WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 17, 1006-1017. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF MICROSPHERES CONTATING LAMAVUDINE

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Article Received on
29 October 2017,

Revised on 19 Nov. 2017,
Accepted on 10 Dec. 2017

DOI: 10.20959/wjpr201717-10420

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ABSTARCT

Lamivudine is an antiretroviral drug, specifically a *nucleoside reverse transcriptase inhibitor*. It is used to treat HIV, a retrovirus. Retroviruses use the genetic material in the body's cells to produce more viruses which can infect other cells. Adverse effect of lamuvidine headache, fever, chills, and muscle aches, dizziness, nausea, vomiting, insomnia, restlessness, and rash. The main objective of this research work was to prepare hydroxypropyl methyl cellulose microspheres loaded with lamuvidine and Invitro release study. In the present study, emulsification heat stabilization method is used in the preparation microspheres. The polymer hydroxyproply methylcellulose was dissolved and added to the solution and stirred it for 2 hrs. In this experiment, lamuvidine 100mg was taken and dissolved in span-60 10-15ml. then 100mg hydroxypropyl methylcellulose is taken and dissolve in 10-15ml. these both beakers are kept aside until the solutions get dissolved. Then these 2 dissolution are taken in one beaker. The drug is added in the polymer solution drop by drop with the help of mechanical stirrer resulting in formation of microspheres. Microspheres were spherical shape and smooth surface. Infrared spectra showed identical peaks of the drug and polymer. Drug entrapment efficiency was determined by uv-spectrophotometer at 254 nm. In vitro release studies were performed by using shaking flask method about drug was released in 6hrs. It is concluded that hydroxlyproply methylcellulose and microspheres of lamuvidine can be prepared by emulsification heat stabilization in vitro release data is satisfactory.

KEYWORDS: Lamivudine, span-60, hydroxyproply methylcellulose, microspheres.

INTRODUCTION

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. These are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. The approach facilities the accurate delivery of small quantity of the potent drugs, reduced drugs concentration at the site other than the target site and the protection of the liable compound before and after the administration and prior to appearance at the site of action. One such approach is using microspheres as carries of drugs.[17] Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers having a particle size ranging from 1-1000 μm.

An intelligent approach to therapeutics employing drug carrier's technology required a detailed understating of the carrier interaction drugs in vivo can be manipulated by coupling the drug to a carrier particle. The clearance kinetics, tissues distribution, metabolism and cellular interaction of the drug are strongly influenced by the behavior of the carrier. The most convenient and commonly employed route of drug delivery has historically been by oral ingestion. Drugs that are easily absorbed from GIT and having a short half-life are eliminate quickly from the blood circulation. Efforts to improve oral drug bioavailability have grown in parallel with the pharmaceutical industry.

DEFINITION AND GENERAL DESCRIPITION

Microspheres are defined a " monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particular dispersion of particles" they can also defined as a structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers having a particle size ranging from 1-1000 μm.[18,19,20]

Microspheres are made of polymeric, waxy, or other protective materials that may be synthetic polymers or modified natural products such as starch, waxes etc. the natural polymers include albumin and gelatin and the synthetic polymers include polylactic acid and polyglycolic acid.

Microspheres are sometimes referred to as micro particles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available. Solid and hollow microspheres vary widely in density and, therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material. Solid microspheres have numerous applications depending on what material they are constructed of and what size they are.

Polyethylene and polystyrene microspheres are two most common types of polymer microspheres. Polystyrene microspheres are typically used in biomedical applications due to their ability to facilitate procedures such as cell sorting and Immune precipitation. Proteins and ligands adsorb onto polystyrene readily and permanently, which makes polystyrene microspheres suitable for medical research and biological laboratory experiments. Polyethylene microspheres are commonly used as permanent or temporary filler. Lower melting temperature enables polyethylene microspheres to create porous structures in ceramics and other materials. High sphericity of polyethylene microspheres, as well as. Availability of colored and fluorescent Microspheres makes them highly desirable for process troubleshooting and numerous Research applications. Charged polyethylene microspheres are also used in electronic paper digital displays. Glass microspheres are primarily used as filler for weight reduction, retro-reflector for highway safety, additive for cosmetics and adhesives, with limited applications in Medical technology. Ceramic microspheres are used primarily as grinding media.

THEORY

Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity. Further, currently available slow release oral dosage forms, such as enteric coated/ double-layer tablets which release the drug for 12-24 hours still result inefficient systemic delivery of the drug and potential gastrointestinal irritation.

Microencapsulation for oral use has been employed to sustain the drug release, and to reduce or eliminate GIT irritation. In addition, multi particulate delivery systems spread out more uniformly in the GIT. This results in more reproducible drug absorption and reduces local irritation when compared to single-unit dosage forms such as no disintegrating, polymeric matrix tablets.

ADVANTAGES OF MICROSPHERES[21,22]

- Microspheres provide constant and prolonged therapeutic effect.
- Reduces the dosing frequency and thereby improve the patient compliance.
- They should be injected into the body due to the spherical shape and smaller size.
- Taste and odor masking.
- For easy handling (e.g. conversion of oils and other liquids to solids).
- Improvement of flow properties of powders.
- Safe handling of toxic substance.
- Prevention or delay of volatilization.
- Separation of incompatible materials (other drugs or Excipient such as buffer).
- For dispensing water-insoluble substance in aqueous media.
- Production of sustained-release, controlled-release, and targeted medications.
- Microspheres have also applications in inject able and inhalation products.

MATERIALS AND METHODS

Lamivudine was purchased from Karnataka antibiotic centre, hydroxy propyl methyl cellulose was purchased from the keerti agencies uv Scientifics, Di-sodium hydrogen phosphate was purchased from the zopinax pharma Ltd(Ahemadabad), potassium di hydrogen phosphate was purchased from the Lab press pharma, diethyl ether was purchased from the Sartourious Lab, tween-60 was purchased from the Sarco chemical ltd, span-60 Sarco chemical ltd.

METHODS

Preparation of microspheres of Lamivudine by emulsification heat

Stabilizing method

300 mg Lamivudine of and polymer (HPMC) were dissolved in 20 ml of deionized water and added 5 ml of egg albumin solution, 0.1% of Tween-80, stirring it for 30 min. The prepared solution was used as aqueous phase. The oil phase was prepared by mixing 20 ml of sunflower oil and 5ml of diethyl ether with 1% span-80 (as emulsifier) and stirred it for 20 mins at 800-1000 rpm on a magnetic stirrer. The primary emulsion was prepared by adding the oil phase drop wise to the aqueous phase stirred it for 30 mins at 800-1000 rpm. The prepared primary emulsion was added to pre-heated (65 to 70oC) sunflower oil (80 ml) by using 21 No. needle and stirred it 1000-1200 rpm for 2 hrs till the solidification of microspheres formed. The suspension was then allowed to cool to room temperature with

continuous stirring using a magnetic stirrer. On cooling, 100 ml of anhydrous ether was added. The suspension containing the micro-spheres was centrifuged for 15 min and the settled microspheres were washed three times with ether to remove traces of oil on microspheres surfaces.[5] The obtained microspheres were then vacuum dried in a desiccators overnight and stored at 4o c in dark.

STANDARD CALIBARATION CURVE

In Phosphate Buffer saline pH 7.4

- Dissolve 2.38g of Disodium hydrogen phosphate. 0.19g of Potassium dihydrogen, Phosphate. 8.0g of sodium chloride in sufficient water to produce up to 1000ml.
- 100mg of Lamivudine was dissolved in small amount of phosphate buffer saline pH 7.4 and volume was made up to 100ml using phosphate buffer saline 7.4 from, The stock solution serial dilutions were done to obtain solutions in the Concentration.
- The absorbance of solution. Were measured 257nm. Using UV-Visible. Spectrophotometer. A graph of concentration vs absorbance was plotted.

Determination of % yield of microspheres

The dried microspheres were collected and weighed accurately. The Percentage yield was then calculated using formula given below.

$$\% \text{ Yield} = \frac{\text{Mass of micro-spheres obtained}}{\text{Total weight of drug and polymer}} \times 100$$

Particle size determination

For size distribution analysis, different sizes in a batch were separated by sieving using a range of standard sieves. The amounts Retained on different sieves were weighed. Optical microscope was used to determine the size of the particle that lies within a range From 0.2 to 100 µm equal divisions and hence, each division is equal to 10 µm and the particles are measured along an arbitrarily chosen fixed line across the center of the particle. The particle size is the important factor to formulation of microspheres.

Invitro release studies - Shaking flask method

Drug loaded microspheres equivalent to 100 mg of drug were weighed and transferred into a 100 ml conical flask. To this 100ml of pH 7.4 phosphate buffer saline was added, then the flasks were kept in a metabolic shaker and the shaker was adjusted to 50 horizontal shakes

per minutes at 37 ± 0.5oC. One ml of the drug releasing media was withdrawn at various time interval of 30 min, 1, 2, 3, 4, 5and 6 hours and replaced by the same volume of phosphate buffer saline. These samples were filtered through 0.45 µm membrane filter. The filtrate was diluted suitably. The drug was estimate in each batch by UV-Visible Spectrophotometer at 257 nm.

FTIR Spectral assay

FTIR apparition assay of complete medication and polymers was accustomed and assay was formed out whether improvements in the admixture foundation of medication consecutive to abutting it with polymers. The samples were crushed with KBr to get pellets by applying pressure on 600 Kg/cm^2 and scanned with the IR instrument (Shimadzu, 8400 Series, Tokyo, Japan) from 400-4000cm.

SEM Analysis

SEM (JEOL 5400, Tokyo, Japan) is a frequently used method for characterizing drug delivery systems, owing in large part to simplicity of sample preparation and ease of operation. Small amount of suspension spreads on the small square plate and coated with a gold ion for 5-6 mins.[18,2] The prepared sample was kept inside the chamber and images captured with different magnifications.

Zeta potential Analysis

The zeta potential was measured using the appropriate instrument (Beckman Coulter Delsa Nano C, Brea, USA).[18,5] The suspension was diluted with double distilled water and taken in the cuvettes and temperature maintained at 25°C.

RESULTS AND DISCUSSION

Table 1: Calibration data of Lamivudine.

CONC	Absorbance
0.2	0.153
0.4	0.497
0.6	0.734
0.8	1.747
1	2.125

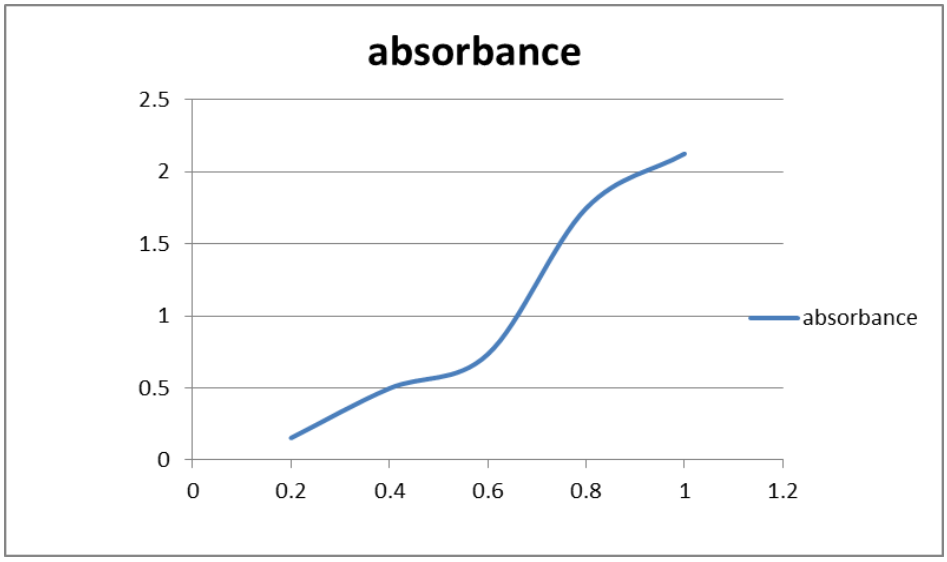

Fig. 1: Standard Graph of Lamivudine.

Table 2: % drug release of lamuvidine microspheres (1:1) ratio.

Time (hrs)	ABS	Concentration	% Drug Release
0.5	0.347	8.2785	8.27%
1	0.685	16.328	16.32%
2	1.055	25.138	25.13%
3	1.390	33.109	33.10%
4	1.644	39.159	39.15%
5	1.905	45.369	45.36%
6	2.301	54.80	54.80%
7	2.747	65.40	65.40%
8	2.995	71.31	71.31%
9	3.328	79.25	79.25%
10	3.675	87.50	87.50%

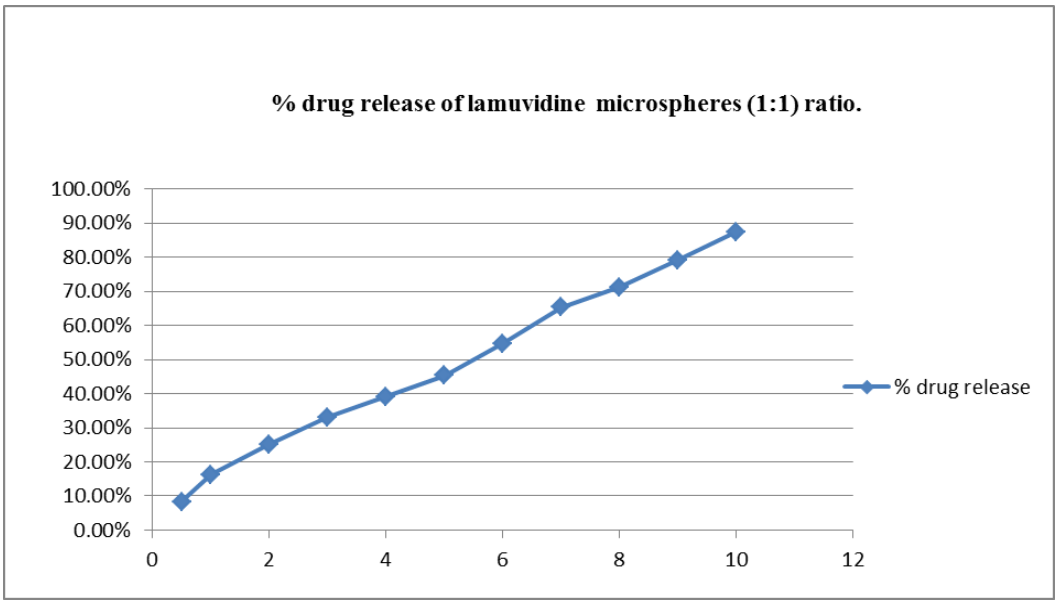

Fig 2: % drug release of lamuvidine microspheres (1:1) ratio.

Table 3: % drug release of lamuvidine microspheres (1:2) ratio.

Time (hrs)	ABS	Concentration	% Drug Release
0.5	0.287	6.83	6.83%
1	0.436	10.40	10.40%
2	0.745	17.75	17.75%
3	1.04	24.92	24.92%
4	1.288	30.07	30.07%
5	1.629	38.80	38.80%
6	1.955	46.55	46.55%
7	2.338	55.68	55.68%
8	2.718	64.71	64.71%
9	3.164	75.33	75.33%
10	3.496	83.25	83.25%

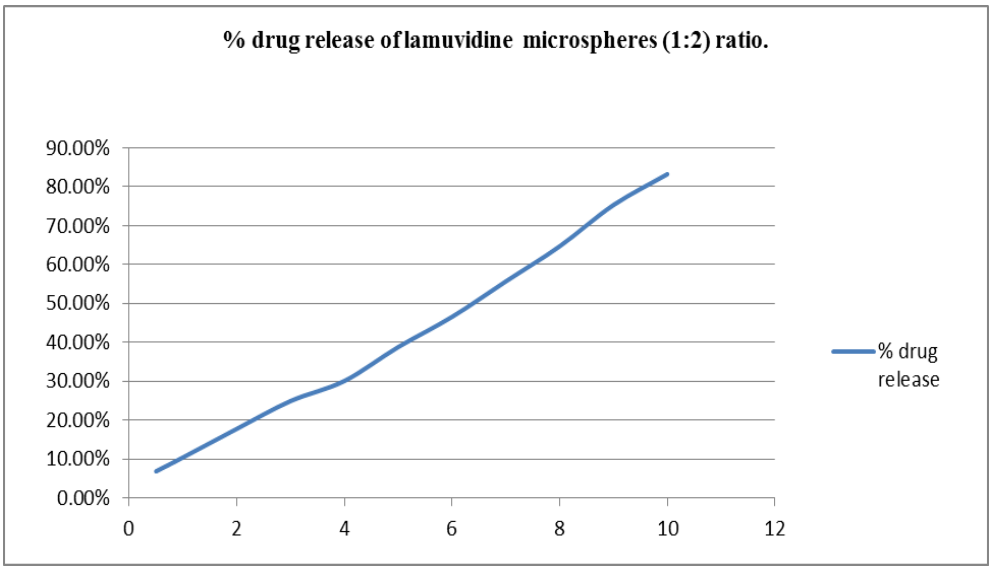

Fig 3: % drug release of lamuvidine microspheres (1:2) ratio.

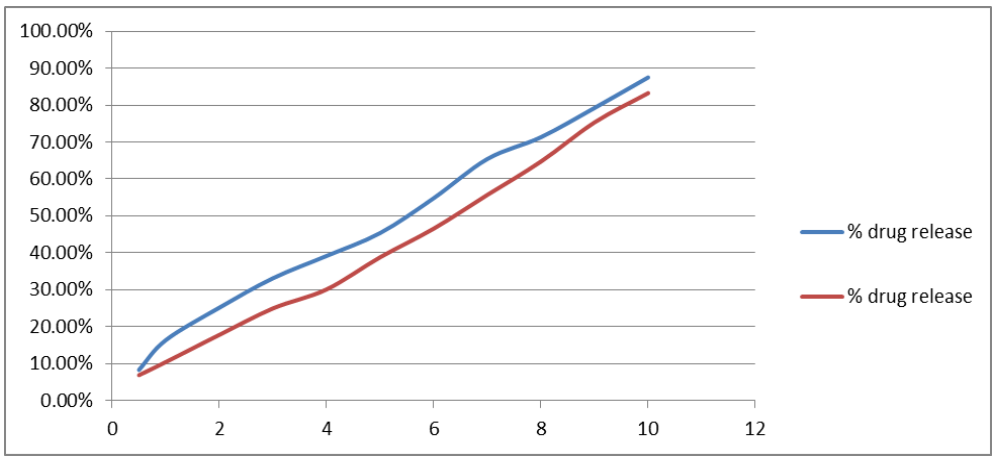

Fig 4: Comparison between (1:1) and (1:2) % drug release.

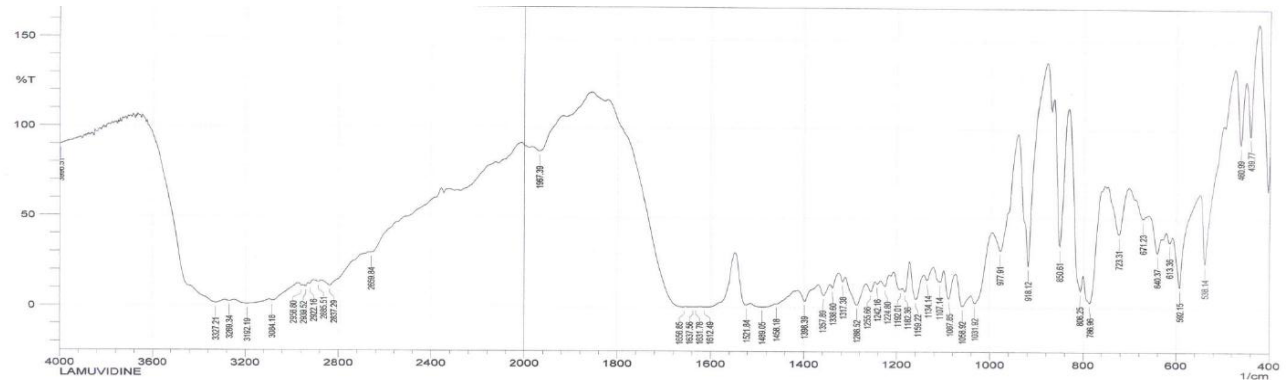

Fig 5: The FTIR graph of drug lamivudine.

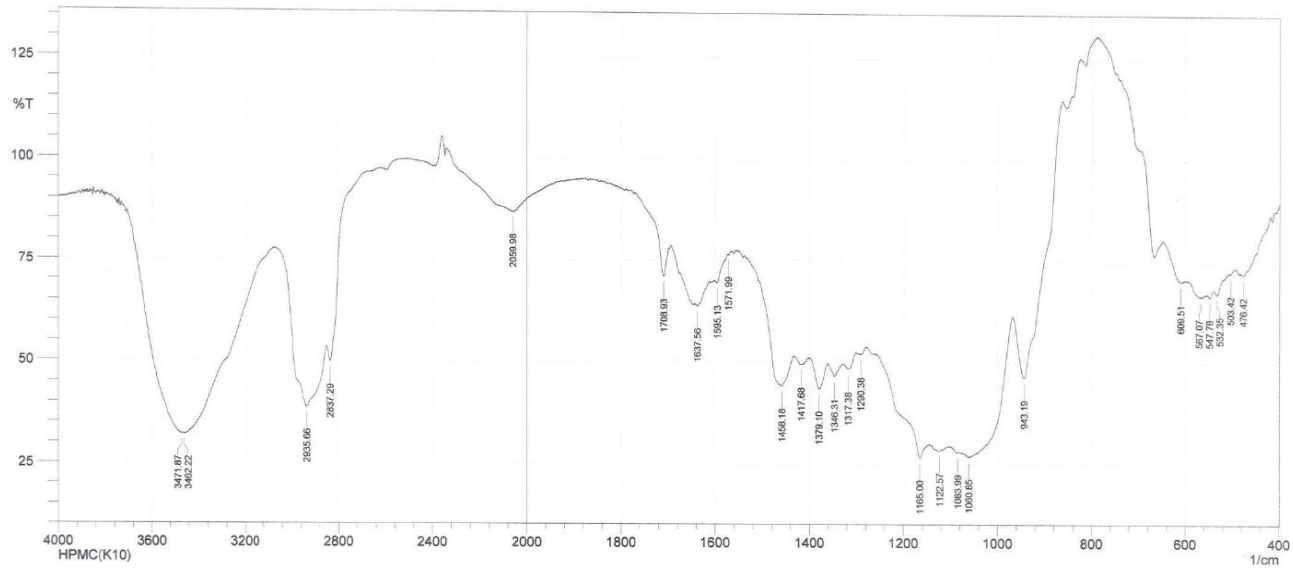

Fig 6: The FTIR graph of polymer HPMC

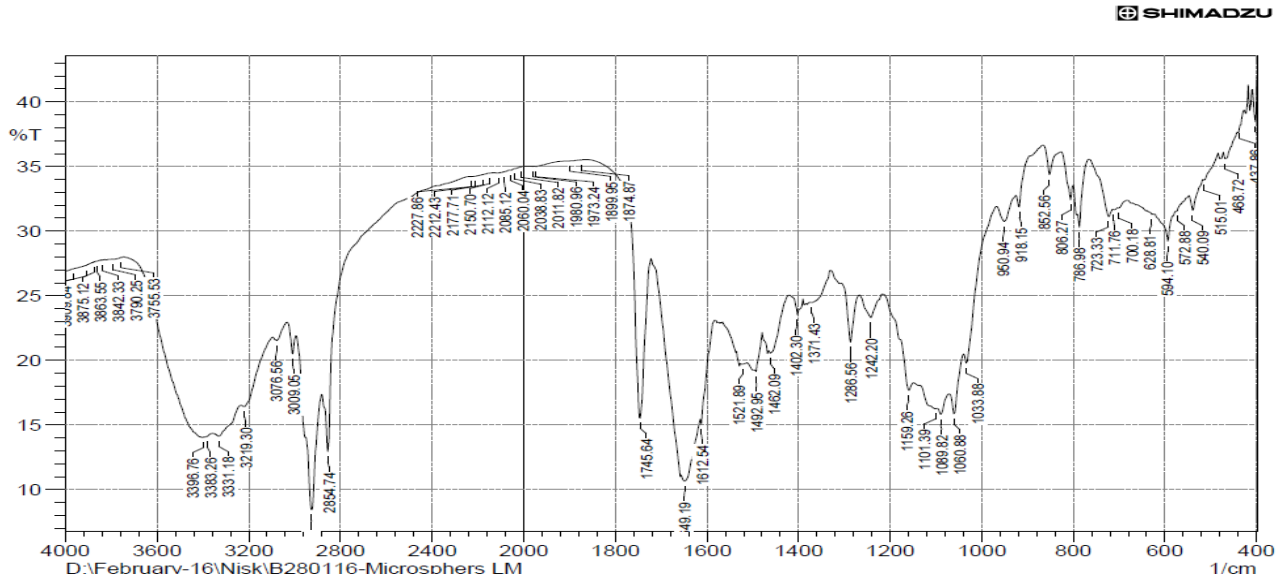

Fig 7: The FTIR graphs shows that there was no chemical interaction between drug-polymer and mixture.

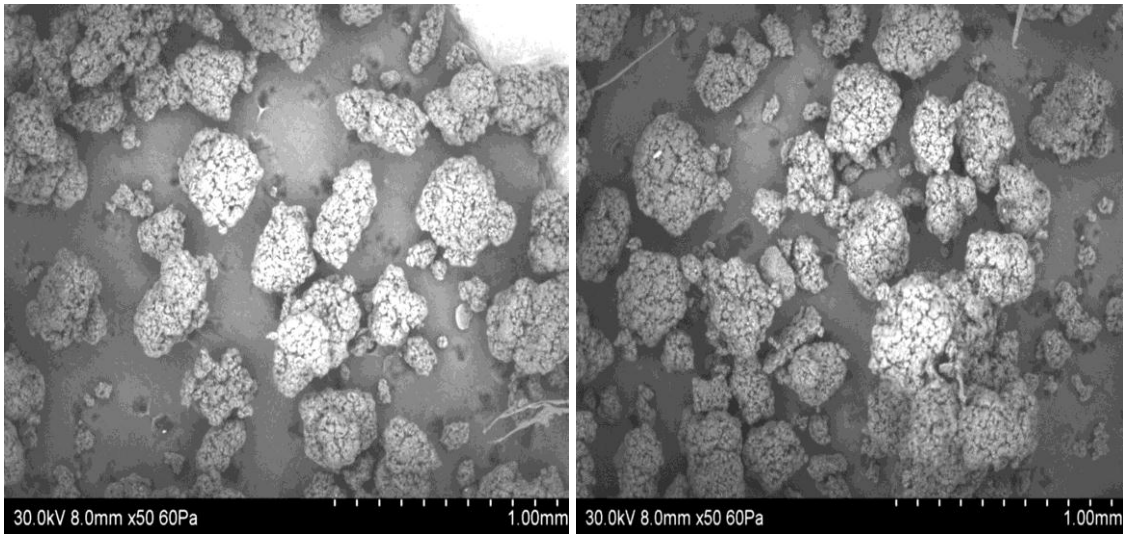

Fig 8: The SEM says that microsphere is spherical and uniform in size. At magnification of 50x.

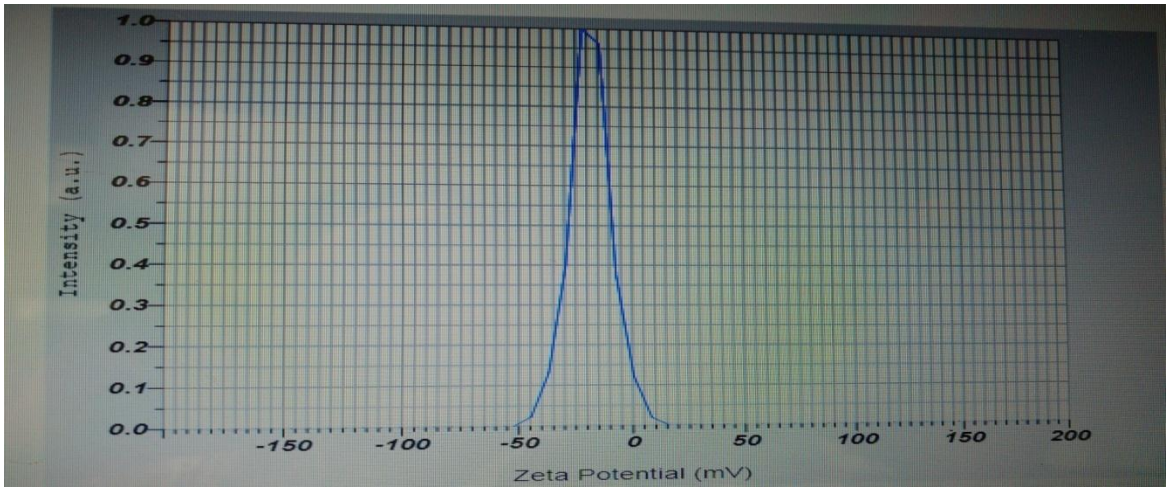

Fig 9: The zeta potential from the graph mean was found to be -18.4m.

CONCLUSION

In the present study a satisfactory attempt was made to develop micro particulate drug delivery system of the lamuvidine with improved bioavailability.

From the experiment results it can be concluded that

- ✓ HPMC polymer is a suitable for the preparation of microspheres of lamuvidine.
- ✓ Particle size analysis reveals that the microspheres were in the range and all the formulation showed surface characters.
- ✓ In vitro studies showed that LM-1 shows 87.50% and LM-2 shows 83.25%;
  So LM-2 shows sustained release activity.

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