Lamivudine Loaded Carbopol-940 Based Nanoparticles For Controlling Drug Release In Aids Therapy: Development, Physico-Chemical Characterization And In-Vitro Evaluation

(Original research article on Nanoparticulate Internal Medicine against HIV and AIDS)

Dr. Basudev Sahana (1st & Correspondence)
Lecturer, Department of Pharmaceutics, Institute of Pharmacy, Jalpaiguri, Govt. of West Bengal, P.O. & Dist. - Jalpaiguri, West Bengal, PIN – 735101, India, Fax No.: +91-3561-221884, Mobile No. +91 9433524325, E-mail: sahanabasu33@rediffmail.com

Ananda Bairagi (2nd author)
Former Researcher, Department of Pharmaceutics, Institute of Pharmacy, Jalpaiguri, Govt. of West Bengal, P.O. & Dist. - Jalpaiguri, West Bengal, PIN – 735101, India, Mobile No. +91 9382709377, E-mail: anandabairagi36@gmail.com

***Abstract*—The aim of the present study was to develop nanoparticles containing lamivudine – an anti-retro viral drug used for the treatment of chronic hepatitis B, human immunodeficiency virus infection and acquired immunodeficiency syndrome. Mucoadhesive polymer carbopol 940 was used to develop nanoparticles of lamivudine by multiple emulsification (w/o/w) and solvent evaporation technique. Drug–polymer ratios, polyvinyl alcohol concentrations and homogenizing speeds were varied at different stages of preparation to optimize the desired size, drug loading efficiency and release profile of drug. The characterization of particles morphology for shape, size and surface was performed by field emission scanning electron microscope and particle size distribution patterns were studied by direct light scattering method using zeta sizer nano-series. The morphological study showed that lamivudine loaded carbopol-940 based nanoparticles were of smooth surface and spherical in shape. The drug-excipients compatibility study using Fourier-transform infrared spectroscopy showed that there was no chemical interaction between drug and excipients and a mild physical interaction was found at the wave number range between 2840 and 2845 cm-1. Drug content varied from 1.49 to 1.72%, drug entrapment efficiency varied from 29% to 39%, and the cumulative percentage of drug release from the different batches of formulations varied in the range from 60% to 82% within 48 hours. This type of release patterns of the formulations might be used for sustaining the action of lamivudine in the treatment of HIV infections or AIDS.**

Keywords—Polymers; Mucoadhesives; Carbopol 940; Lamivudine; Nanoparticles.

I. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a chronic, potentially life-threatening condition caused by the human immunodeficiency virus (HIV) by damaging the immune system and affecting women and girls with increasing numbers in the world [1]. An important anti-retro-viral drug Lamivudine [2] is used for the treatment of chronic hepatitis B, AIDS and acting efficiently against HIV infections [3]. Mucoadhesion [4, 5] in drug delivery system has recently gained interest as a means of promoting dosage form [6, 7] especially for oral delivery and its residence time [8, 9] as well as improving intimacy of contact using polymers [9,10] with various absorptive membranes [11] of the biological system especially in the gastrointestinal tract wall [12, 13]. Nanoparticles have been used for sustaining the release of drugs [13, 14] which needs long term contact in the absorptive site for contributing therapeutic advantages**.** In order to fulfill the need of a long-term contact [8] and treatment with anti-HIV agents mucoadhesive polymer [14] based nanoparticles can be used to overcome the suffering from the drawbacks of frequent administration, plasma concentration fluctuation, and significant adjustment in the lifestyle [15,]. Mucous is an aqueous gel complex [16] with a constitution of about 95% water, high molecular weight glycoprotein (mucin), lipid, salts, etc [17]. Mucoadhesive agents having sufficient strength [18,19] evaluated by various in-vitro method, like shear stress, falling ball, Wilhelmy plate and Robinson's methods [11,20,21] such as Carbopol-940 based nanoparticles containing Lamivudine can be prepared for sustaining the drug action through the enhancement of gastrointestinal tract residence time [22] and controlling the release of drug [23]. Nanoparticles includes nanospheres in which the drug is adsorbed, dissolved, or dispersed throughout the matrix and nanocapsules in which the drug is confined to an aqueous or oily core surrounded by a shell

[24,25]. These carriers can also be designed to enable controlled the drug release from the matrix [26]. The potentiality of nanotechnology is improving the current treatment, advancing new therapeutic strategies as well as providing alternatives in the quest for vaccine and microbicide developments for HIV/AIDS [25,26,27]. Nanoparticles oligonucleotide bio-barcode assay has been used to detect small levels of the cancer marker prostate-specific antigen (PSA) in Serum [28,29], topical cosmetic and pharmaceutical formulation[30], drug carrier for photodynamic therapy has been developed [31]. The carrier can provide stable aqueous dispersion of hydrophobic photosensitizer[32]. Nanoparticles have also found application as nonviral gene delivery system [33]. Developed mucoadhesive micro-spheres consist of a drug and an adhesive polymer powder cross – linked polyacerylic acid derivative dispersed in a waxy base with ability to adhere the stomach wall in rats and there by remain in gastrointestinal tract for an extended period [34,35]. It is expected that prepared mucoadhesive micro- spheres containing anti-H. pylori agents amoxicillin will provide potent and prolong anti-H. pylori activity[26]. Famotidine microcapsules employing mucoadheive polymer is developed to enhance gastro retention for oral delivery [35]. The development of safe and efficient chitosan-based nano-particulate drug delivery systems[36] and poly (ethylene oxide)-modified poly (epsilon-caprolactone) nanoparticulate system [37] were intracellular delivery vehicle for saquinavir, an anti-HIV protease inhibitor by a solvent displacement process. Nanoparticles were characterized for size, surface charge, and surface presence of PEO chains. The prepared microspheres of Lamivudine by microencapsulation method using varying concentration of drug, polymer (ethyl cellulose) and solvent methanol to obtain sustained release action and to compare it with conventional Lamivudine tablet after administering orally to New Zealand white rabbit species [38]. The preparations of prolong release Poly (lactic acid)/Chitosan Nanoparticles for anti-HIV application and Eudragit based microspheres [40] containing Lamivudine by solvent evaporation method varying concentration of drug, polymer (Eudragit RSPO and Eudragit RLPO) and solvent acetone, liquid paraffin, n-hexane enriched to prepare for sustaining and obtaing relatively constant effective level of Lamivudine and characterized by particles size distribution, entrapment capacity, entrapment efficiency and in-vitro-release behavior. The aim of our research work was to design, develop and evaluate (in-vitro) Carbopol – 940 based controlled release nanoparticles containing Lamivudine for the HIV/AIDS therapy. Different formulations were prepared by varying concentration of drug and polymer. The best formulation was screened out of the experimental formulations in terms of drug content, drug entrapment efficiency, particles size and distribution and drug release profile. It was concluded that drug polymer ratio concentration 1:3 are found to control the release with good dissolution upto 12 hours exhibiting 86 percent drug entrapment efficiency.

II. MATERIALS AND METHODS

A. Materials

The study utilized the Lamivudine as an active ingredient collected as a gift sample from Hetero drugs, Hyderabad, Carpool 940 was purchased from LOBA Chemie Pvt Ltd, Mumbai, India and others laboratory supplied chemicals such as dichloromethane (DCM), methanol, dimethyl sulfoxide (DMSO), polyvinyl alcohol (PVA), sodium hydroxide pallets, sodium dodecyl sulphate and hydrochloric acid.

B. Methodss

1. The light absorption in the UV- range of a 0.002% w/v solution in distilled water exhibits one maxima at about 271nm in spectroscopic analysis. The solubility study of Lamivudine in different solvents was performed using different solvent like water, methanol, dichloromethane, and optimized the desired solvent for the drug according to need for the formulation in the study and the selected solvents used for drug solubility was methanol. The pure drug Lamivudine (active ingredient), Carbopol-940 (mucoadhesive polymer), a mixture of Lamivudine and Carbopol-940 were separately mixed with IR grade Potassium Bromide (KBr) in the ratio of 1:100 and corresponding pellets were prepared by applying 5.5 metric ton pressure with a hydraulic press. The pellets were scanned in an inert atmosphere over a wave number range of 4000-400cm^{-1} in Magna IR 750 series II (Nicolet, USA) FTIR Instrument.

2. Nanoparticles were prepared by double emulsification (w/o/w) and solvent evaporation technique[23]. Desired amount of drug and polymer (in specific ratio) was dissolved in specified amount of dichloromethane-methanol mixture with the help of magnetic stirrer (table 1). Previously prepared PVA-water solution (specific concentration) was added to it in desired quantity with continuous stirring for the preparation of w/o primary emulsion. Sufficient amount of different strength PVA-water solution (specific concentration) was taken in 500ml beaker and prepared w/o primary emulsion was added to it drop wise under continuous homogenization with the help of homogenizer in specific speed for 10mins. Then the prepared emulsion w/o/w was placed on magnetic stirrer in room temperature for 12 hrs to evaporate the solvent (dichloromethane-methanol). The prepared nanopraticles were filtered and washed using double distilled water by centrifugation in 5000 r.p.m (30mins x2) and collected in a watch glass. It was dried in vacuum condition at room temperature using vacuum desicator. Then the prepared nanoparticles were stored in refrigerator for characterization and evaluation.

Table-1. The composition of Lamivudine loaded Carbopol-940 based nanoparticles.

Formulations	Lamivudine (mg)	Carbopol 940 (mg)	PVA-Water solution (2.5%w/v) (ml)	Methanol (ml)	DCM (ml)	PVA-Water solution (1.5%w/v) (ml)
C-940 (1:2)	50	100	5	5	5	200
C-940 (1:3)	50	150	5	5	5	200
C-940 (1:4)	50	200	5	5	5	200
C-940 (1:5)	50	250	5	5	5	200
C-940 (1:6)	50	300	5	5	5	200

The physicochemical characterization and evaluation of the nanoparticles were needed to observe any morphological changes of the particles and to determine the chemical stability of the drug in the formulation in short term and long term storage of the products.

3. The surface morphology of nanoparticles was investigated using Field Emission Scanning Electron Microscope (FESEM). The prepared batches of samples were mounted on the stubs using double-sided adhesive tapes. The stubs were then vacuum-coated with platinum using JEOL JFC 1600 (JEOL, Tokyo, Japan) Autofine coater. Then the platinum coated samples were observed and examined with the help of FESEM (JEOL JSM 6700F, Tokyo, Japan) and photographs were taken of different batches.

4. The characterization of particle size and size distribution were determined by Direct Light Scattering (DLS, Zeta Sizer Nano ZS) and analyzed using DTS software (Malvern Instrument Limited, UK), Average particle size was calculated and expressed in nanometer and the graphs of this study were taken.

5. Drug content and drug loading or drug entrapment efficiency studies using 5%w/v sodium dodecyl sulphate in 0.1M NaOH were performed with accurately weighed 2mg of each product sample taken in separate centrifuge (eppendorf) tube of 2ml volume. 2ml of prepared 5%w/v sodium dodecyl sulphate in 0.1M NaOH solution was added to each tube carefully with the help of micropipette. The closed tubes were placed on incubator shaker for shaking for 3 hours at 37ºC. Then the closed tubes were placed in SPINWIN centrifuge instrument for centrifugation for 10 minutes at 5000 r.p.m for the settlement of insoluble material. After that the supernatant liquid were withdrawn with the help of a micropipette carefully and observed in spectroscope at 271nm for the determination of concentration as well as the content of drug and drug loading or drug entrapment efficiency of the different batches of Carbopol-940 based Lamivudine loaded nanoparticles. The solution were then read against 5%w/v sodium dodecyl sulphate in 0.1M NaOH as blank using UV absorption spectroscope (Beckman, USA) at 271nm. The data were read from the calibration curve.

6. Dissolution studies of 3mg nanoparticles products containing drug Lamivudine were weighed and kept in 2 ml 0.1 (N) HCl solution as dissolution medium in 2 ml centrifuge tube (eppendorf) in incubator shaker at 37ºC ± 2 ºC [23] for maximum 48 hours in certain interval and after the time period the samples were filtered to separate the insoluble part by using centrifuge (SPINWIN) at 5000 r.p.m for 10 minutes. After that the supernatant liquid (in which the released drug was present) samples were estimated at 271nm using UV absorption spectroscope (Beckman, USA).

II. RESULTS AND DISCUSSION

In this study, the particles size, shape, drug entrapment efficiency, drug loading and drug release pattern were optimized by varying the drug polymer ratio, speeds of homogenizer, amount of stabilizers, speeds of centrifuge for filtration. After optimization of the condition sufficient quantity of the formulations were prepared and characterized. The prepared nanoparticles were of spherical, smooth, size ranges 10-30nm, the average particles size was 13.69nm, the polydispersity index ranges 0.9-1, the drug entrapment efficiency ranges 30.97-46.24%, the drug content ranges 1.5-2.12% and the drug release pattern followed almost zero order kinetics upto 48hrs release after initial fast release within 8hrs and within 48hrs in gastrointestinal pH approx 60-82% of content drugs were released. This type of release patterns of the formulations might be used for extending or sustaining the action of lamivudine in the treatment of HIV infections or AIDS.

1. The results obtained from UV Spectroscopic analysis for the determination of maximum absorbance of Lamivudine was 271nm. So all the analytical evaluation of the different batches formulations were done in 271nm. The solubility study of Lamivudine using various solvents showed that the drug was freely soluble in water, dichloromethane, methanol, PVA-water solution. Dichloromethane and methanol were used as solvent for the benefit of our desired formulations before that FTIR spectra analysis for drug-polymer interaction studies were done.

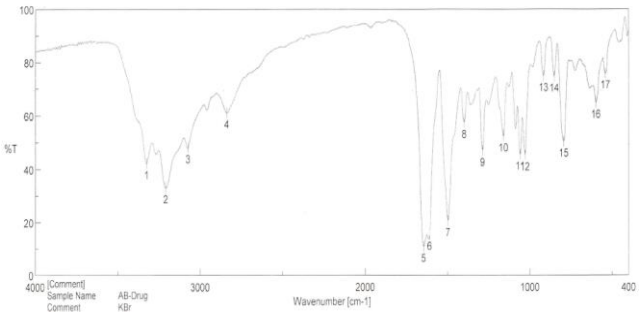

Figure 1 FTIR Spectra of Lamivudine

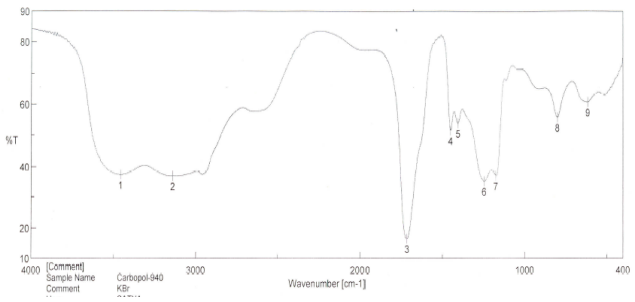

Figure-2 FTIR Spectra of Carbopol 940

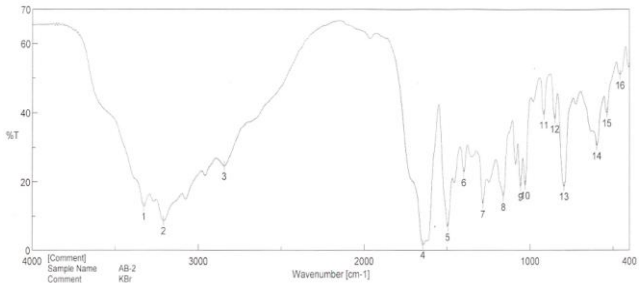

Figure-3 FTIR Spectra of mixture of Lamivudine and Carbopol 940

Drug excipients interaction has been studied using FTIR spectroscopy to understand interaction between the drug and excipients at the level of functional groups. In this study FTIR spectra of Carbopol 940, Lamivudine and mixture of Lamivudine and Carbopol 940 were (fig no. 1,2,3) compared and a mild physical interaction was found at the wave number range between 2840 to 2845 cm^{-1} .The wave numbers in the region between 2840 to 2845 cm^{-1} is the stretching vibration zone of medium intensity CH (aldehyde) and strong intensity-OH. The drug lamivudine content NH_2, CO, OH, N as reactive functional groups and in case of carbopol OH,COOH,H are present. Thus there may be some physical interactions between the functional groups by information of week H-bond or bond due to Van der Waal force of attraction or dipole-dipole interaction etc. Since there was no shifting of characteristic peaks of drug as well as excipient in the spectra of drug excipient mixture, this may be suggested that there was no chemical interaction.

2. Characterization of nanoparticles were seen very clearly in the figure-4,5,6 C-940(1:2) by FESEM photographs. The particles size range around from 10nm to 30nm was found in the photographs. The average particles size range 14nm to 15nm. The particles were very fine and spherical observed by 1,50,000 to 3,00,000 magnifications. Those particles were obtained in optimized speed of homogenizer, drug–polymer ratio, PVA-water concentration, speed of centrifugation for filtration of the product which can alter the particles nature. The FESEM photographs were taken from our some screened formulations only

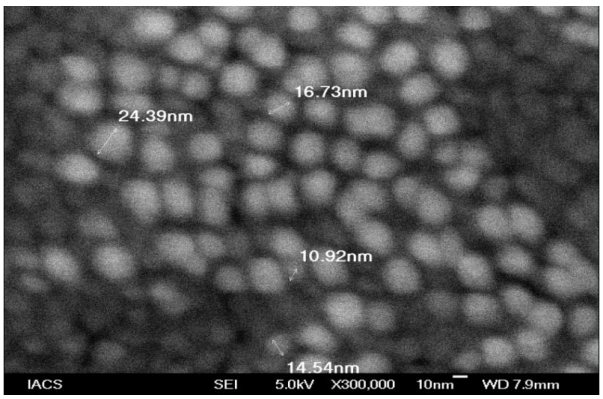

Figure 4 C-940 (1:2)

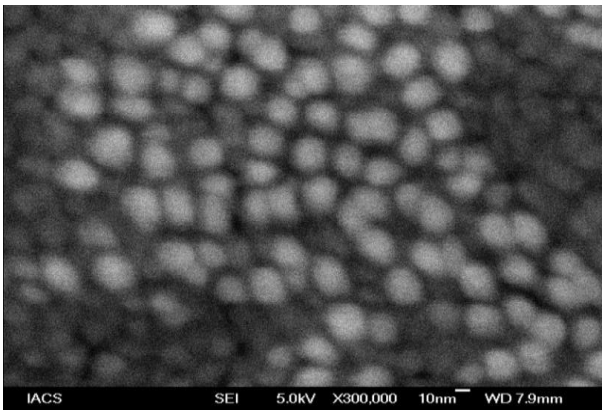

Figure 5 C-940 (1:2)

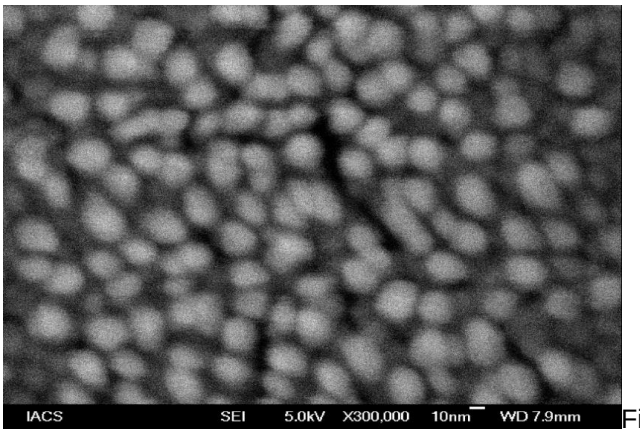

Figure 6 C-940 (1:2)

The Field Emission Scanning Electron Microscopic Photographs for characterization in high magnification of particles' photographs showed the rough surface of particles but practically it was smoothed (fig.no. 4,5,6).

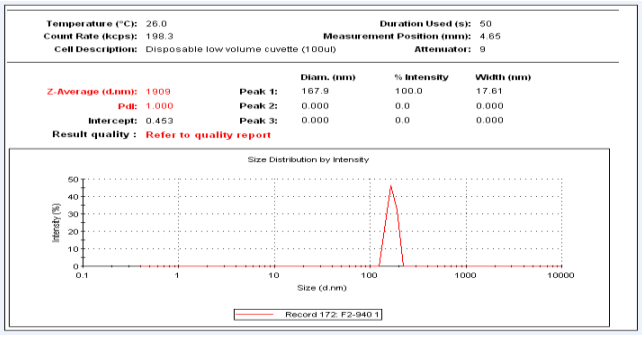

Figure-7 C-940 (1:2)

3. The figure-7 shows that the particles size distribution varied from 10nm to 28nm and the average particles size was about 14nm with poly dispersity index (PDI) 01 of the sample formulation obtained from low speed centrifugation. The poly dispersity indices suggest the particles were with nano ranges only and uniform mono-disperse particles size distribution.

4. Lamivudine content and entrapment efficiency studies were calculated based on equation y = 0.366x+0.6691, R^2 = 9992 standard calibration curve of Lamivudine in 5%w/v Sodiumdodecyl sulphate in 0.1(M) Sodium hydroxide solution and equation y =

0.0475x+0.1002, R^2 = 9997 standard calibration curve of Lamivudine in 0.1(N) HCl solution.

5. Product obtained in different batches of formulations was near about same when the amount polymer was gradually increased the amount of product obtained was not remarkably changed. So the amount of product did not depend on the polymer taken for formulation. We got better amount of product when the drug polymer ratio was 1:6 in batch C-940(1:6). The amount product might be increased with proper separation process or centrifugation speed. Drug content and drug entrapment efficiency of the different batches of formulations were determined after extraction of drug from the capsules using 5%w/v Sodium Dodecyl Sulphate in 0.1M Sodium Hydroxide solution. The extracted amount of drug from different batches of formulations was given in the table-2. The table showed the variation of percentage of drug content and percentage of drug entrapment efficiency of different batches of formulations, it might be due to variation of drug-polymer ratio and capability of drug entrapment of Carbopol-940.

The percentage of drug content and percentage of drug entrapment efficiency was higher in case of the lower drug- polymer ratio was taken for formulation. Drug content and entrapment efficiency was determined using following formula a and b.

Formulations	Products Obtained (mg)	Taken Sample (mg)	Drug content (µg)	Drug content (%)	Drug Entrapment Efficiency (%)
C-940 (1:2)	72	2	35	1.72	39.26
C-940 (1:3)	69	2	33	1.69	37.49
C-940 (1:4)	56	2	25	1.49	29.19
C-940 (1:5)	75	2	31	1.59	31.79
C-940 (1:6)	78	2	28	1.56	30.97

a. **Percentages of Drug Content = (weight of drug in the products ÷ Total weight of the products) × 100.**

b. **Percentage of Drug Entrapment Efficiency = [(weight of the drug in 1mg .product × Total products)/Total drug taken for formulation of a particular batch] × 100.**

6. **In-vitro Drug Release Study**

The in-vitro drug release profiles of Lamivudine from Carbopol-940 based nanoparticles of different batches of formulations were reported in the figure no. 8. The figure showed the gradual, uniform and slower drug release from the experimental nanoparticles formulations from batch C-940(1:2) to C-940(1:6). The figure no. 8 showed the initial faster drug release from carbopol-940 based nanoparticles following almost first order kinetic pattern and after 8 hours the drug release pattern was changed from faster to slower and followed almost zero order kinetics pattern. The Carbopol-940 based nanoparticles released the drug slowly and uniformly and the cumulative percentage of drug release from the different batches of formulations varied in the range from 60% to 82% within 48 hours. This type of release patterns of the formulations might be used for extending or sustaining the action of lamivudine in the treatment of HIV infections or AIDS with this drug.

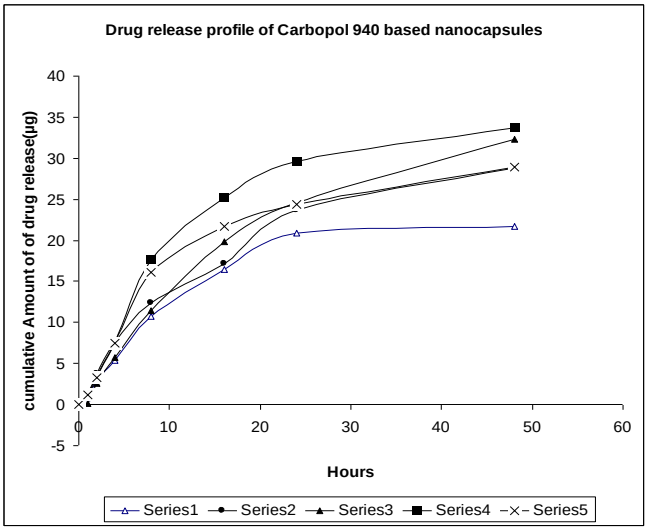

Figure-8. Series1- C-940 (1:2), series 2-C-940 (1:3), series 3-C-940(1:4), series 4-C-940 (1:5), series 5-C-940 (1:6)

CONCLUSION

Extensive efforts have recently focused on targeting a drug delivery system in a particular region of the body for extended period of time not only local targeting of drug but also for the better control of systemic drug delivery. The concept of mucoadhesive has alerted many investigations to the possibility that these polymers can used to overcome physiological barrier in long term drug delivery. They render the treatment more effective and safe, not only for topical but also for systematic problems. The research work was done to design, develop and evaluate the mucoadhesive polymer carbopol-934 and carbopol-940 based nanoparticles containing the drug Lamivudine used for the treatment of HIV infections or AIDS. This particular pharmaceutical product development was formulated with the mucoadhesive polymer with desired specific characters of mucoadhesion for the enhancement of G I transit time, successful entrapment of drug, reasonable drug loading, controlled and prolonged drug release from the nanoparticles for the betterment of AIDS therapy. Thus the mentioned nanoparticles formulations containing lamivudine could be beneficial system to deliver the drug and it would be the potential alternative dosage form for the treatment of HIV infections or AIDS with a great advantage over conventional controlled release dosage forms. However, further studies are required.

Acknowledgement: The authors are very much grateful to Prof. Biswajit Mukherjee, Division of

Pharmaceutics, Dept of Pharm. Tech., Jadavpur University, Kolkata, other institutes of Kolkata and University Grants Commission (Government of India) as the work has been supported to complete properly.

Conflicts of interest: No conflicts of interest have been declared in relation to this paper.

REFERENCES

1. DR. SCOTT C. LITIN M.D. (EDITOR), MAYO CLINIC FAMILY HEALTH BOOK, 5TH EDITION, COMPLETELY REVISED AND UPDATED HARDCOVER – MAY 29, 2018
2. Öztürk AA, Namlı İ, Güleç K, Görgülü S,Design of Lamivudine Loaded Nanoparticles for Oral Application by Nano Spray Drying Method: A new approach to use antiretroviral drug for lung cancer treatment. Combinatorial Chemistry & High Throughput Screening Vol. 23, 2020 **DOI** : 10.2174/1386207323666200325155020
3. Chatterjee .A, Bhowmik. B. B, Solanki. D; Drug release efficacy of Zidovudine loaded microencapsulated bioadhesive vaginal tablet. Journal of Pharmacy Research 2009,2(6),1124-1128
4. Ahuja A, Khar RK, Ali J, Mucoadhesive drug delivery systems, Drug Dev. Ind. Pharm 1997; 23: 489-515.
5. V.V. Khutoryanskiy, Advances in mucoadhesion and mucoadhesive polymers, Macromol. Biosci. 11 (2011) 748-764.
6. Longer MA, Ch'ng HS, Robinson JR, Bioadhesive polymers as platforms for oral controlled drug delivery III: oral delivery of chlorothiazide using a bioadhesive polymer. Journal of pharmaceutical Sciences 1985; 74: 406-11.
7. Sahana B, Design and development of oral mucoadhesive drug delivery systems with some natural mucoadhesive agents, Jadavpur University 2002.
8. Sahana B, Bhaduri K, Special Approach to Linger Residence Period of Drug Formulations for Enhancement of Bioavailability and Therapeutic Activity Based on Mucoadhesive Polymers. International Journal for Pharmaceutical Research Scholars 2019b; 8: 1-18.
9. Basudev Sahana*, Kuhu Bhaduri, Screening and evaluation of edible herbal polymers for development of oral mucoadhesive gastro-retentive tablets: an approach to improve bioavailability and action of drugs, International journal of recent scientific research vol. 11, issue, 01(e), pp. 36987-36996, january, 2020
10. Basudev Sahana*, Kuhu Bhaduri, Studies on mucoadhesiveness of edible plants' extracted mucoadhesive polymers for designing, development and in-vivo evaluation of gastro-retentive tablets international, International journal of current advanced research, volume 9; issue 01 (D); pp 21081-21090 january 2020
11. Ballard BE, Sustained and Controlled Release Drug Delivery System. (J.R. Robinson ed.). Marcel Dekker 1978.
12. Basudev Sahana*, Kuhu Bhaduri, Polymers: Excellent Formulations Devising Agent, International Journal for Pharmaceutical Research Scholars V-8, I-2, 2019
13. Asane GS, Mucoadhesive gastro intestinal drug delivery system- an overview. 2007; 5: http// www.pharmainfo.net. Accessed on 06/07/2010.
14. Borchard.G, Lueben.H .L. The potential of Mucoadhesive polymers in enhancing intestinal peptide drug absorption .Journal of control release Vol 39 Issue 2-3 May 1996 page 131-138.
15. Bender .A, Schafer .V, Steffam.A.M, Royer.C, Krcuter.J, Briesen.V.H Inhibition of HIV in vitro by antiviral drug targeting using Nanoparticles. Research in Virology Vol145, 1994 pages 215-220.
16. Tamburic .S ,Craig.D .O.M . An investigation into the Reheological-Dielectric & mucoadhesive Properties of poly (acrylic acid) gel system. Journal of controlled release Vol 37 ,Issues 1-2 November 1995, Page 59-68.
17. Tangri P, Oral mucoadhesive drug delivery system: A review. Int. J. of biopharm 2011; 2: 36- 46.
18. Nagai T, R. J. Konishi, Buccal / gingival drug delivery systems. J Control Release. 1987; 6: 353–60.
19. Huang Y, Leobandung W, Foss A, et al., Molecular aspects of mucoadhesion and bioadhesion: tethered structures and site specific surfaces. J Control Release 2000; 65: 63-71.
20. Duchene D, Touchard F, Peppas NA, Pharmaceutical and medical aspects of bioadhesive systems for drug administration. Drug Development and Industrial Pharmacy 1988; 14: 283-18.
21. Mahajan P, Kaur A, Aggarwal G, et al., Mucoadhesive Drug Delivery System- A Review. Int. J. Drug Dev. Res 2013; 5: 11-20.
22. Akiyama Y, Yoshioka M, Horibe H, Inada Y, Hiarai S, Kitamori N, et al. Antihypertensive effect of oral controlled release microspheres containing an ACE inhibitor (Delapril hydrochloride) in rats. J Pharm Pharmacol 1994;46:661-5.
23. Sahana B, Santra K, Basu S, B. Mukherjee, Development of biodegradable polymer based tamoxifen citrate loaded nanoparticles and effect of some manufacturing process parameters on them: a physicochemical and in-vitro evaluation. International Journal of Nanomedicine 2010; 5: 621-630.

24. Glperina.S,Kisich.K,D.Michael & Heifets.L.The potential advantages of Nanoparticle drug delivery system in chemotherapy in Tuberculosis. American journal of respiratory & critical care medicine, 2005.
25. Naoki. N, Yohko. A, masafumi.N, Mayumi.T, Megumi. K and Yasuyuki. O, Mucoadhesive Microsphere containing Amoxicillin for clearance of Helicobacter pylori. Antimicrobial agent & chemotherapy, American Society, Oct 1998 p-2492 to 2494 vol.42 no.10.
26. Mamo T, Moseman A E, Kolishetti N, Morales S C, Shi J, Kuritzkes D R, Langer R, Andrian U V, Farokhzad. Emerging Nanotechnology Approaches for HIV/AIDS Treatment and Prevention Nanomedicine (Lond.) 2010, 5(2) 269-285, 2010.
27. Sumit Basu,[1,2] Biswajit Mukherjee,[1] Samrat Roy Chowdhury,[1] Paramita Paul,[1] Rupak Choudhury,[3] Ajeet Kumar,[1] Laboni Mondal,[1] Chowdhury Mobaswar Hossain,[1] and Ruma Maji[1],Colloidal gold-loaded, biodegradable, polymer-based stavudine nanoparticle uptake by macrophages: an in vitro study, Int J Nanomedicine. 2012; 7: 6049–6061. Published online 2012 Dec 13. doi: 10.2147/IJN.S38013
28. Nam RK, Diamandis EP, Toi A, et al. Serum human glandular kallikreine-2 protease levels predict the presence of prostate cancer among men with elevated prostate – specific antigen. J Clin Oncol 2000;18:1036-42.
29. Nam RK, Zhang WW, Trachtenberg J, et al. Comprehensive assessment of candidate genes and serological markers for the detection of prostate cancer. cancer Epidemiol Biomarkers prev 2003; 12:1429-37.
30. Muller R H, Rattke.M, Wissing S A, Influence of solid lipid nanoparticles on skin hydration and viscoelasticity in vivo study,Adv.Deliv.Rev.S131
31. Indrajit Roy, Tymish Y Ohulchanskyy, Haridas E Pudavar, Earl J Bergey, Allan R Oseroff, Janet Morgan, Thomas J Dougherty, Paras N Prasad (2003), Ceramic-based Nanoparticles Entrapping Water-Insoluble Photosensitizing Anticancer Drugs: A Novel Drug-Carrier System for Photodynamic Therapy J Am Chem Soc, 2;125(26):7860-5. doi: 10.1021/ja0343095. Affiliations expand PMID: **12823004**
32. Roy .I Ohuchanskyy.T.Y, Pudavar.H.E, Bergey. E. T, Oseroff. A. R, Morgan. J, Dougherty. T J, Prasad. P. N, The Carrier can provide stable aqueous dispersion of hydrophobic photosensitizer. Soc 125, 7807, 2003
33. Bergemann F, Kruger A, Plank C, Non viral gene delivery system, Gene.Ther.9, 102,2002
34. Chowdary. K. P. R, Suresh. B, Sangeeta.B and Kamalakara, Reedy. G, Design & evaluation of DIltiazem Mucoadhesive Tablets for oral controlled Release.Saudi Pharmaceutical Journal,Vol.11 NO.4 Oct 2003
35. Nayak . B, Ghosh. S .K, Tripati. K, Putro. B. Preparation & characterization of Famotidine Microcapsule employing Mucoadhesive polymer in combination to enhance gastro retention for oral delivery.International Journal of Pharmacy & pharmaceutical sciences. Vol.1 Issue 2.Oct-Dec.2009.
36. Sunil A Agnihotri[1], Nadagouda N Mallikarjuna, Tejraj M Aminabhavi, Recent advances on chitosan-based micro- and nanoparticles in drug delivery, PMID: **15491807**,DOI: 10.1016/j.jconrel.2004.08.010
37. Shah.L.K, Amiji.M.M. Intracellular delivery of saquinavir in bio degradable polymeric Nanoparticles for HIV/ AIDS. Pharmaceutical research Vol 23 no 11 Nov 2006.
38. Ghosh A, Nayak Udaya Kumar & Roy P, Development, Evaluation and Method Selection for the Preparation of Lamivudine Microspheres, January 2007, Pharma Times 9:67-71
39. Dev A, Binula N S, Anitha A,Nair S V,Furuike-T, Tamura H, Jayna Kumar R; Preparation of Poly (lactic acid)/Chitosan Nanoparticles for anti-HIV Drug DeliveryApplication. Volume 80, Issue 3, 5 May 2010 pages-833-838.
40. Yash Chavda, B. Bhimani, Dhiren Daslaniya, Preparation and evaluation of lamivudine microspheres with eudragit ® polymers by solvent evaporation method for lymphatic, Chemistry, Materials Science, Published 2013, Corpus ID: 34858970.