight the potential applications, technical and economical limitations associated with these approaches.

Keywords: Inclusion complexes, Cyclodextrins, Aqueous solubility, Dissolution rate, Bioavailability.

INTRODUCTION

Recent high through put screening, associated with combinatorial chemistry and parallel synthesis are continuously increasing the number of lipophilic drug molecules, which are difficult to deliver due to bioavailability problems[1]. Formulation of such a difficult molecules are being tried to improve their solubility by physical modification. For such physical modifications, various excipients such as cyclodextrins, carbohydrates, hydro tropes, polyglycolized glycerides, and dendrimers are utilized. Nearly one-third of drugs in development are water insoluble and one-half in trials because of under privileged pharmacokinetics[2]. These poorly water soluble drugs are allied with slow drug absorption leading to inadequate and variable bioavailability and gastrointestinal mucosal toxicity[3].Therefore, most of new chemical entities under development these days are intended to be used are solid dosage form that originate an effective and reproducible *in-vivo* plasma concentration after oral administration[4,5].

As the oral drug delivery is the simplest and easiest way of drug administration, because of the greater stability, lesser bulk, accurate dosage, cheaper cost of production and easy process, solid oral dosage forms have advantages over other dosage forms[6,7].Infact, all the poorly water soluble drugs after oral administrations are not well absorbed[8]. And thus leads to decreased inherent efficiency of drugs[9-11]. Therefore, the improvement of drug solubility thereby its oral bio-availability remains one of most challenging aspects of drug development process especially for oral drug delivery system.

There are numerous approaches available and reported in literature to enhance the solubility of poorly water soluble drug. The techniques are chosen on the basis of certain aspects such as properties of drug under consideration, nature of excipients to be selected and nature of intended dosage form. Among these approaches salt formation, solubulization, particle size reduction, solid dispersion, and solvent deposition technique are most frequently used. But, there are practical limitations of these techniques[12].

Cyclodextrins (CDs) are belongs to the category of carbohydrates and are cyclic oligosaccharides discovered just over 100 years ago. They are called "Cellulosine", when first discovered by A.Villiers in 1891. F. Schardinger identified the three naturally occurring Cyclodextrins α, β and γ. And these were referred to as "Schardinger Sugars". For 25 years, between 1911 and 1935, Pringsheim in Germany was the leading researcher in this area, demonstrated that CDs formed stable aqueous complexes with many other chemicals. CDs are produced from starch by means of enzymatic conversion. Over the last few years, an application of CDs is expanded into food, pharmaceutical, chemical, agricultural, and environmental engineering fields. Due to specific structure and the orientation of the hydroxyl groups made the CDs capable of solubylizate in aqueous medium and to encapsulate the lipophilic molecules into their interior cavity[13-15].

This review is intended to discuss the detailed information on use of cyclodextrins (CDs) as complexing agents, the various technologies adopted to prepare the inclusion complexes of poorly water soluble drugs with CDs, and an attempt was also made to highlights the potential applications, technical and economical limitations associated with these approaches.

APPROACHES FOR MAKING OF INCLUSION COMPLEXES

1. Physical blending method

A solid physical mixture of drug and CDs are prepared simply by mechanical trituration. In laboratory scale CDs and drug are mixed together thoroughly by trituration in a mortar and passes through appropriate sieve to get the desired particle size in the final product. In industry scale, the preparation of physical mixtures is based on extensive blending of the drug with CDs in a rapid mass granulator usually for 30 minutes. These powdered physical mixtures are then stored in the room at controlled temperatures and humidity conditions.

2. Kneading method

This method is based on impregnating the CDs with little amount of water or hydroalcoholic solutions to converted into a paste. The drug is then added to the above paste and kneaded for a specified time. The kneaded mixture is then dried and passed through sieve if required[16]. Parik et al.[17] have reported the dissolution enhancement of nimesulide using complexation method. In laboratory scale kneading can be achieved by using a morter and pestle[18-20]. In large scale the kneading can be done by utilizing the extruders and other machines. This is the most common and simple method used to prepare the inclusion complexes and it presents very low cost of production.

3. Co-precipitation technique

This method involves the co-precipitation of drug and CDs in a complex. In this method, required amount of drug is added to the solution of CDs. The system is kept under magnetic agitation with controlled process parameters and the content is protected from the light. The formed precipitate is separated by vacuum filtration and dried at room temperature in order to avoid the loss of the structure water from the inclusion complex. Moyano et al.[21] have studied the solid-state characterization and dissolution characteristics of gliclazide-bete-cyclodextrin inclusion complexes. This technique leaves a drug-CD solution in very close conditions to the saturation and through abrupt changes of temperature with addition of organic solvents. It is obtained to the precipitation of the material forming inclusion complex. The powders are obtained by rotation or filtration with heat while stirring the solution[22]. However, due to low yield, risk of using organic solvents, and longer time required for the preparation in larger scale, this method is attaining little attraction in the industrial scale[23].

4. Solution/solvent evaporation method

This method involves dissolving of the drug and CDs separately in to two mutually miscible solvents, mixing of both solutions to get molecular dispersion of drug and complexing agents and finally evaporating the solvent under vacuum to obtain solid powdered inclusion compound. Generally, the aqueous solution of CDs is simply added to the alcoholic solution of drugs. The resulting mixture is stirred for 24 hours and evaporated under vaccum at 45 ºc. The dried mass was pulverized and passed through a 60-mess sieve. This method is quite simple and economic both on laboratory and large scale production and is considered alternative to the spray drying technique.

5. Neutralization precipitation method

This method is based on the precipitation of inclusion compounds by neutralization technique and consists of dissolving the drug in alkaline solutions like sodium/ammonium hydroxide and mixing with an aqueous solution of CDs. The resultant clear solution is then neutralized under agitation using hydrochloric acid solution till reaching the equivalence point. A white precipitate is being formed at this moment, corresponding to the formation of the inclusion compound. This precipitate is filtered and dried. Doijad et al. [24] have studied the enhancement of solubility of piroxicam by complexation with beta-cyclodextrin. Acid and alkaline susceptible drugs can undergo degradation during this process is the limitation associated with this method.

6. Milling/Co-grinding technique

A solid binary inclusion compounds can be prepared by grinding and milling of the drug and CDs with the help of mechanical devices. Drug and CDs are mixed intimately and the physical mixture is introduced in an oscillatory mill and grinded for suitable time. Alternatively, the ball-milling process can also be utilized for preparation of the drug-CD binary system. The ball mill containing balls of varied size is operated at a specified speed for a predetermined time, and then it is unloaded, sieved through a 60-mesh sieve. This technique is superior to other approaches from economic as well as environmental stand point in that unlike similar methods it does not require any toxic organic solvents[25]. This method differs from the physical mixture method where simple blending is sufficient and in co-grinding it requires to achieve extensive combined attrition and impact effect on powder blend.

7. Atomization/Spray drying method

Spray-drying is a common technique used in pharmaceuticals to produce a dry powder from a liquid phase. Another application is its use as a preservation method, increasing the storage stability due to the water elimination[26]. This method represents one of the most employed methods to produce the inclusion complex strating from a solution. The mixture pass to a fast elimination system propitiate solvent and shows a high efficiency in forming complex. Besides, the product obtained by this method yield the particles in the controlled manner which in turn improves the dissolution rate of drug in complex form. Vozone et al.[27] have developed complexation of budesonide in cyclodextrins and particle aerodynamic characterization of the complex solid form for dry powder Inhalation.

The sufficient and efficient interaction between drug and CDs to form a perfect complex is the added advantage of atomization/spray drying method where as thermal stress and low yield of the final product are the limitations associated with this technique.

8. Lyophilization/ Freeze drying technique

In order to get a porous, amorphous powder with high degree of interaction between drug & CD, lyophilization/ freeze drying technique is considered as a suitable[28,29]. In this technique, the solvent system from the solution is eliminated through a primary freezing and subsequent drying of the solution containing both drug & CD at reduced pressure. Thermolabile substances can be successfully made into complex form by this method. The limitations of this technique are long time process and yield poor flowing powdered product. Lyophilization/ freeze drying technique is considered as an alternative to solvent evaporation and involve molecular mixing of drug and carrier in a common solvent[30].

9. **Microwave irradiation method**

This technique involves the microwave irradiation reaction between drug and complexing agent using a microwave oven. The drug and CD in definite molar ratio are dissolved in a mixture of water and organic solvent in a specified proportion into a round bottom flask. The mixture is reacted for short time of about one to two minutes at 60 ºc in the microwave oven. After the reaction completes, adequate amount of solvent mixture is added to the above reaction mixture to remove the residual, uncomplexed free drug and CD. The precipitate so obtained is separated using whatman filter paper, and dried in vaccum oven at 40 ºc for 48 hrs. Deshmukh et al.[31] have developed inclusion complexes of ziprasidone hydrochloride with beta-cyclodextrin and hydroxypropyl beta-cyclodextrin to design the fast dissolving formulation using various superdisintegrants. Microwave irradiation method is a novel method for industrial scale preparation due to its major advantage of shorter reaction time and higher yield of the product [32-34].

10. Supercritical antisolvent technique

This method has been introduced in the late 1980s[35]. Since the first experiences of Hannoy et al in 1879, a number of techniques have been developed & patented in the field of supercritical fluid-assisted particle design. In the supercritical fluid antisolvent technique, carbon dioxide is used as anti-solvent for the solute but as a solvent with respect to the organic solvent. The use of supercritical carbon dioxide is advantageous as its low critical temperature and pressure makes it attractive for processing heat-labile pharmaceuticals. It is also non-toxic, non-flammable, inexpensive and is much easier to remove from the polymeric materials when the process is complete, even through small amount of carbon dioxide remains trapped inside the polymer, it poses no danger to the consumer. Supercritical particle generation processes are new and efficient route for improving bioavailability of pharmaceutically active compounds[36]. In addition, supercritical fluid processes were recently proposed as a new alternative method for the preparation of drug-cyclodextrin complexes. Supercritical carbon dioxide is suggested as a new complexation medium due to its properties of improved mass transfer and increased solvating power[37-41]. This method constitutes one of the most innovators methods to prepare the inclusion complex of drug with CD in solid state. This is a non-toxic method as it is not utilizing any organic solvent, fast process, maintenance cost is low with promising results, but it requires a quite high initial cost. In this technique, first, drug and CD are dissolved in a good solvent then the solution is fed into a pressure vessel under supercritical conditions, through a nozzle (i.e. sprayed into supercritical fluid anti-solvent). When the solution is sprayed into supercritical fluid anti-solvent, the anti-solvent rapidly diffuses into that liquid solvent as the carrier liquid solvent counter diffuses into the anti-solvent. Because of the supercritical fluid expanded solvent has lower solvent power than the pure solvent, the mixture becomes supersaturated resulting in the precipitation of the solute and the solvent is carried away with the supercritical fluid flow[42,43].

Cyclodextrins as permeation enhancers

In spite, the solubility enhancement application, CDs can also be used as membrane permeability enhancer and stabilizing agents[44,45]. The permeability through biological membrane is enhanced by the presence of cyclodextrins. Masson[46] reported about the permeation enhancement property of poorly water soluble drugs in presence of the CDs. These acts as permeation enhancers by carrying the drug through the aqueous barrier which exists before the lipophilic surface of biological membranes[47]. This can also be achieved through the double characteristics of the CDs, thus present character much lipophilic as hydrophilic. CDs can also be used as nasal permeation enhancers acting by interaction with nasal epithelium by modifying tight junction & lipid and protein content of the membrane, which enhances the permeation of the membrane[48].CDs can also be utilized as permeation enhancer in pulmonary drug delivery systems.Rifampicin is a so- called concentration-dependent antibiotic, the rate and extent of bacterial kill is related to the attainment of high maximum concentration relative to the minimal inhibitory concentration. The rifampicin-CD inclusion compound can improve the lung transport of drug when nebulized with compatible pulmonary deposition and achieve required concentration of drug in broncho-alveolar epithelium lining-fluid when administered as aerosolized solution[49-52].

CONCLUSION

Among the emerging new chemical entities, most are poorly water soluble drugs putting impact on their bio-availability and therapeutic effect. The solubility enhancement techniques also play an important role in getting the excellent dissolution properties of poorly soluble drugs. Successful improvement in aqueous solubility is mainly depends on the method which we choose. Inclusion complex with cyclodextrins is the most attractive technique to enhance aqueous solubility of poorly soluble drugs. CDs, act as the useful solubilizer enabling both solid/liquid oral and parenteral dosage forms. Solid binary system of drug and CDs are capable to modify the physicochemical properties of drugs such as solubility, particle size, crystal habit, thermal behavior, and there by forming a highly water soluble amorphous forms. The CDs, due to their extreme high aqueous solubility, they became capable to enhance the dissolution

rate and bio-availability of the poorly soluble drugs. The permeation of insoluble drugs through various biological membranes can also be enhanced by preparing drug- CD inclusion compounds.

REFERENCES

1. Varma M, Khandavilli S, Ashokraj Y, Jain A, Dhanikula A, Sood A, Thomas N, Pillai O, Sharma P, Gandhi R, et al. Biopharmaceutics classification System; a scientific framework for Pharmacokinetic optimization in drug research. Curr. Drug Metab.5: 2004:375-86.
2. Lobenberg R, Amidon GL. Modern Bio-availability, Bio-equivalence and Biophamacuetics Classification System; New Scientific Approaches to International Regulatory Standards. Eur. J. Pharm. Biopharm.50:2000:3-12.
3. Rinaki E, Valsami G, and Macheras P. Quantitative Biopharmacuetics Classification System; the central role of dose/solubility ratio. Pharm. Res.20: 2003:1917.
4. Ikegami K, K Tagawa, T Osawa. Bioavailability and in vivo release behavior of controlled-release multiple-unit theophylline dosage forms in beagle dogs, cynomolgus monkeys, and göttingen minipigs. J. Pharm. Sci.95:2006:1888-95.
5. Adley AN, Lima, Jose LS, Sabcinho, Roberto AC, Correa JR, Pedro J. R. N. Alternative Technologies to Improve Solubility of Poorly Water Soluble Drugs. Am. J. Pharm.27(5): 2008:789-97.
6. Youn YS, JY Tung. Shoh, SD Yoo, K C Lee. Improved intestinal delivery of salmon calcitonin by Lys18-amine specific PEGylation: Stability, permeability, pharmacokinetic behavior and in vivo hypocalcemic efficacy. J. Contr. Rel.144:2006:334-42.
7. Sugawara M, S Kadomura, H Xin, Y. Takekuma, N Khori, K Miyazaki. The use of an in vitro dissolution and absorption system to evaluate oral absorption of two weak bases in pH-independent controlled-release formulations. Eur. J. Pharm. Sci.26:2005: 1-8.
8. Van Drooge, DJ, WLJ Hinrichs, MR Visser, HW Frijlink. Characterization of the molecular distribution of drugs in glassy solid dispersions at the nano-meter scale, using differential scanning calorimetry and gravimetric water vapour sorption techniques. Int. J. Pharm.301: 2006:220-29.
9. Vippagunta S R, Zaren W, Hornung S, Krill SL. Factors affecting the formation of eutectic solid dispersions and their dissolution behavior. J. Pharm. Sci.96: 2006:230- 294.
10. Ponton CW. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. Eur. J. Pharm. Sci.29:2006:278-87.
11. Bogdanova S, Pajera I, Nikolova P, Tsaskovska I. Interactions of Poly(vinylpyrrolidone) with Ibuprofen and Naproxen: Experimental and Modeling Studies Pharm. Res.22:2005:806-15.
12. Wadke, DA Serajuddin, ATM, Jacobson H. In: pharmaceutical dosage forms: Tablets, Vol 1; Lieberman H A; Lachman L; Schwartz JB, Eds; Marcel Dekkar: New Yark, 1989, pg 1-73.
13. Vekama K. Design and Evaluation of Cyclodextrin-Based Drug Formulation. Chem.Pharm.Bull.52: 2004:900-15.
14. Rajewski RA, Stella VJ. Pharmaceutical applications of cyclodextrins. 2. in vivo drug Delivery. J. Pharm. Sci.85:1996:1142-69.
15. Loftsson T, Brewster M E. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci.85:1996:1017-25.
16. Baboota S, Bhaliwal M, Kohli K. Physicochemical Characterization, *in-vitro* Dissolution Behaviour, and Pharmacodynamic Studies of Reficoxib-Cyclodextrin Inclusion Compounds. Prepration and Properties of Reficoxib hydroxypropyl β-Cyclodextrin Inclusion Complex: a technical note. AAPS Pharm. Sci. Tech. 6(1); Article 14: 2005;E 83-E 89.
17. Parikh RK, Mansuri NS, Gohel MC, Sonlwala MM. Dissolution enhancement of Nimesulide Using Complexation and Salt Formation Techniques. Indian Drugs.42:2005:149-53.
18. Fernandes CM, Veiga FJB. Effect of the Hydrophobic Nature of Triacetyl-β-cyclodextrin on the Complexation with Nicardipine Hydrochloride: Physicochemical and Dissolution Properties of the Kneaded and Spray-dried Complexes.Chem. Pharm. Bull.50(12):2002:1597-1602.
19. Cirri M, Rangoni C, Maestrelli F, Corti G, Mura P. Development of Fast-Dissolving Tablets of Flurbiprofen-Cyclodextrin Complexes. Drug Dev. Ind. Pharm.31:2005:697-707.
20. Cunha-Filho, MSS, Dacunha-Marinho B, Torres-Labandeira JJ, Martinez-Pacheco R, Landin M. Characterization of β-Lapachone and Methylated β-Cyclodextrin Solid-state Systems. AAPS Pharm Sci. Tech.8:2007:1-10.
21. Moyano JR, Blanco MJA, Gines JM, Giordano F.Solid State Characterization and Dissolution Characteristics of Gliclazide Beta-Cyclodextrin Inclusion Complexes. Int. J. Pharm.48:1997:211-17.
22. Miro A, Quagila F, Sorreutino R, D Emmanuele di VB, Varricchio G, La Rotonda MI. Proceedings of the International Symposium on Controlled release of Bioactive Materials:11-13:Control Rel. Society:Paris: July 2000: 1270-1.
23. Hedges AR. Industrial Applications of Cyclodextrins. Chem. Rev. 1998; 98:2035-44.

24. Doijad RC, Kanakal MM, Manvi IV. Studies on Piroxicam-beta-Cyclodextrin Inclusion Complexes. Indian Pharmacists.VI:2007: 94-98.

25. Friedrich H, Nada A, Bodmier R. Solid State and Dissolution Rate Characterization of Co-ground Mixtures of Nifedipine and Hydrophillic Carriers. Drug Dev. Ind. Pharm.31: 2005:719-28.

26. Broadhead J, Edmond RK, Rhodes CT. The spray drying of pharmaceuticals. Drug Dev. Ind. Pharm.18:1992:1169.

27. Vozone CM, Marques HMC. Complexation of Budesonide in Cyclodextrins and Particle Aerodynamic Characterization of the Complex Solid Form for Dry Powder Inhalation. J. Incl. Phenom. Macroc. Chem.44:2003:111-15.

28. Cao FT, Guo J, Ping Q. The Physicochemical Characteristics of Freeze-Dried Scutellarin-Cyclodextrin Tetracomponent Complexes. Drug Dev. Ind. Pharm.31:2005:747-56.

29. Rodriquez-Perez AI, Rodriquez-Tenreiro C, Alvarez-Lorenzo C, Concheiro A, Torres Labardeira J J. J. Nanosci. Nanotecnol.6: 2006:3179-86.

30. Tsinontides SC, Rajnaik P, Pham D, Hunke WA, Placek J, Reynolds SD. Freeze drying-Principles and Practice for Sucessful Scale up to Manufacturing. Int. J. Pharm.28(1): 2004:1-16.

31. Deshmukh SS, Potnis VV, Shelar DB, Mahaparale PR. Studies on Inclusion Complexes of Ziprasidone Hydrochloride with beta-cyclodextrin and Hydroxypropyl beta-cyclodextrin. Indian Drugs.44:2007:677-682.

32. Wen X, Tan F, Jing Z, Iiu Z. Prepration and study of the 1:2 Inclusion Complex of Carvedilol with β -Cyclodextrin. J. Pharm. Biomed. Anal.34:2004:517-523.

33. Shin-ichi Y, Katsuhiro I, Keiichi M, Hideo T, Akira O.Evaluation of ophthalmic suspensions using surface tension. Eur. J. Pharm. Biopharm.57:2004:377-382.

34. Saharan VA, Kukkar V, Kataria M, Gera M, Choudhary PK.Dissolution enhancement of drugs.Part I: Technologies and effect of carriers. Int. J. Health.Res.2(2):2009:107-124.

35. Muhrer G, Meier V, Fusaro F, Albano S, Mazzotti M. Use of Compressed Gas Precipitation to Enhance the Dissolution Behaviour of a Poorly Water-Soluble Drug: Generation of Drug Microparticles and Drug Polymer Solid Dispersions. Int. J. Pharm.308:2006:69-83.

36. J Fages, H Lochard, J-J Letourneau, M Sauceau, E Rodier. Phase Behavioral Effects on Particle Formation Processes Using Supercritical Fluids. Powder Technol.141:2004:219-26.

37. G S Tirucherai, A K Mitra. Effect of hydroxypropyl beta cyclodextrin complexation on aqueous solubility, stability, and corneal permeation of acyl ester prodrugs of ganciclovier. AAPS Pharm. Sci. Tech.4:2003: E45.

38. T Van Hees, G Piel, B Evrard, X Otte, L Thunus, L Delattre. Application of supercritical carbon dioxide for the preparation of piroxicam- β-cyclodextrin inclusion compound. Pharm. Res.16:1999: 1864-1870.

39. M Charoenchaitrakool, F Dehghani, N R Foster. Utilization of supercritical carbon dioxide for complex formation of ibuprofen and methyl- beta-cyclodextrin. Int. J Pharm.239: 2009:103-112.

40. N Bandi W Wei C B Roberts, L P Kotra, U B Kmpella. Preparation of budesonide and indomethacin- hydroxipropyl β-cyclodextrin (HPBCD) complexes using a single- step, organic solvent free supercritical fluid process. Eur. J Pharm Sci.24: 2004: 159-168.

41. E Rodier, H Lochard, M Sauceau, J-J Letourneau, B Freiss, J Frages. A three step supercritical process to improve the dissolution rate of Eflucimibe. Eur. J Pharm Sci.26: 2005;184-193.

42. Al-Marzouqui AH, Jobe B, Dowaidar AF, Maestrelli F, Mura P. Evaluation of supercritical fluid technology as preparative technique of benzocaine-cyclodextrin complexes-Comparison with conventional methods J. Pharm. Biomed. Anal.43:2007:566-74.

43. Vamsi KM, Gowrisankar D. Role of Supercritical fluids in the Pharmaceutical Research-A Review. Indian J. Pharm. Edu. Res.41(1): 2007:10-17.

44. 44. Loftsson T, Brewster ME. Pharmaceutical Applications of Cyclodextrin. Drug Solubilization and Stabilization. J. Pharm. Sci.85:1996:1024-74.

45. Loftsson T, Brewter ME. “Cyclodextrin as Pharmaceutical Excipients”. Pharma Manager.5:1997:22-31.

46. Masson, Thorstein, Loftsoon, Gisli Masson, Einar Stefansson. Cyclodextrins as Permeation Enhancers; Some Theoretical Evaluations and *in-vitro* Testing. J. Contr. Rel.59:1997:107-18.

47. Prakash Rao B, Sarasija S, Narendra C. Recent Advances in Cyclodextrin Complexes; Mechanistic Analysis as Permeation Enhancers. Indian J. Pharm. Edu. Res.41(2):2007:102-13.

48. Marttin E, Verhoef JC, Spies F, Van der Meulen J, Nagelkerke JF, Koerten HK, Merkus FWHM. The Effect of Methylated β -Cyclodextrins on the tight Junctions of the rat nasal Respiratory epithelium: Electron Microscopic and Confocal Laser Scanning Microscophic Visualization Studies. J. Contr. Rel.57:1994:205-13.

49. Gumbo T, Louie A, Deziel MR, Liu W,Parsons LM, Salfinger M, DrusanoGL.Concentration-dependent Mycobacterium tuberculosis killing and prevention of resistance by rifampicin, Antimicrob. Agents. Chemother.51(11):2007:3781-3788.

50. Louey MD, Garcia-Contreras L. Controlled release products for respiratory delivery. American Pharm. Rev.7(4):2004:82-87.

51. Fores B, Ehrhardt C. Human respiratory epithelial cell culture for drug delivery applications. Eur. J. Pharm. Biopharm.60(2):2005:193-205.

52. Frederic T, Julien B, William C, Jean-Christophe O. Formulation of rifampicin-cyclodextrin complexes for lung nebulization. J. Contr. Rel.129:2008:93-99.

****