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Original Article

PREPARATION, CHARACTERIZATION AND EVALUATION OF POLY (LACTIDE–CO–GLYCOLIDE) MICROSPHERES FOR THE CONTROLLED RELEASE OF ZIDOVUDINE

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ABSTRACT

Objective: The purpose of this research work was to develop and evaluate microspheres appropriate for controlled release of zidovudine (AZT).

Methods: The AZT loaded polylactide-co-glycolide (PLGA) microspheres were prepared by W/O/O double emulsion solvent diffusion method. Compatibility of drug and polymer was studied by Fourier-transform infrared spectroscopy (FTIR). The influence of formulation factors (drug: polymer ratio, stirring speed, the concentration of surfactant) on particle size encapsulation efficiency and *in vitro* release characteristics of the microspheres was investigated. Release kinetics was studied and stability study was performed as per ICH guidelines.

Results: Scanning electron microscopy (SEM) images show good reproducibility of microspheres from different batches. The average particle size was in the range of 216-306 µm. The drug-loaded microspheres showed 74.42±5.08% entrapment efficiency. The cumulative percentage released in phosphate Buffer solution (PBS) buffer was found to be 55.32±5.89 to 74.42±5.08 %. The highest regressions (0.981) were obtained for zero order kinetics followed by Higuchi (0.968) and first order (0.803).

Conclusion: Microsphere prepared by double emulsion solvent diffusion method was investigated and the results revealed that 216-306 µm microsphere was successfully encapsulated in a polymer. FT-IR analysis, entrapment efficiency and SEM Studies revealed the good reproducibility from batch to batch. The microspheres were of an appropriate size and suitable for oral administration. Thus the current investigation show promising results of PLGA microspheres as a matrix for drug delivery and merit for *In vivo* studies for scale up the technology.

Keywords: Zidovudine, PLGA, Microspheres, W/O/O double emulsion, Higuchi model, Diffusion-Controlled Mechanism

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INTRODUCTION

Human Immunodeficiency Virus infection and Acquired Immune Deficiency Syndrome commonly referred to as HIV/AIDS has emerged as being the most serious and challenging public health problems in the world. Since the first case of AIDS occurred in 1981 with a report of *Pneumocystis carinii* pneumonia and Kaposi's sarcoma in homosexual men in the United States, AIDS has become a major worldwide epidemic. Today an estimated 30.6 million people are living with HIV infection or AIDS [1].

HIV begins its infection of a susceptible host cell by binding to the CD4 receptor on the host cell. Following fusion of the virus with the host cell, HIV enters the cell. The virus RNA is released and undergoes reverse transcription into DNA by an enzyme in HIV called reverse transcriptase. Once the genetic material of HIV has been changed into DNA, this viral DNA enters the host cell nucleus where it can be integrated into the genetic material of the cell. Once the viral DNA is integrated into the genetic material of the host, it is possible that HIV may persist in a latent state for many years [2-5]. Activation of the host cells results in the transcription of viral DNA into messenger RNA (mRNA), which is then translated into viral proteins. The new viral RNA forms the genetic material of the next generation of viruses. The viral RNA and viral proteins assemble at the cell membrane into a new virus. Following assembly at the cell surface, the virus then buds forth from the cell and is released to infect another cell [6].

AZT is a strong inhibitor of reverse transcriptase and is effective in the treatment of AIDS. Although orally administered AZT is rapidly absorbed from intestinal mucosa it loses considerable potency during its first pass metabolism (40%) and is rapidly eliminated from the body with a half-life of 1 hour. Orally administered AZT often show strong side effects on bone marrow resulting in leukopenia which may be attributed to an excessive plasma level of AZT immediately after administration [7]. Therefore an adequate Zero order release system is desired to decrease the high daily dose of AZT (5-10 mg/kg every 4 H) to maintain an expected antiviral effect, to reduce the strong side effect and to improve patient compliance [8-10]. The development of a sustained release device and formulation of AZT would be beneficial in comparisons with the recent intermittent dose regimens. Biodegradable sustained release microspheres have been developed for the numerous bioactive reagents. The most significant candidate for biodegradable polymeric controlled release system is the poly (Lactide–Co–Glycolide) due to its controllable biodegradability and good biocompatibility. Synthetic biodegradable polymers have gained more popularity than natural biodegradable polymers. The major advantages of synthetic polymers include high purity of the product, more predictable lot-to-lot uniformity, and free of concerns of immunogenicity [11-12]. Therefore the undertaken study aims at the development of AZT loaded PLGA microspheres for controlled release of the drug [13-14].

MATERIALS AND METHODS

Materials

AZT was obtained as a gift sample from Cadila Pharmaceutical Ltd, Ahmedabad (Gujrat), Light liquid paraffin, Heavy liquid paraffin, Glutaraldehyde have obtained from Himedia laboratories Mumbai (Maharashtra). PLGA was obtained from Sun Pharma, Vadodara (Gujrat) span 80 from SD Fine chemicals Mumbai (Maharashtra) and all other chemicals were of analytical grade.

Compatibility studies

Compatibility of drug and polymer was studied by obtaining IR spectra's of drug and mixture of the drug with the polymer by FTIR spectrophotometer (Shimadzu 8400, Japan). The pure drug and mixture of drug-PLGA in the ratio of 1:1 were kept at room

temperature for 30 d. Samples were subjected to FTIR studies using KBr as a blank and the IR spectrum of pure drug and excipients mixtures were compared to find any interaction between drug and excipients used for formulation and also on prepared microspheres were studied and shown in fig. 1-3.

Preparation of AZT loaded PLGA microspheres

Microspheres were prepared using a solvent evaporation method with O/O and conventional W/O/O systems. In O/O method 100 mg of PLGA polymer was dissolved in 10 ml acetonitrile and to it AZT (25 mg) was added. This organic phase obtained was emulsified in 100 ml continuous phase containing 1% w/v span 80. The mixture was stirred at 1500 rpm and kept overnight at different temperatures (25 ˚C and 50 ˚C) to evaporate the acetonitrile. The resulting microspheres were washed three times in n-hexane to remove excessive surfactant on the surface and light mineral oil. The resulting AZT-loaded microspheres were dried at–20 ˚C for 24 h by using freeze drier and then stored in a vacuum oven.

Characterization

Scanning electron microscopy (SEM) analysis

The surface morphology of the microspheres was investigated by Scanning Electron Microscopy (SEM)). The microspheres were mounted in metal stubs using a double-sided adhesive tape. After being vacuum coated with a thin layer (100-150 A °) of gold, the microspheres were examined by SEM at different magnification.

Percentage yield and particle size of AZT loaded PLGA microsphere

The particles were grossly separated. Particle size analysis was carried out by using a compound microscope. Dried microspheres were first re-dispersed in distilled water and placed on a glass slide and the number of divisions of the calibrated eyepiece was counted by using a stage micrometre. The average size of around 100 particles was determined. The prepared microspheres were collected and weighed. The yield was calculated for each batch by dividing the measured weight by the total weight of non-volatile components

Loading efficiency

Microspheres equivalent to 100 mg of pure drug were crushed and added to 50 ml of ethanol. The resulting mixture was shaken in a mechanical shaker for 3 h to completely extract the drug. The solution was filtered through Whatman filter paper and 1 ml of this solution was appropriately diluted to 25 ml with ethanol and analyzed spectrophotometrically at 266 nm using UV-Vis spectrophotometer (Chemito-1600, Japan). Loading efficiencies were calculated using the given formula (table 3).

$$\text{Drug entrapment efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theortical drug loaded experimental}} \text{x100}$$

Zeta potential study

The surface charge of microspheres was determined by the electrophoretic mobility of microspheres in a U type tube at 25℃ , using a zeta sizer (Malvern, UK) (fig. 5).

***In–vitro* drug release studies**

A fabricated Franz diffusion cell was used. A dialysis membrane was placed on the upper donor chamber of the diffusion cell, separating this compartment from the receptor chamber. An accurately weighed quantity of AZT-loaded microspheres (10 mg) was placed on the membrane using a slab with an area of 1.77 cm^2 and thickness of 1.2 mm. One ml of pH 7.4 150 ml PBS buffer containing 0.1% w/v sodium azide and 0.05% w/v Tween 80 (PBS-T buffer) to prevent microsphere contamination and agglomeration, respectively, was added on the membrane. At fixed intervals, aliquots were withdrawn from some cells and replenished with freshly prepared buffer. Tests were performed in triplicate at 37±0.5 °C and 500 rpm. Samples were analyzed using the UV spectrophotometer (Chemito-1600, Japan) (fig. 7).

Stability study

The stability study of the selected formulation of the AZT loaded microsphere was carried out by storing 1 gm of microparticles, in an amber coloured screw capped bottle for a period of 1months at 40±2 °c temperature and 75±5% RH Using stability chamber (Thermo lab, Mumbai). Sampling was carried out a 10days interval and examined for percent entrapment. These samples were collected on 10, 20, 30 d and checked at regular intervals for Drug content.

Statistical analysis

All experiments were performed in triplicate manner. The data were expressed as mean values±SEM using InStat software and tested with analysis of variance followed by the multiple comparison test of Tukey–Kramer with $p<0.01$ was considered significant.

RESULTS AND DISCUSSION

Compatibility studies

The FT-IR spectra of the free drug and the microspheres were recorded. The drug-excipients compatibility studies reveal that there are no physical changes observed in the drug and polymer mixtures. The IR spectrum of the drug, drug-PLGA mixture and microspheres formulation were compared to find any change in the frequency of functional group in microspheres with a respective functional group of the drug. The spectral observations indicated that the prominent IR absorption peaks observed in the spectra of the drug were close to those in the spectra of the microspheres indicates that there is no interaction between the drug and the polymer. It indicates that neither the polymer nor the method of preparation has affected the drug stability.

Formulation and characterization of PLGA microspheres

Non-aqueous solvent evaporation method was used to prepare AZT loaded PLGA microsphere. The first trial was made to prepare microspheres by using a solvent evaporation technique in the water phase, using acetonitrile-water system but although many formulations were investigated, no spherical particles could be obtained.

Then acetonitrile/liquid paraffin system was used and various formulations with different Drug: Polymer ratios were tried; stirring speed was also changed to obtain spherical particle. Stirring speed plays an important role in the microspheres size distribution and drug loading, Microspheres were prepared by the method described the optimized ratio of drug and the polymer (1:4), keeping surfactant concentration (0.4%), utilizing three different speeds i.e. 1000, 1500, 2000rpm. It is an important parameter, which needs to be optimized for optimum particle size and stability of the microspheres.

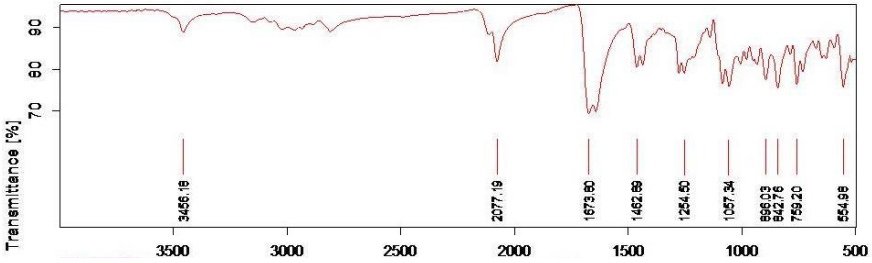

Fig. 1: FTIR spectra of zidovudine

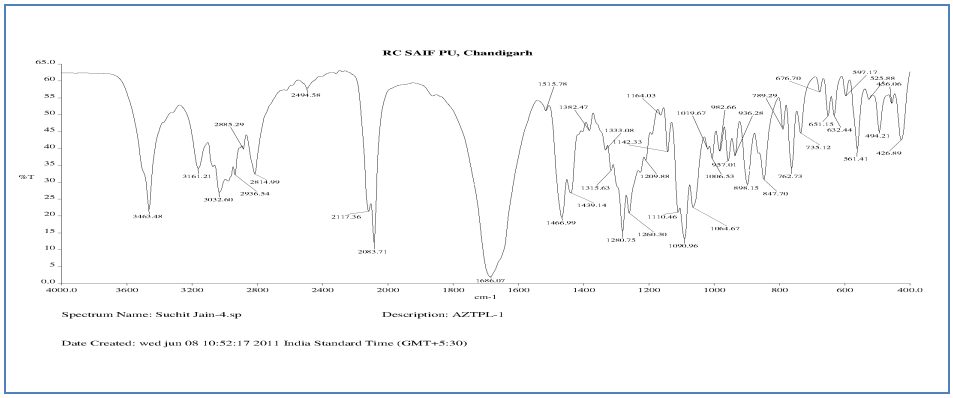

Fig. 2: FTIR spectra of drug and polymer

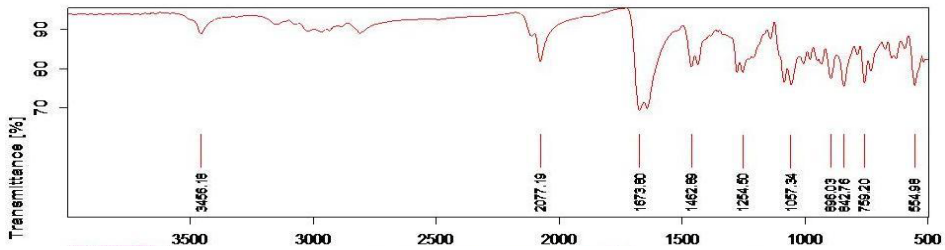

Fig. 3: FTIR spectra of prepared microparticles

Span 80 was used as an emulsifier. Microspheres were prepared according to the method described above with optimized drug-polymer ratio i.e. 1:4 and stirring speed 1500 rpm with various concentrations i.e. 0.2%, 0.3%, 0.4% and 0.5% v/v of span 80. Among various D: P ratios 1:4 ratio and stirring speed was 1500 rpm, the best spherical particles with good surface characteristic were obtained. SEM of the microsphere prepared is shown in fig. 4.

The entrapment efficiency increased with an increase in polymer concentration. At 1:4 drug-polymer ratio (formulation A1) entrapment efficiency was found to be maximum 74.57%. Size range of microspheres in the 200-300 µm by all formulation (table 1). Increase the D: P ratio caused the mean particle size to shift towards a higher particle size. On increasing the stirring speed, the particle size of microspheres decreased. The yield of the preparation and AZT entrapment efficiencies was high for all formulations and maximum for optimized formulation. The values of the optimized formulation were shown in table 2.

These optimised parameters help to regulate the properties of the resulting biodegradable products which govern the release pattern of the drug [15-16].

Table 1: Process variables for PLGA microspheres

Formulation variables	Particle size	% entrapment efficiency
Drug: Polymer ratio	µm	%wt/wt
1:01	216±5.29	65.58±4.19
1:02	238±3.84	70.42±5.27
1:03	266±3.41	73.12±4.24
1:04	285±3.78	74.57±4.19*
1:05	294±3.19	72.68±4.67
Emulsifier concentration		
0.2	306±3.64	68.42±3.21
0.3	301±4.98	69.62±3.11
0.4	282±4.52	74.42±3.87*
0.5	258±4.52	68.2±5.17
Stirring speed		
1000	300±3.71	66.5±4.36
1500	286±3.94	75.1±4.41*
2000	256±4.04	68.3±5.67

SD; Standard Deviation for n=3.

Table 2: Formula for microspheres after optimization

Optimized parameters	Values
Drug: Polymer	1:4
Emulsifier Conc (%w/v)	0.4
Stirring speed (rpm)	1500

Table 3: Effect of various emulsifiers on morphology of PLGA microspheres

Emulsifier	AZT	Solvent	Continuous phase	Morphology
Span 80 (0.4 ml)	25 mg	Acetonitrile (5 ml)	Light mineral oil 40 ml	Very good
Soyabean lecithin (0.4 ml)	25 mg	Acetonitrile (5 ml)	Light mineral oil 40 ml	Good
Span80 (0.4 ml)	25 mg	Acetonitrile (5 ml)	Soyabean oil: 40 ml	Not Good
Soyabean lecithin (0.4 ml)	25 mg	Acetonitrile (5 ml)	Soyabean oil: 40 ml	Not Good

Scanning electron microscopy (SEM) analysis

The surface topography of different formulation of microspheres was investigated by SEM. As seen in fig. 4, they were spherical in shape and exhibited porous surfaces. The SEM of Formulation B microspheres had rough surface due to a higher concentration of drug as compared to the other microspheres. SEM images of Formulation C and D showed rough and aggregated clump formation. Surface study of the microspheres after release study showed bigger pores suggesting that the drug was released through pores and the mechanism of drug release was diffusion controlled. The *in vitro* release of a drug from a biodegradable microparticulate system is affected by the properties of polymer and particles [17]. Controlling the release is to controlling the properties affecting release, including particle size, size distribution, drug content, polymer properties and surface properties [18].

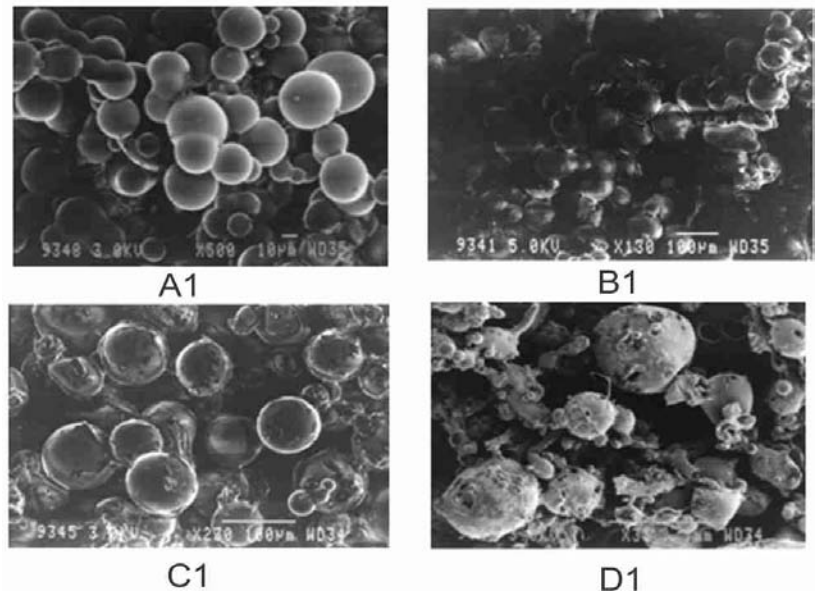

Fig. 4: SEM Microphotograph of AZT loaded PLGA Microsphere prepared at different emulsifiers and continuous phases (1) Formulation A1 (2) Formulation B1 (3) Formulation C1 (4) Formulation D1

Particle size and % yield of AZT loaded PLGA microsphere

The results of particle size and percentage yield mentioned in table 4. The % yield and the subsequent particle size of the respective formulations with the assigned product code were determined. Particle size was measured using an optical microscopy and mean particle size was calculated by measuring 200-300 particles with a calibrated ocular micrometre. The % yield was calculated using the following formula.

% Yield = (Total weight of floating microparticles/Total weight of drug and polymer) × 100

Table 4: Percentage yield and Particle size of AZT loaded PLGA microsphere

S. No.	Product Code	% Yield	Particle Size (μm)
1	A1	80.20	282±3.8 μm
2	C1	85.30	231±3.2 μm
3	E1	75.75	264±4.2 μm

SD; Standard Deviation for n=3.

It has been well known that particle size and its distribution will significantly affect the *in vitro* release in microspheres system. For hydrophobic drugs, the removal of drugs out from boundary layer was crucial during drug release.

A high ratio of surface area and volume such as a decrease in the particle size always lead to a higher release. The particle size obtained was considered to be beneficial for faster drug release rate [19].

Entrapment efficiency

To 10 ml of ethanol, 50 μg of microparticles were crushed, suspended and was kept for 12 h for extraction of the drug. RG content was determined from the filtrate after suitable dilution spectrophotometrically at 300 nm. The percentage drug entrapment was calculated as follows: % Drug entrapment = (Calculated drug content/Theoretical drug content) × 100

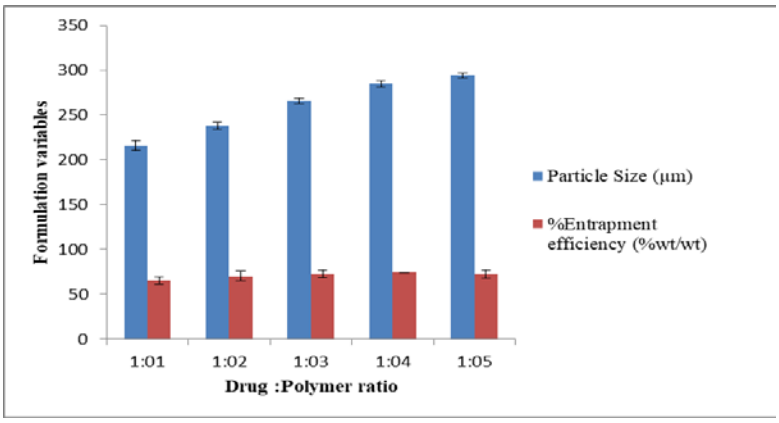

Fig. 5: Effect of a drug-polymer ratio on average particle size and percent drug entrapment (X-axis–Drug Polymer ratio, Y axis-Drug Entrapment efficiency). Error bars indicate SD for n=3

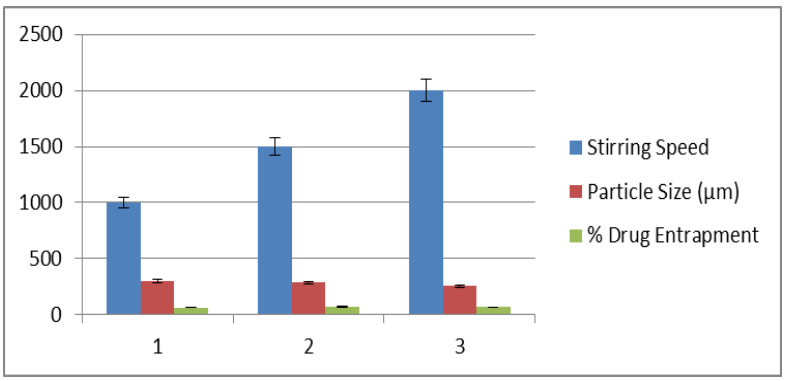

Fig. 6: Effect of stirring speed on particle size and percent drug entrapment for batch 1, batch 2 and batch 3. Error bars indicate SD For n=3

Zeta potential study

Microparticles formulations were characterized also in terms of Zeta Potential because as well known it can influence particle stability. The electrostatic repulsion between particles with the same electric charge prevents the aggregation of the microsphere. In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. On increasing the (D: P) 1:1, 1:2, 1:3, 1:4, 1:5 the zeta potential become-11.07, -18.20, -26.20-39.80, -39.80 (table 5) and particle size 300.00, 282.00, 249.00, 202.20, 290.20. On increasing D: P the zeta potential turned more negative but when it came to 1:5 the zeta potential increased. Similar kind of change was observed in particle size; it increases on further increase in D: P ratio. The surface charge reversal can be attributed to the transfer of a proton from the bulk solution to the surface of the microsphere. A similar charge reversal with the change in pH has been observed for polystyrene nanoparticles with carboxyl functional groups on the surface [20-21].

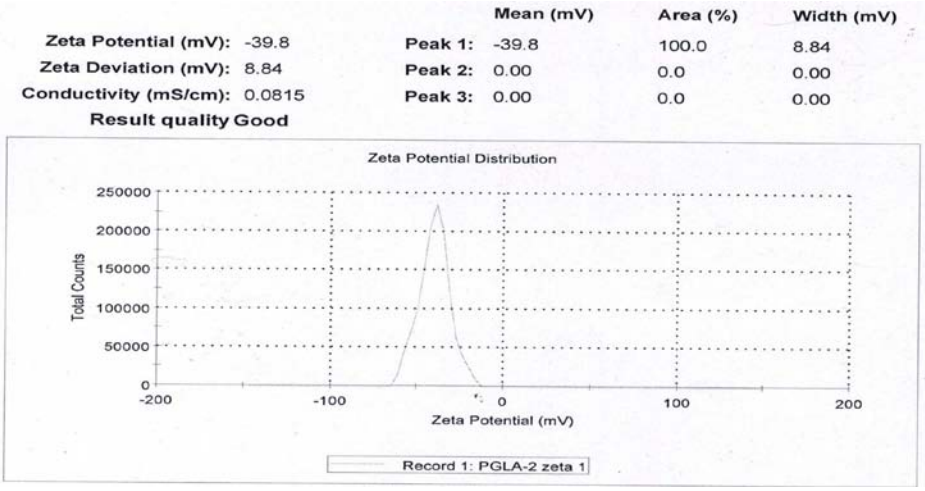

Fig. 7: Elaborating zeta potential of the optimized batch

Table 5: Zeta potential study

S. No.	D P Ratio	Zeta Potential (mV)	Particle Size
1	1:1	-11.07	300.00
2	1:2	-18.20	282.00
3	1:3	-26.20	249.00
4	1:4	-39.80	202.20
5	1:5	-21.02	290.20

In vitro release and kinetics study

The cumulative percentage released in PBS buffer was 55.32±5.89 to 74.42±5.08 %. PLGA copolymer undergoes degradation by hydrolysis or biodegradation through cleavage of its backbone ester linkages into oligomers and finally monomers. This has been demonstrated in both *in vivo* and *in vitro* for various drug types and proteins with different polymer ratios (Ramchandani *et al.*, 1998; Amann *et al.*, 2010). The degradation process for these polymers is mainly through uniform bulk degradation of the matrix where the water penetration into the matrix is higher than the rate of polymer degradation. The degradation of PLGA copolymer is the collective process of bulk diffusion, surface diffusion, bulk erosion and surface erosion. Since there are many variables such as (molecular weight concentration, interaction with the carrier, diffusion and polymer-related variables such as size and density of particle, amount and type of matrix material surface erosion etc) that influence the degradation process, the release rate pattern is often unpredictable. Formulation clearly showed initially burst release followed by sustained release, matrices having higher drug content possess a larger initial burst release than those having lower content because of their smaller polymer to drug ratio. The initial burst of drug release is related to drug type, drug concentration and polymer hydrophobicity. Drug on the surface, in contact with the medium is released as a function of solubility as well as penetration of water into the polymer matrix. Random scission of PLGA decreases the molecular weight of polymer significantly, but no appreciable weight loss and no soluble monomer product are formed in this phase. Then the drug is released progressively through the thicker drug depleted layer. The water inside the matrix hydrolyzes the polymer into soluble oligomeric and monomeric products. This creates a passage for the drug to be released by diffusion and erosion until complete polymer solubilisation. The best-selected *in vitro* release data of optimized formulation was fitted to various mathematical models such as zero order, first order, Higuchi and Peppas kinetic models. The highest regressions (0.981) was obtained for zero order kinetics followed by Higuchi (0.968) and first order (0.803) and Peppas (0.709) model. The drug release mechanism was found to be diffusion controlled and nearly zero order sustained release was observed.

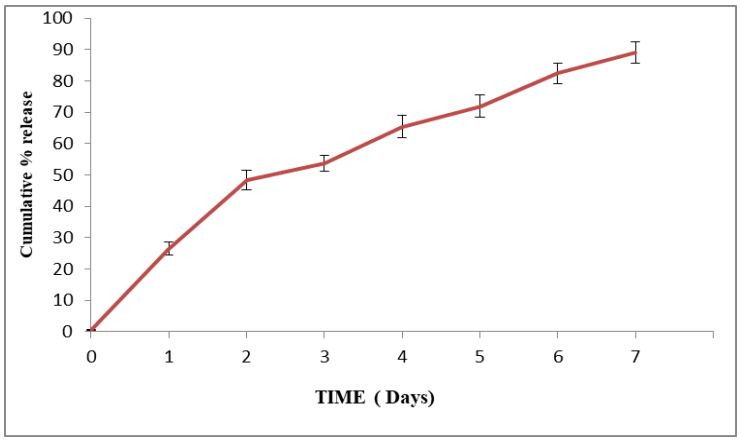

Fig. 8: Cumulative *in vitro* release of PLGA microspheres using Franz diffusion cells and pH 7.4 PBS at 37 °C. Error bars indicate SD For n=3

Table 6: Correlation coefficient of the optimized batch

S. No.	Zero order	First order	Higuchi	Krosmeyer Peppas
1	0.981	0.803	0.968	0.709

Stability study

International Conference on Harmonization (ICH) Guideline on drug stability Q1A (R2) requires that analysis of stability samples should be done by the use of validated stability-indicating analytical methods. It also recommends carrying out of stress testing on the drug substance to establish its inherent stability characteristics and to support the suitability of the proposed analytical procedure. The stress testing encompasses the influence of temperature, humidity, and light, oxidizing agents as well as susceptibility over a wide range of pH values.

We also try to study degradation of zidovudine under different ICH recommended stress conditions, and to establish a validated stability-indicating HPLC method. Zidovudine is chemically 3-azido-3-deoxythymidine. It is active against human immunodeficiency virus (HIV-I and-II) and human T-celllymphotropic virus (HTLV-I and-II). The drug is official in USP and BP. The drug is reported to exhibit acid and water catalyzed degradation and remained stable in alkali. Recently, its decomposition behaviour in hydrolytic and photolytic conditions was compared with stavudine. The stability of the drug in pharmaceutical dosage forms. However, there is no report yet on the development of stability-indicating assay method for the drug, employing ICH suggested approach. Otherwise, a number of reports are available for the determination of drug in the intracellular milieu and from biological fluids, such as plasma and urine.

A validated stability-indicating HPLC assay method was developed for zidovudine, using the stress-testing route suggested by ICH. The

developed method is simple, accurate, precise, specific, and could separate drug from degradation products. It is suggested for use in analysis of samples generated during stability studies on zidovudine and its formulations. The stability study of the selected formulation of the AZT loaded microsphere was carried out by storing 1 gm of microparticles, in an amber colored screw capped bottle for a period of 1months at 40±2 °c temperature and 75±5%RH Using stability chamber (Thermolab, Mumbai) Sampling was carried out a 10days interval and examined for percent entrapment (table 7).

Table 7: Stability data of the optimized batch

Sampling time	0 D	10 D	20 D	30 D
Optimized Batch	68.40±0.10	76.26±0.17	82.34±0.34	81.34±0.24

SD; Standard Deviation for n=3.

The drug decomposed under hydrolytic stress upon refluxing, and on exposure to light. It was stable to oxidation and thermal stress. The same major decomposition product could be seen in all the decomposed solutions, which was identified as thymine through comparison with the standard. Separation of drug from major and minor degradation products was successfully achieved on a C-18 column utilizing water–methanol in thereto of 75:25. The detection wavelength was 265 nm. The method was validated and the response was found to be linear in the drug concentration range of 25-500 µg/ml.

The mean values (±R. SD) of slope and correlation coefficient were 20, 659 and 0.9995, respectively. The R. SD values for intra-and inter-day precision were<0.6 and<1.2%, respectively. The method was established to have sufficient intermediate precision as similar separation was achieved on another instrument handled by a different operator. The recovery of the drug from a mixture of degraded samples ranged between 102.2 and 100.6%. PDA peak purity test confirmed the specificity of the method. The method was also successful in analysis of drug in marketed tablets subjected to stability testing under accelerated conditions of temperature, humidity, and to thermal and photolytic stress. The drug contents of the samples were analyzed after 10, 20 and 30 d of storage and there were no significant changes in the drug content. Hence, it can be concluded that the developed Zidovudine microsphere were stable and retained their pharmaceutical properties over a period of its shelf life.

CONCLUSION

Microsphere coating or encapsulation with polymer using the double emulsion solvent diffusion method was investigated in this research. The results revealed that 216-306 µm microsphere was successfully coated or encapsulated in polymer. FT-IR analysis is another valuable qualitative analysis method of material characterization. PLGA microsphere has high percentage yield, entrapment efficiency, SEM Study with good reproducibility from batch to batch. This coating process is a promising environmentally friendly technique for microsphere coating/encapsulation with polymer with applications of pharmaceuticals and other products where chemical interactions must be avoided. The method used for the preparation of AZT loaded microparticles is a rapid and simple technique. The microspheres were of an appropriate size and suitable for oral administration. Thus the current investigation show promising results of PLGA microspheres as a matrix for drug delivery and merit for *In vivo* studies for scale up the technology.

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AUTHORS CONTRIBUTION

Kohli S makes substantial contributions to acquisition of data, analysis and interpretation of data and drafting the article. Jain S participates in concept, and data analysis and interpretation. Pal A participates in concept, guidance, critical review, and article revision.

CONFLICT OF INTERESTS

The authors have declared that there is no conflict of interest

REFERENCES

1. Athanasiou KA, Niederauer G, Agrawal CM. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials 1996;17:93-102.
2. Bodmeier R, Chen H. The effect of the addition of low molecular weight poly(dl-lactide) on drug release from biodegradable poly(dl-lactide) drug delivery system. Int J Pharm 1989;51:1-8.
3. Brook I. Basic principles of drug discovery and development. J Am 1987;258:1517.
4. Cho JC, Khang GJ, Rhee MY, Kim S, lee JS, Lee HB. Preparation and characterization of Poly(lactide-co-glycolode) Microsphere for the sustained release of AZT. Korea Polym 1999;8:80-8.
5. Chow FPR, Hamburger. *In vivo* evaluation of the anemia induced by azidothymidine (AZT) in a murine model of AIDS. Eur J Haematol 1991;47:91-7.
6. Christoper G, Peter V, Raghavan C, Vijaya S, Hari K, Prasad R. Formulation and optimization of coated PLGA-Zidovudine nanoparticles using factorial design and *in vitro in vivo* evaluations to determine brain targeting efficiency. Saudi Pharm J 2014;22:133-40.
7. Devita VT, Broder S, Fauci AS, Kovacs JA, Chabner BA. Developmental therapeutics and the acquired immunodeficiency syndrome. Ann Intern Med 1987;106:568–81.
8. Gopferich A. Polymers degradation and erosion mechanisms and applications. Eur J Pharm Biopharm 1996;42:1-11.
9. Gottlieb MS, Schroff R, Schanker HM. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 1981;305:1425-31.
10. ICH, Stability Testing of New Drug Substances and Products, International Conference on Harmonization IFPMA, Geneva; 2003.
11. Ramchandani M, Robinson D. *In vitro* and *in vivo* release of ciprofloxacin from PLGA 50:50 implants. J Controlled Release 1998;54:167-75.
12. Rohit RB, Riyaz Ali MO, Padmaja C, Afrasim M. Formulation and evaluation of sustained release dosage form using modified cashew gum. Int J Pharm Pharm Sci 2015;7:141-50.
13. Chawla SJ, Amiji MM. Biodegradable polycaprolactoe nanoparticles for tumor targeted delivery of tamoxifen. Int J Pharm Pharm Sci 2002;249:127-38.
14. Amann LC, Gandal MJ, Lin R, Liang Y, Siegel SJ. *In vitro–in vivo* correlations of scalable PLGA-risperidone implants for the treatment of schizophrenia. Pharm Res 2010;27:1730–7.
15. Sandrap P, Moes AJ. Influence of manufacturing parameters on the size characteristics and the release profiles of nifedipine from poly (DL-lactide-co-glycolide) microspheres. Int J Pharm 1993;98:157-64.
16. George C, Park TG. Preparation of porous and nonporous biodegradable polymeric hollow microspheres. J Controlled Release 1995;35:91-105.
17. Bodmer D, Kissel T, Traechslin E. Factors influencing the release of peptides and proteins from biodegradable system. J Controlled Release 1992;21:129-38.
18. Duan Y, Sun X, Gong T, Wang Q, Zhang Z. Preparation of DHAQ loaded MPEG-PLGA and evaluation of drug release behaviour *in vitro*/*in vivo*. J Mater Sci Mater Med 2016;17:509-16.

19. Zhang P, Chen L, Gu W. *In vitro* and invivo evaluation of donepezil–sustained release microparticles for the treatment of Alzheimers disease. Biomaterials 2007;28:1882-8.
20. Stolnik S, Garnett MC. The colloidal properties of surfactant free biodegradable nanospheres from (ß-malic acid–co-benzyl malate) s and poly(lactic acid-co-glycolide). Colloids Surf 1995;97:235-45.
21. Makino K, Ohshima H, Kondo T. Transfer of protons from bulk solution to the surface of poly(L-lactide) microsphere. J Microencaps 1986;3:195-202.