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Review Article

Micro Particulate Drug Delivery System for Anti- Retroviral Drugs: A Review

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ABSTRACT

The sustained release of drugs in slow and controlled manner is one of the major challenges in drug delivery system. Recent drug discovery using advanced techniques such as combinatorial chemistry, genomics and silico three dimensional drug designs has yielded drug content with low water solubility and low mucosal permeability which makes the difficult development to the pharmaceutical formulations. To overcome these, particulate systems like microparticles have been used physically to improve the pharmacokinetic and pharmacodynamics properties of various types of drug molecules and targeting drug to the particular site. In this formulation different polymers are used to the antiretroviral microparticulate drug delivery research to increase therapeutic activity and minimizing side effects. The current aim of this review is to study various aspects of the antiretroviral microparticulate drug delivery system including method of formulation, evaluation and characterization.

Keywords: microparticles, antiretroviral therapeutic agent, evaluation, characterization.

INTRODUCTION

Each drug has characteristic 'minimum effective concentration' below which no therapeutic effect is observed and a characteristic 'minimum toxic concentration' above which undesired side effects occur. The range is called 'therapeutic range' or therapeutic window which could be narrow for most drugs [2]. The optimum effect of many medical treatments is obtained by maintaining the drug concentration in the therapeutic range over a sustained period of time. This is especially true for highly potent drugs, such as anti-viral drugs. By administration of the entire drug dose at once use, i.e., by conventional pharmaceutical dosage forms (e.g. Tablets, capsules, microspheres), the whole amount is rapidly released in to the stomach, absorbed in to the blood and distributed throughout the human body. As a result the rate at which the drug reaches the site of action, determines the therapeutic action. As no continuous drug supply is provided and as the human body eliminates the active agent, so the concentration decreased. The result is a fluctuating concentration of the drug levels in the plasma and the therapeutic range is maintained only for very short time periods. Various processes, such as diffusion, erosion and swelling are involved in the control of the overall drug release, which can be modified by novel drug delivery systems resulting in a prolonged release profiles.

Micro particulates are small solid particles within the size range of 1-1000µm[1]. Depending upon the method of preparation, the drug is dissolved, entrapped, and encapsulated to the micro particle matrix. Microspheres are systems in which the drug is surrounded by a polymer membrane. In recent years, biodegradable polymers used as a microparticulate drug delivery systems, to target a particular organ for sustained period of time.

Micro particles gives easy administration way to deliver macromolecules by various routes and effectively control the release of drugs over the periods ranging from few hours to months, because of effective protection of encapsulated drug against degradation. It is important part of novel drug delivery system as it is prepared for prolonged or controlled drug delivery and to improve the bioavailability of the drug and also to target the specific sites in the body[3-6]. Microspheres also have advantages like limiting the fluctuation within therapeutic range, reducing side effects, reducing dosing frequency and improving patient compliance [7,8].

Advantages

- Particle size and its effective surface area of micro particles helps to achieve the drug targeting to the specific site in the body.
- Micro particles will increase the therapeutic efficacy and reduce the side effects.
- It can be administered for various routes like, oral, parenteral, nasal, intra – ocular etc.
- Drug entrapment is high. Drugs can be incorporated in to the system without any chemical reaction.

Limitations

- The particles will be aggregated, because of small particle size and large surface charges.
- Physical handling of micro particles is difficult in liquid and dry forms.
- Small particle size and large surface area will cause the limited drug loading and burst release effect.

Polymer used in micro particle preparation-

Material: The coating material can be selected from a

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Table 1 List of polymers used for micro particle formation

Coating material	Solvent for coating material	Non-solvents
Acrylonitrite styrene	Methyl ethyl ketone	Polybutadiene
Benzyl cellulose	Trichloroethylene	Propanaol
Cellulose nitrate	Methyl ethyl ketone	Polybutadiene
Epoxy resin	Toluene	Polybutadiene
Ethyl cellulose	Methyl ethyl ketone	Polydimethyl siloxone
Natural rubber	Benzene	Methanol
Polyethylene	Xylene	Ethanol
Polymethyl methacrylate	Benzene	Polybutadiene siloxone
Polystyrene	Xylene	Petroleum ether
polyvinyl acetate	Chloroform	Isopropanol
Polyvinyl formaldehyde	Nitropropane	Polybutadiene
Styrene maleic acid	Ethanol	Isopropyl ether
Vinyl diene chloride acrylonitrite	methyl ethyl ketone	Polybutadiene

variety of natural and synthetic polymers depending on the core material to be encapsulated. The amount of coating material used ranges from 3% to 30% of the total weight, which corresponds to a dry film thickness of less than 1–200 μm, depending on the surface to be coated.

Natural or synthetic hydrophilic colloids:These are large molecules that are soluble or dispersible in aqueous solutions. Some examples of natural and synthetic hydrophilic colloids are agar acrylic polymers, polyacrylic acid, poly acryl methacrylate, gelatin, poly (lactic acid), pectin (poly glycolic acid), waxes (poly hydroxyl butyrate-co-valerate), cellulose derivatives, cellulose acetate phthalate, cellulose acetate Nitrate, Ethyl cellulose, Hydroxy ethyl cellulose, Hydroxypropylcellulose, Hydroxy propyl methyl cellulose, Hydroxypropylmethylcellulose phthalate, Methyl cellulose, Sodium carboxymethylcellulose, Poly (ortho esters) ,Polyurethanes, Poly (ethylene glycol), Poly (ethylene vinyl acetate), Polydimethylsiloxane, Poly (vinyl acetate phthalate), Polyvinyl alcohol, Polyvinyl pyrrollidone, shellac. Here the capsule wall presents a good barrier to oily and hydrophobic materials, but it is usually a poor barrier to hydrophilic substances. Hydrophobic colloids are realized in encapsulating water-soluble drugs. Soluble starch and its derivatives including Amylodextrin, Amylopectin and Carboxy methyl starch is used as wall forming material in solid microsphere preparation.

Biocompatible polymer: This includes poly (lactic) acid (PLA), poly (glycolic acid) (PLGA) etc. PLGA is a water-insoluble polymer, high strength and hydrophobicity. As a polymeric vehicle, biocompability, biodegradability, predictability of degradation, ease of fabrication, and regulatory approval are features that make PLGA desirable for medical applications. Some Natural polymers such as Albumin, Chitin, Starch, Collagen, Chitosan, Dextrin, Gelatin, Hyaluronic acid, Dextran, Fibrinogen, Alginic acid ,Casein, Fibrin, Poly (ortho esters), Polyalkylcyanoacrylate, Polyanhydrides are also used as biocompatible polymers. The bioavailability enhancers used are lysophatide, lysophosphatidyl choline. (Table 1)

Methods of microspheres preparations:

Emulsion – solvent evaporation method

Phase separation

Spray drying

Interfacial polymerization

Emulsion extraction method

Fluidizing and solvent precipitation method

1. Emulsion - Solvent evaporation method

1.1. Single emulsion method: This method involves oil-in-water (o/w) emulsification [1]. The o/w emulsion system consists of an organic phase of a volatile solvent with dissolved polymer in an aqueous phase containing a dissolved surfactant.

A surfactant is included in the aqueous phase to prevent the organic droplets from coalescing once they are formed. The polymer – drug solution is emulsified (with appropriate stirring and temperature conditions) to yield an o/w emulsion. The emulsion created by using a propeller or magnetic stirrer for mixing the organic and aqueous phases. Once the emulsion is formed, the solvent removed by either evaporation or extraction process to solidify the polymer droplets. One of the disadvantages of the o/w emulsification method is the poor encapsulation efficiency with water soluble drugs.

1.2 Double emulsion method: It has been usually applied for drugs not soluble in organic solvents. A solid-in -oil-in-water emulsion (s/o/w) method could be used to encapsulate a drug, provided its in the form of small size. Smaller crystals will be homogeneously distributed throughout the organic droplets created in emulsion, so hydrophilic drug has been used in this method for encapsulation. The problem with encapsulating hydrophilic drug is loss of drug to the external aqueous phase during the formation of the micro particle. To minimize these problems, the organic droplets should be solidified in to micro particles as quickly as possible following their formation.

Another alternative to encapsulate hydrophilic drugs is to employ the water-in-oil-in-water (w/o/w) emulsion method. An aqueous solution of the drug is added to an organic phase consisting of the polymer and organic solvent with vigorous stirring to form the first w/o emulsion. The emulsion is then dispersed in another aqueous phase containing more surfactant to form the w/o/w emulsion. A number of hydrophilic drugs like the peptide leuprolide acetate, luteinizing hormone, vaccines, protein/ peptides have been successfully encapsulated by this method.

The problem with this type of emulsion occurs, when the inner emulsion is not sufficiently stabilized, resulting in loss of aqueous droplets containing drug to the external aqueous phase.

2. Phase Separation

The method yields two liquid phases such as polymer containing coacervative phase and polymer containing supernatant phase [2]. The drug which is dispersed / dissolved in the polymer solution is coated by the coacervation. This method includes the following 3 steps.

The 1st step consists of formulation of three immiscible chemical phase. The core material is dispersed in polymer solution.

The 2nd steps consist of deposition of coating polymer absorbed on the liquid vehicle phase.

The final step comprising the rigidity of coating material by thermal, cross-linking or desolvation techniques to form micro particles.

This method is suitable to encapsulate both water-soluble drugs as well as water-insoluble drugs [10]. However, the coacervation method is mainly used to encapsulate water-soluble drugs like peptides, proteins and vaccines.

First non-solvent is added such that the polymer solvent is extracted slowly, allowing sufficient time for the polymer to deposit and coat evenly on the drug particle surface during the coacervation method. The concentration of the polymer used is important, because high concentration would result in rapid phase separation and non uniform coating of the drug particles. To rectify this problem the stirring rate and temperature can be adjusted. Dichloromethane, acetonitrile, ethyl cellulose and toluene have been used as non-solvents in this method. The non-solvents should not dissolve the polymer or the drug, and should be miscible with the polymer solvent, So that the non-solvent affects both aqueous separarion and coacervation process.

3. Spray drying: Spray drying is a widely used method in the pharmaceutical industry. The method typically use the drug being dissolved or suspended in a polymer solution (depending upon the polymer used either organic or aqueous solvent). The solution/ suspension is then fed in to the spray drying apparatus through the nozzle and polymer / drug solution is mixed with in the air and forced through the small diameter orifice and resultant droplets are very quickly dried by the evaporation of the micro particles.

4.Interfacial polymerization method: Interfacial polymerization is the one in which oil soluble and another one is water soluble drug, are employed and the polymer is formed on the droplet surface to formed micro particles.

5.Emulsion extraction method: The drug and polymer used in forming the micro particles are mixed with a suspension of proteins like agar, gelatin or albumin. One method is alginate plus ca^{+2} to produce the micro particles. Then the mixture is dispersed to produce desired sized particles.

If the drug is insoluble in gas and gas is soluble in liquid. The drug is dissolved in suitable solvents for polymeric solvents to form the micro particles.

Formulation considerations

1. Stabilizer: Stabilizer plays an important role in the formulation of microparticles. In the absence of an appropriate stabilizer, the high surface energy of micro-sized particles can induce agglomeration or aggregation of the drug crystals. The type and amount of stabilizer has a pronounced effect on the physical stability and in-vivo behavior of microparticles. In some cases, a mixture of stabilizers is required to make stable microparticles. The drug-to stabilizer ratio in the formulation may vary from 1:20 to 20:1. Stabilizers that have been explored include cellulosics, poloxamers, polysorbates, lecithins. Lecithin is the stabilizer of choice if one intends to develop a parenterally acceptable and autoclavable microparticles.

2. Organic solvents: Organic solvents may be required in the formulation of microparticles. As these techniques are still in their infancy, elaborate information on formulation considerations is not available. The acceptability of the organic solvents in the pharmaceutical area, their toxicity potential and the ease of their removal from the formulation need to be considered when formulating micro particles using emulsions or microemulsion as templates. The pharmaceutically acceptable and less hazardous water-miscible solvents, such as ethanol and isopropanol, and partially water miscible solvents, such as ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate and benzyl alcohol, are preferred in the formulation over the conventional hazardous solvents, such as dichloromethane. Additionally, partially water miscible organic solvents can be used for the microemulsion when the microparticles are to be produced using a microemulsion as a template.

3. Co-surfactants: The choice of co-surfactant is critical when using micro emulsion to formulate micro particles. Since co-surfactants can greatly influence phase behavior, the effect of co-surfactant on uptake of the internal phase for selected microemulsion composition and on drug loading should be investigated.The literature describes the use of bile salts and dipotassium glycerrhizinate as effective surfactants, and various solubilizers such as transcutol, glycofurol, ethanol and isopropanol can be safely used as co-surfactants in the formulation of microemulsions.

4. Other additives: Microparticles may contain additives such as buffers, salts, polyols, osmogent and cryoprotectant depending on either the route of administration or the properties of the drug moiety.

Evaluation of Microspheres

1. Particle shape and size determination: It can be done by microscopy, sieve analysis, laser light scattering, coulter counter method, photon correlation spectroscopy in which the former two are most common. In sieve analysis the micro particles are passed through the standard set of sieves ranging from 10-100 meshes and the amount retained on each sieve is weighed. In microscopy technique, the sample is loaded in the stage calibrated with eye piece micrometer at the dimensions of micro particles are measured.

- Size and surface morphology can be studied by freeze fracture microscopy and freezes etch electron microscopy.
- Micro particles size range can be effectively evaluated by laser diffractometer and light microscope.

2. Bulk and tap density: Porosity, specific area can also be evaluated by Mercury or Helium intrusion potensiometry. Flow properties of micro particles can be evaluated by determining the angle of repose by fixed funnel & free standing cone method & the compressibility index by tapped density method.

3.The thermal properties: The thermal properties are detected using Differential Scanning Calorimetry and Thermo gravimetric analysis.

3.1 In Differential scanning calorimetry or DSC: The amount of heat required for causing a change in sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. The temperature is gradually increased to find the changes in samples. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.

3.2 Thermo gravimetric analysis or thermal gravimetric Analysis (TGA) : The TGA detected on samples to determine changes in both weight and temperature. The analysis is carried out by raising the temperature gradually and against the temperature to plotting the weight (percentage). There are many testing methods of temperature routinely to reaches 1000^{o}C or greater. After the data, the smooth curve are obtained.

Electrostatic interaction: Is detected by rheological & FTIR assays (Fourier Transform Infra red spectroscopy) using potassium bromide pellet technique.

Drug loading and drug entrapment efficiency: Micro particulate have high drug-loading capacity reduces the quantity of drug for administration. Loading of drug can be done by two methods:

- Incorporation method
- Absorption method.

Drug content and encapsulation efficiency depend on the solubility of drug in polymer composition, molecular weight, drug- polymer interaction. For ionic interaction between the drug and polymer can be a very effective way to increase the drug loading of small molecules.

The Drug release studies: It was evaluated by USP type-II using dissolution media with Phosphate buffer pH 7.4 with the temperature maintained at 37 ±0.5 & then analyzing the drug release samples spectophotometrically.

Classification of Antiretroviral drugs

I. Nucleoside reverse transcriptase inhibitors (NRTIs)
II. Nucleotide inhibitors
III. Non- nucleoside reverse transcriptase inhibitors (NNRTIs)
IV. Protease inhibitors
V. Fusion inhibitors

Available dosage form of antiretroviral microspheres

1. Zidovudine – nucleotide reverse transcriptase inhibitors (NRTIs)
2. Stavudine – nucleotide reverse transcriptase inhibitors (NRTIs)
3. Lamivudine – nucleotide reverse transcriptase inhibitors (NRTIs)
4. Indinavir – protease inhibitors
5. Nelfinavir – protease inhibitors

Anti- Retroviral Microspheres: Different method are used to prepared the microspheres for antiviral drugs like Zidovudine (emulsification stabilizing heat method, ionic gelation method, emulsion solvent evaporation method, dry-in-oil method, emulsion solvent diffusion method), Stavudine (solvent evaporation method, emulsion solvent diffusion method), Lamivudine (solvent evaporation method, ionic gelation method), Indinavir (emulsion / solvent evaporation method), Nelfinavir (solvent evaporation method).

In antiviral microspheres are prepared by using different polymers such as

HPMC (Phalguna Y)[11] used zidovudine by emulsification heat stabilizing method and result of this prepared microspheres is spherical in shape, infrared spectra showed identical peaks of drug and polymer and drug entrapment efficiency (69%), *in-vitro* drug release was found to be 87.5% at 10 hrs.

Chitosan (Usha yohendra nayak, Dhanaraju MD)[12] with Zidovudine microspheres using ionic gelation method and resuls was found to be particle size (60 – 210µm), drug entrapment efficiency (60%) and *in-vitro* release (75%) at 12 hrs.

Chitosan (Dhanaraju MD)[13] with Lamivudine microspheres using ionic gelation method and characterization was done for drug loading (54.70 – 63.70%), particle size (5 - 40µm), encapsulation effiency (54.83 – 63.70%) and in-vitro release (23.32 – 68.72%).

Eudragit RS100, RL100 (Bipul nath)[14] and Zidovudine microspheres using emulsion solvent evaporation method and evaluation was done by particle size (1000 - 3000µm), drug entrapment efficiency (56.4 – 87.1%) and release rate of Zidovudine from Eudragit RS100 microspheres much lower than from Eudragit RL100.

Eudragit RS100 (Josephine LJ)[15] using Stavudine microspheres by emulsion solvent evaporation method. The prepared microspheres was found to be entrapment efficiency (88%) and buoyant for more than 12 hrs.

Eudragit RS100 and ethyl cellulose (Sanjay Dey)[16] using Stavudine microspheres by emulsion solvent diffusion method and results was found to be particle size (206 - 290µm to 201 - 413µm) percentage yield (56 - 79% to 69 – 87%), drug entrapment efficiency (47 – 70% to 56 – 72%).

Ethyl cellulose (Jains S)[17] and Zidovudine microspheres using dry-in-oil method and the good bioadhesive property was observed by the *in-vitro* release .

Ethyl cellulose (Rama Rao K)[18] using zidovudine microsphers by double emulsion solvent diffusion method and results was found to be drug content (41-55%) of drug entrapment and release was extended up to 18 – 20hrs. The FTIR, DSC, DTA showed stable character of Zidovudine microspheres.

Eudragit E100 (Diego A Chiappetta)[19] with Indinavir microspheres using double emulsion solvent evaporation method and result of the prepared microspheres was done by particle size distribution (210 - 420µm), drug content (67.28 – 89.71%) and *in-vitro* release (67.3 – 89.8%).

Ethyl cellulose, bibytyl phthalate (Rawal Tanvi) [20]and Stavudine microspheres using solvent evaporation method and evaluation was done for particle size (225 - 253µm), entrapment efficiency (48 – 58.5%) and FTIR, DSC confirmed the stability of the Stavudine microspheres.

Ethyl cellulose (Sanjay Dey)[21] and Stavudine microspheres using emulsion solvent diffusion method and results was found to be particle size (290µm), drug entrapment efficiency (70%) and *in-vitro* release (80%).

Acrylic and methacrylic acid (Sunit kumar Sahoo)[22] using stavudine microspheres by solvent evaporation method and evaluation was done for drug loading (67 – 91%) of encapsulation efficiency and *in-vitro* release at 8 hrs. The FTIR, DSC, XRD showed stable character of Stavudine.

Acryl coat, LSOD,S100(Nayak Bhabani Shankar)[23] using Lamivudine microspheres by solvent evaporation method and characterization was done for particle size (23 - 31µm), encapsulation efficiency (95.36 – 99.01%), loose crystal study (16.66 – 22.25%) and *in-vitro release* (17.38 – 71.16%).

Cellulose acetate phthalate, Ethyl cellulose (prakash K) [24]with Lamivudine microspheres by using solvent evaporation method and results was found to be percent drug content (34.72 – 40.12%), drug entrapment efficiency (76 – 86%) and FTIR, DSC showed the stability of the Lamivudine microspheres.

cellulose polymers like ethyl cellulose, cellulose acetate phthalate, cellulose acetate (Narasimha rao) [25]with Lamivudine microspheres using emulsion solvent evaporation method and results are found to be particle size (27.89 – 41.28µm), drug content (93 – 99.10%), drug entrapment efficiency (95.3 – 98.45%), drug release (60.22 – 71.16%) and FTIR, DTA was done by stability study of Lamivudine microspheres.

cellulose acetate (Pheeba Mary Philip)[26] and Nelfinavir using solvent evaporation method and evaluation was done by particle size distribution (80 - 100µm), % yield (60.30 – 86.03%), in-vitro release (81.68%) at over a period of 8th hrs and FTIR showed all the peaks are confirming that no interaction with drug and polymer.

Nature gum moi (Nayak Bhabani Shankar)[27] and Lamivudine microspheres using solvent evaporation method and evaluation was done for % yield (69.1 – 86.65%), particle size (27.76 – 31.34µm), drug entrapment efficiency (70.12 – 91.09%), loose crystal surface study (18.21 – 91.09%) and in-vitro release (76.46 – 76.98%).

CONCLUSION

The microparticles of drug delivery system are physically to improve the pharmacokinetics and pharmacodynamics of drug molecules. This can be used to protect the drug entity in the systemic circulation and the drug chosen sites and delivered at extended rate to the site of action with enhanced therapeutic activity, while minimizing side effects. The review embracing various aspects of antiretroviral micro particulate formulations, characterization, and their applications in site specific delivery of drug molecules is better insight of micro particulate drug delivery for better management of life threatening diseases.

REFERENCES

1. Abhey Padalha N, Sadhana Shahi R, Mahesh W. Micro particles: an approach for betterment of drug delivery system. Int. J. Pharm. Res&dev.2011; 3(1): 99 – 115.
2. Satheesh Madhav NV, Shivani Kala. Review of Microparticulate drug delivery system. Int. J.Pharm tech res.2011; 3(3): 1242 – 1254.
3. Sudhamani T, Naveen Kumar Reddy K, Ravikumar VR, Revathi R, Ganesan V, et al. preparation and evaluation of ethyl cellulose microspheres of ibuprofen for sustained drug delivery. Int. J. pharma Res and dev. 2010; 1:119-125.
4. Selvakumar Dhanasingh, Shanmuga Kumar Nalaperumal. Chitosan /Casein micro particles: Preparation, characterization and drug release studies. Int. Journal of engineering and applied sciences.2010; 6(4).
5. Morinto Y, Fujimoto S. "Albumin microspheres as drug carrier" CRC critical reviews in therapeutic drug carrier systems. 1985; (1)2:19-63.
6. Gupta P, Fung C. Targeted delivery of low dose doxorubicin hydrochloride administered via magnetic albumin microspheres in rats. J.microencap. 1990; 7(1): 85-94.
7. Venkatesan P, Muralidharan C, Manavalan R, Valliyappan K. selection of better method for the preparation of microspheres by applying analytic hierarchy process. J.pharm.sci & Res, 2009; 1 (3): 64-78.
8. Fedreic Lagarce, Pascal Renaud, Nathalie Faisant, Guillaume Nicolas, Annie Cailleux, Joel Richard, Philipp menei and Jean- pierre benoit. Eur. J. pharm biopharm. (2004); 59: 449-459.
9. Ambati Brahma Reddy, Karunanidhi M, Sudheer karna k, Samyuktha Rani B, Effect of SLS on ethyl cellulose containing Cyclobenzaprine Hcl floating microspheres. Research journal of pharmaceutical, biological and chemical sciences. 2011; 1(4): 898-908.
10. Jalil R, Nixon J, Microencapsulation with biodegradable materials. In: Microencapsulation of Drugs, 1992; 177–188.
11. Phalguna Y, Venkateshwarlu BS, Ganesh Kumar Gudas, Subal Debnath. HPMC microspheres of Zidovudine for sustained release. Int.J.pharm & pharm.sci. 2010; 2(4): 41-43.
12. Usha Yohendra Nayak, Shavi Gopal, Srinivas Matalik, Averinen Kumar Ranjith et.al. Glutaraldehyde cross – linked Chitosan microspheres

for controlled delivery of Zidovudine. J.microencapsulation. 2009; 26(3): 214-222.
13. Dhanaraju MD, Mani Kumar R, Nithya P, Kishan JVVN, Thirumurugan G. controlled delivery of anti retroviral drug loaded chitosan cross- linked microspheres. Archives of applied Sci.Res. 2009; 1(2): 279-286.
14. Bipul Nath, Lila Kantanath, Pradeep Kumar. Preparation and *in-vitro* dissolution profile of Zidovudine loaded microspheres made of Eudragit RS100, RL100 and their combinations. Acta poloniae pham. Drug res. 2011, 68(3): 409 – 415.
15. Josephine LJ, Mohul RJ, Wilson B, Shanaz B, Bincy R. formulation and *in-vitro* evaluation of floating microspheres of anti-retro viral drug as a gastro retentive dosage form. Int.J.Res.Pharm&Chem. 2011, 1(3): 519-527.
16. Sanjay Dey, Bhaskar Mazumder, Mrinal Kanti Sarkar. Comparative study of Stavudine microspheres prepared using ethyl cellulose alone and in combination with Eudragit RS100. Malaysian J.Pharm.sci. 2010, 8(2): 45 – 57.
17. Jain S, Biswal I, Dinda A, Moitra SK, Kohli S. Modified sustained release chitosan - coated Zidovudine microspheres. Int. J.pharm &pharm.sci. 2011, 3(4): 95 – 97.
18. Rama Rao K, Prakash Senapati, Dos MK. Formulation and *in-vitro* evaluation of Ethyl cellulose microspheres containing Zidovudine. J.microencapsulation. 2005; 22(8): 863 – 876.
19. Diego A Chiappetta, Angel M. Carcaboso, Carlos Bregni, Modesto Rubio et.al. Indinavir – loaded pH sensitive Micro particles for taste masking: toward extemporaneous pediatric Anti – HIV/AIDS liquid formulation with improved patient compliance. AAPS pharm sci.tech. 2009; 10(1): 1-6.
20. Rawal Tanvi, Diwan Anupama. Novel polymeric combinations for gastroretentive microspheres of Stavudine. Int.J. Drug Dev&Res. 2011; 3(2): 211-216.
21. Sanjay dey, Soumen Pramanik, Ananya Malgope. Formulation and optimization of sustained release Stavudine microspheres using response surface methodology. ISRN pharmaceuticals. 2011; 1-7.
22. Sunit kumar Sahoo, Abdul Arif Mallick, Barik BB, Prakash CH Senapati. Formulation and *in-vitro* evaluation of Eudragit microspheres of stavudine. Tropical J.Pharm. Res. 2005; 4(1): 369 – 375.
23. Nayak Bhabani Shankar, Nayak Udaya Kumar. Lamivudine loaded microspheres for oral use: design development and establishment of *in-vitro* correlation. Asian J. Pharm & clinical Res. 2009; 2(1): 55-60.
24. Prakash K, Raju PN, Shanta KK, Lakshmi MN. Preparation and characterization of Lamivudine microcapsules using various cellulose polymers. Tropical J. Pharm. Res. 2007; 6(4): 841-847.
25. Narasimha Rao R, Prakash K. Preparation and evaluation of Lamivudine microspheres using various cellulose polymers. Int.J.pharm & tech. 2011; 3(1): 1397-1410.
26. Pheeba Mary Philip, Ganesh Shankar Arya. Formulation and *in-vitro* evaluation of Nelfinavir mesylate microcapsules using cellulose acetate. Int.J.pharm & pharm.sci. 2010; 2(3): 169 – 171.
27. Nayak Bhabani Shankar, Nayak Udaya Kumar, Patrok Balakrishna, Rout Prasant Kumar. Preparation and *in-vitro* evaluation of Lamivudine entrapped MOI microspheres for oral administration. Res.J.Pharm & tech. 2008; 1(4): 437-440.