Fabrication and Characterization of Lamivudine Embedded Gum Sterculia Microspheres by Emulsification-Internal Gelation Technique

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ABSTRACT

The present study was aimed to fabricate and characterize controlled release microspheres of lamivudine by using a natural gum sterculia as microencapsulating agent. An emulsification-internal gelation technique was employed for design of different batches which can be made industrially feasible. The batches designed were characterized *in vitro* for particle morphology, microencapsulation efficiency, production yield, swelling properties, micromeritic properties,

release profile and release kinetics etc. Physico-chemical characteristics of lamivudine and lamivudine loaded microspheres were evaluated by infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) technique. The drug loaded microspheres were found to be roughly spherical, discrete and free flowing in nature. Microencapsulation efficiency was in a narrow range suggesting an identical distribution of drug in different batches. DSC and XRD results revealed a partial modification in lamivudine's solid state. The drug release from the optimized batches were found to be slow and extended up to 24 hours depending on the core: coat ratio. Finally after kinetic fitting the release mechanism was found to be following non-Fickian diffusion from the drug loaded microspheres.

Keywords: Lamivudine, Controlled release, Gum, Microspheres, Sterculia

INTRODUCTION

Drug discovery alone is insufficient in treating diseases; often correct dosing and targeting is equally important for clinical success. Controlled drug delivery systems are aimed at controlling the release of the drug at a therapeutically effective rate, prolonging the duration of drug delivery & therapeutic response and targeting the delivery of the drug to a tissue[1-4].

One of the very common types of orally administered controlled release system is microparticles which includes microcapsules and microspheres, produced by a process known as microencapsulation. Moreover these are multiunit systems that spread over a large surface area of GIT and release the drug more uniformly instead of vagaries of gastric emptying and different transit rates through the gastrointestinal tract[5-7].

Natural biodegradable polymers remains attractive to serve as a release retarding microencapsulating agent primarily because they are readily available in the nature, relatively inexpensive, products of living organisms, readily undergoes *in-vivo* degradation, non-toxic and

capable of chemical modifications[8]. In the present study gum sterculia was used as a natural microencapsulating agent to retard the release of the drug. It is the dried exudation obtained from the stems and branches of "*Sterculia Uren's*", belonging to the family *Sterculiaceae*, a tree native to India. It was successfully evaluated for its suitability in the preparation of hydrophilic matrices, mini-matrices, and microspheres etc[9, 10].

In the current investigation lamivudine loaded microparticulate beads were formulated using the emulsification-internal gelation technique in order to improve the drug loading and for achieving controlled drug delivery[11, 12].

Lamivudine (The (-)-enantiomer of 2′,3′-dideoxy-3′-thiacytidine), is a synthetic nucleoside analog which hinders HIV reverse transcriptase and is being increasingly used as a powerful antiretroviral agent for treatment of AIDS[13]. Lamivudine, chemically $C_8H_{11}N_3O_3S$ is soluble in water with a molecular weight of 229.256 g/mol. Because of its rapid absorption, it produces a bioavailability of around 82% following oral intake. Conventional oral dosage forms of lamivudine are administered several times a day (150 mg bid), because of its moderate half-life (5-7 hours)[14, 15]. However these conventional oral dosage forms are associated with several limitations, like adverse side effects producing from drug buildup during multidose therapy, high cost of therapy, poor patient adherence, etc. Therefore the inception of controlled release of lamivudine would be advantageous in contrast with the conventional dosage regimens[16]. In the present study, anti retroviral drug lamivudine loaded microspheres were developed by using natural, biodegradable, non-toxic gum sterculia for controlled release.

MATERIALS AND METHODS

Lamivudine was obtained as gift sample from HETERO DRUGS Ltd. (Hyderabad, India). Gum sterculia was obtained as a gift sample from Girijan Corporation, (Viasakhapatnum, India).

Glacial acetic acid **(**Finnar chemicals**)**, petroleum ether (Qualigens), Light liquid Paraffin (Qualigens), span 80 (Finnar chemicals), Barium carbonate (Ranbaxy Fine chemicals) etc. were used. All reagents were of pharmaceutical grade and used as received.

Preparation of microspheres

Microspheres containing lamivudine were prepared employing gum sterculia in combination with sodium alginate as supportive coat materials. Different batches of lamivudine-loaded microspheres were prepared by emulsification internal gelation technique. Sodium alginate and gum sterculia were dissolved in 50mL of distilled water, stirred magnetically with gentle heat to form a homogeneous polymer solution. The active substance lamivudine and cross linking agent were added to the polymer solution and mixed thoroughly to form a viscous dispersion. The drug to polymer ratio was varied keeping the amount of drug and sodium alginate constant in all cases, but changing the amount of gum sterculia. Then the dispersion was extruded through a 23 gauze needle into light liquid paraffin containing 1% of span-80 and 0.2% of glacial acetic acid being kept under magnetic stirring (Remi MS-301) at 500 rpm to undergo emulsification and to form the microspheres. The formed microspheres were allowed to keep as such for 30minutes to produce rigid discrete particles. The microspheres were decanted (after sedimentation), filtered and washed with petroleum ether to remove the traces of paraffin oil. The product thus obtained was dried at 45 ^{0}C for 24 hours. The microspheres thus formulated with their different core to coat ratio are enlisted in Table 1.[17].

Estimation of lamivudine

Accurately weighed microspheres equivalent to 100 mg of drug were crushed and suspended in 100 ml of pH 7.4 phosphate buffer. The resulting mixture was stirred at 1000 rpm for 2 hours

and kept overnight. Then the solution was filtered, diluted suitably and analyzed for drug content at 270.01 nm using UV-visible spectrophotometer (Carry 60, Agilent, Australia)[18].

Production Yield and Microencapsulation efficiency

Production yield or percentage yield is calculated by using the following Equation[19]:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of microspheres obtained}}{\text{Weight of raw materials}} \times 100$$

Microencapsulation efficiency[20] was calculated using following formula:

$$\text{Microencapsulation efficiency (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Micromeritic properties

Micromeritic properties, such as angle of repose, tapped density and bulk density were measured. The angle of repose[20] was measured using static funnel method. The experiments were carried out in triplicate.

Determination of particle size distribution by sieve analysis

Separation of the microspheres into various size fractions was carried out using a mechanical sieve shaker. A series of five standard stainless steel sieves (Geologists Syndicate Pvt. Ltd, India) having mesh size of #14, #18, #20, #25 and #30 were arranged in an order of decreasing aperture size. A specific quantity of drug loaded microspheres was placed on the uppermost sieve. The sieves were shaken for a period of 10 min, and then the particles on each screen were weighed[21]. The procedure was carried out three times for each product.

Characterization of lamivudine microspheres

FT-IR studies

Drug-polymer interactions were studied by FT-IR spectroscopy using the instrument Shimadzu, Japan, FTIR-8400S. The spectra were recorded for pure drug lamivudine and the microspheres containing drug. Samples were prepared in KBr discs (2 mg sample in 200 mg KBr) with a hydrostatic press at a force of 5.2 N/m^2 for 3 min. The scanning range was 400–4000 cm-1 and the resolution was 4 cm-1[18].

Surface scanning electron microscopy (SEM)

The surface morphology of the microspheres was observed by using scanning electron microscope (LEO 440i, England). The samples were mounted on an aluminum sample stub using adhesive carbon tape and placed in a low humidity chamber for 12 h prior to analysis. Samples were coated with gold-palladium for 60 sec under an argon atmosphere using ion sputter coater in a high vacuum evaporator equipped with a rotary stage tray. Images were taken at an acceleration voltage of 20 kV[18].

Differential scanning calorimetry

The thermal behavior of the microspheres was investigated using differential scanning calorimeter (DSC 60, Shimadzu, Japan). Samples of about 5 mg were placed in 50 μm perforated aluminium pans and sealed. All samples were run at a heating rate of 10^0/min over a temperature range of 5–300^0C in atmosphere of nitrogen as purging gas at a flow rate of 25 ml/min[18].

X-ray diffraction analysis

Microspheres were subjected to X-ray diffraction analysis, using Philips PW 170 system (Philips USA) with Cu-Kα radiation (400 kV, 30 mA, and scan speed 1˚/min) to investigate the physical state of lamivudine entrapped in the microspheres[18].

***In-vitro* drug release studies**

The *in-vitro* release rate study of lamivudine from gum sterculia-coated microspheres were carried out for 24 hours using paddle type dissolution apparatus (USP-XXIII, ETC-11L, Electrolab, Mumbai) containing 900 ml of dissolution medium maintained at 37±0.5 ^{0}C and speed of agitation at 100 rpm[22]. An accurately weighed quantity of microspheres containing around 100mg of drug were suspended in dissolution medium consisting 900 ml of phosphate buffer pH 7.4, and the process was continued up to 24 hours. The system was adjusted to ensure sink conditions. Aliquots (5 ml) of the dissolution medium were withdrawn at predetermined time intervals, filtered by using Whatman No. 42 filter and were replenished immediately with the same volume of fresh medium. Withdrawn samples were assayed spectrophotometrically at 270.01 nm, the detected wavelength of maximum absorbance of lamivudine in pH 7.4 phosphate buffer (Cary 60, Agilent Technologies). Gum sterculia and sodium alginate did not interfere with lamivudine absorption in pH 7.4 phosphate buffer at this wavelength. The analysis was carried out in triplicate.

Kinetic models and the analysis of the release profiles

The *in vitro* release profiles were fitted on various kinetic models like Higuchi, first-order, Peppas and zero-order equations in order to find out the mechanism of drug release. The rate constants were calculated from the slope of the respective plots. The data obtained were also put in Korsemeyer-Peppas model in order to find out *n* value, which describes the drug release mechanism. The mechanism of drug release from spherical polymeric devices may be Fickian diffusion when the value of $n = 0.43$ or less, anomalous (non-Fickian) transport when the value of *n* lies between 0.43 and 0.85, and case II transport when $n = 0.85$. An exponent value of '*n*' greater than 0.85 signifies super case II transport mechanism[23].

RESULTS AND DISCUSSION

Preparation of microspheres, production yield (%), Estimation of drug content and microencapsulation efficiency (%)

In an attempt to modify the release of lamivudine from the microspheres, different batches of formulations were prepared in which the increasing amounts of gum sterculia were added to the fixed weight of lamivudine and sodium alginate. When hydrophilic drugs like lamivudine are encapsulated using an aqueous phase as the harvesting medium, preferentially they partition out in to the aqueous medium leading to low encapsulation efficiency[24]. It has been reported that as much as 80% lamivudine can partition out in to the outer aqueous processing medium depending on the processing conditions[25]. In the present study an attempt was made to encapsulate lamivudine with sufficiently high encapsulation efficiency employing a natural biodegradable gum like sterculia and using a non-aqueous processing medium (liquid paraffin) with the expectation that for lamivudine it would be non favorable to diffuse out of the microspheres before they form as rigid and discrete particles. Span 80, a non-ionic surface active agent having HLB value 4.3 was used to stabilize the emulsification process by reducing the interfacial tension and preventing the droplets coalescence. The emulsification-internal gelation technique use an oil soluble acid (0.2% glacial acetic acid) in the external oil phase, which diffuse through the oil-water interface into the polymeric dispersed globules containing barium carbonate, resulting in the release of free Ba2+. The sodium ion (Na+) of alginate is exchanged with Ba2+ initiating gelation reaction to form barium alginate gel beads. It was observed that as the drug to polymer concentration increases, the product yield also increases. The low percentage yield in some formulations may be due to the microspheres lost during the washing process. An increase in the encapsulation efficiency was achieved by increasing the polymer ratio (Table 1). It was

observed that the encapsulation efficiencies were within a narrow range suggesting an identical distribution of drug in different batches.

Keeping the drug-polymer ratio constant (LK4), there was a there was a statistically significant ($P < 0.05$, student's t-test) decrease in the encapsulation efficiency of lamivudine with increasing concentration of surfactant (span-80) for the emulsification process (Table 2). This may be due to the fact that the increase in the surfactant concentration permits the remarkable reduction in the size of alginate gel beads as a result of decreasing the interfacial tension and preventing the droplets coalescence, yielding smaller particles with higher drug leaching out into the processing before hardening. When 0.5% span-80 was incorporated, microspheres were not formed because the lower emulsifier content failed to prevent coalescence of the gel beads. Thus it concludes that the emulsifier has a key role to play in the preparation of microspheres.

The volume of the processing medium also significantly influences the encapsulation efficiency of the lamivudine microspheres (Table 2). As the volume of the processing medium was increased from 100 ml to 250 ml and to 500 ml, the encapsulation efficiency significantly decreased ($P < 0.05$, student's t-test) from 81% to 68 and 55%, respectively. This may be due to the fact that increase in the volume of the processing medium allows the gel beads to move freely in the medium, thus reducing the chances of collision induced aggregation and resulting small and uniform microspheres. This could also be a reason of the higher rate of drug extraction into the processing medium and a result of low encapsulation efficiency.

The change in the stirring speed of the processing medium (rpm) also significantly influences the encapsulation efficiency of the lamivudine microspheres (Table 2). The highest encapsulation efficiency was observed with the stirring speed of 500 rpm. The change of stirring speed from 250 rpm to 500 rpm and 750 rpm significantly decrease ($P < 0.05$, student's t-test) the

entrapment efficiency due to the formation of larger and smaller gel beads respectively, ensuring drug diffusion out of the microspheres before their hardening. With a speed of less than 250 rpm, the microspheres use to settle and stick at the bottom of the container forming a solid cake.

Table 1. Data showing core: coat ratio, production yield and microencapsulation efficiency

Formulation Codes	Core: Coat ratio*	% Yield	Microencapsulation Efficiency
LK1	1: 0.7	71.65	76.87
LK2	1: 0.9	74.47	78.51
LK3	1: 1.1	77.49	79.62
LK4	1: 1.3	86.42	80.64
LK5	1: 1.5	85.23	83
LK6	1: 1.7	86.18	87.29

*Coat consists of a combination of sodium alginate and gum sterculia where concentration of sodium alginate is kept constant for all the batches.

Table 2. Effect of various processing parameters on microencapsulation efficiency of optimized batch (LK4)

Processing parameters		Theoretical drug content (%)	Experimental drug content (%)	Microencapsulation efficiency (%)
Surfactant concentration (%)	1	43.478	35.061	80.64
	1.5	43.478	30.341	69.784
	2	43.478	24.435	56.2
Volume of	100	43.478	35.061	80.64

processing medium (ml)	250	43.478	29.614	68.112
	500	43.478	23.795	54.728
Stirring speed (rpm)	250	43.478	31.013	71.33
	500	43.478	35.061	80.64
	750	43.478	27.438	63.107

SEM and micromeritic studies

The SEM photomicrographs of the optimized formulation of lamivudine indicated that the microspheres were discrete, spherical, free flowing, multinucleate, and uniform in shape (Figure 1). Surface of the microspheres appear to be rough, may be due to the presence of drug. The different batches of lamivudine loaded microspheres were assessed for parameters like angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio. The results were given in the table 2. The flow properties of different batches of microspheres were excellent as the angle of repose values were found to be less than 25, compressibility index less than 15% and Hausner's ratio less than 1.25 in case of all the batches. It suggests that microspheres don't require a glidant.

Figure 1. Scanning electron micrographs of lamivudine loaded microspheres (LK4)

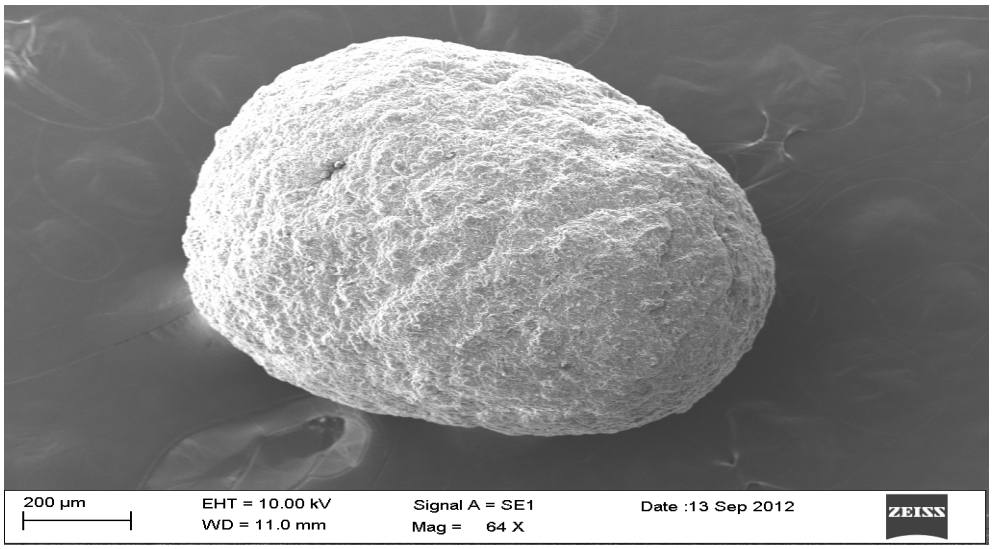


Table 3. Flow properties of microspheres from different batches

Formulation Codes	Angle of Repose ±S.D.	Loose Bulk Density (g/cm^3) ±S.D.	Tapped Bulk Density (g/cm^3) ±S.D.	Carr's Index (%)	Hausner's Ratio
LK1	22.44 ± 0.51	0.517 ± 0.83	0.564± 0.21	8.333	1.09
LK2	24.25 ±0.31	0.529 ±0.91	0.586 ±0.40	9.726	1.107
LK3	23.57 ±0.22	0.527 ±0.42	0.567±0.37	7.054	1.075
LK4	21.84 ±0.52	0.516 ±0.67	0.567 ±0.44	8.994	1.098
LK5	24.53 ±0.65	0.524 ±0.33	0.599 ±0.25	12.52	1.143
LK6	23.59 ± 0.24	0.523 ±0.19	0.586 ±0.22	10.75	1.12

S.D.: Standard deviation; n=3

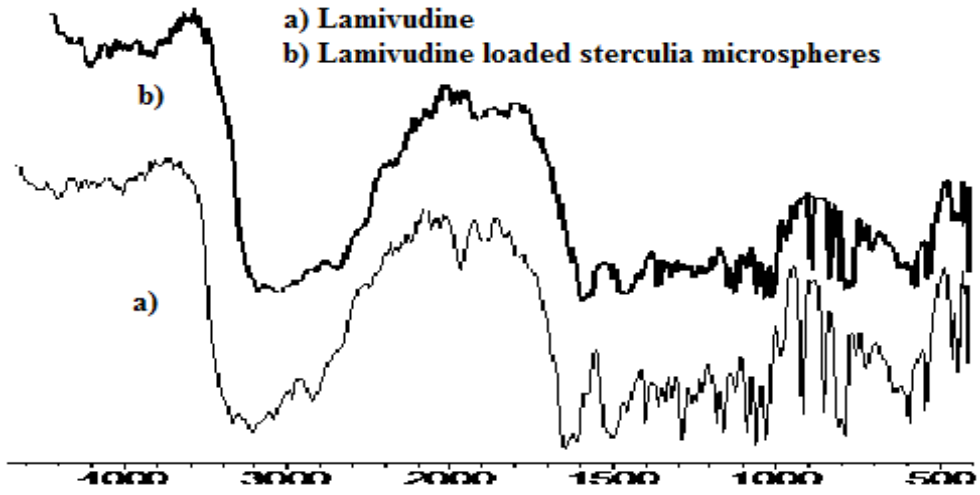
Sieve analysis results of microspheres

Microspheres from different batches presented a narrow particle size distribution and Size analysis showed that a large proportion of microspheres in different batches were in the size range of -18+20 (925 µm) and -20 + 25 (780µm) mesh.

FT-IR studies

The results of FTIR spectral studies showed that there was no significant interaction between the drug and gum. It was observed that there are no major degenerative interactions and hence the gum could be used safely to formulate the microspheres. Lamivudine proved sharp characteristic peaks of carbonyl group at 1650 cm^{-1} (present in the cytidine nucleus). Band peaks at 3319, 3271, and 3197 cm^{-1} remaining to amino and hydroxyl groups. Band Peaks at 1286 and 1161 cm^{-1} remaining to asymmetrical and symmetrical stretching of the C-O-C system (present in the oxathiolane ring). Absence of any modification or interaction between drug and gum were confirmed from the characteristic peaks appeared in the spectrum.

Figure 2. FTIR spectra of pure lamivudine and lamivudine loaded microspheres (LK4)

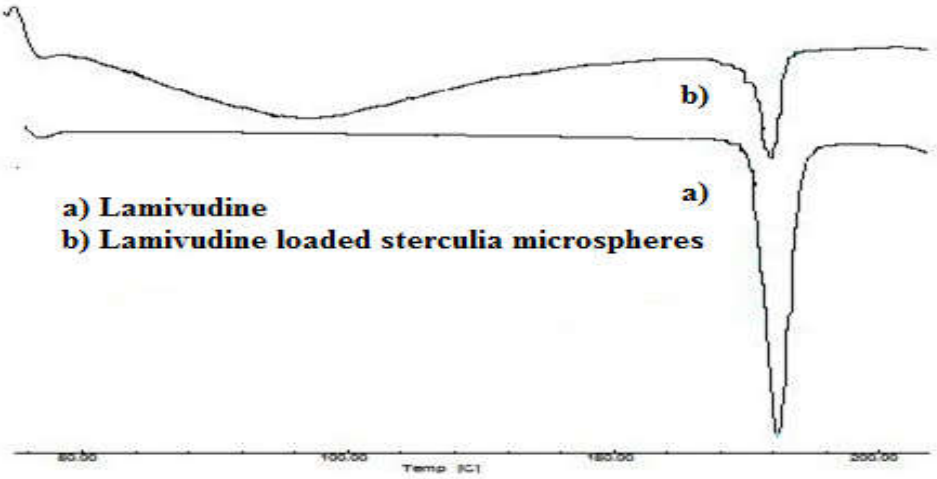


Differential scanning calorimetry

The compatibility of lamivudine in gum sterculia microspheres was evaluated through DSC analysis. The DSC thermograms of pure lamivudine and lamivudine-loaded sterculia microspheres are presented in Figure 4. It was evident from the DSC profile that lamivudine exhibited a sharp endothermic peak associated with crystal melting at a temperature of 180.82 ^0C, which corresponds to the reported melting temperature of the drug. A similar DSC profile

(Figure 4) of the drug appeared at the temperature corresponding to its melting point in the lamivudine-loaded microspheres but with a slight change in its sharp appearance. It appears that there is a minor reduction of drug crystallinity within the microspheres. The DSC study apparently revealed that the drug was compatible with the gum and neither drug decomposition nor drug-polymer interactions occurred in the freshly prepared microspheres.

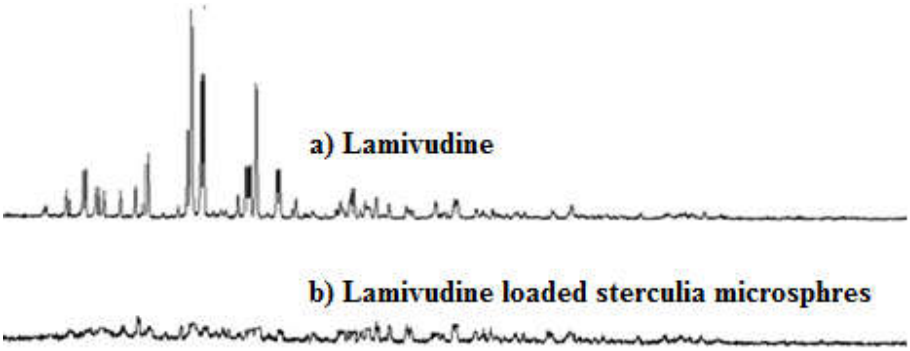
Figure 3. DSC curves of pure lamivudine and lamivudine loaded microspheres (LK4)



X-ray diffraction analysis

The thermal behavior coupled with the X-ray crystallographic data suggested that the diffractogram of pure lamivudine indicates the crystalline structure of the drug. The diffractogram of lamivudine-loaded sterculia microspheres shows a similar pattern with a slight decrease in the intensity of the peaks, which suggests that the drug was able to disperse almost homogenously in the microspheres. This result confirms a partial change in the solid state of lamivudine from crystalline to amorphous. Similar results reported for other sustained release microsphere studies had the same interpretation for lamivudine, famotidine etc[26, 27].

Figure 4. X-ray diffractograms of lamivudine and lamivudine loaded microspheres (LK4)

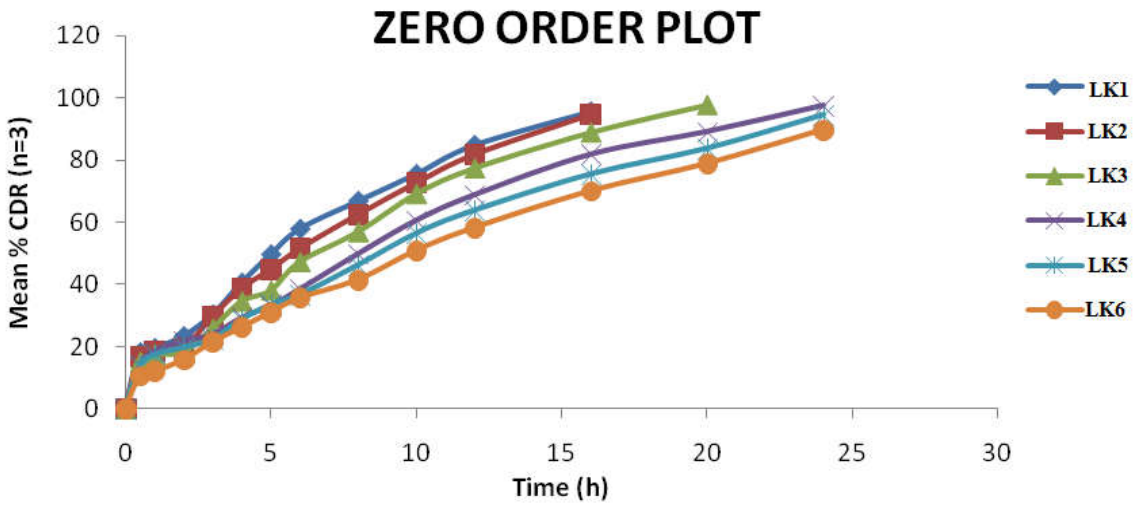


***In Vitro* drug release behavior**

The *in vitro* drug release study of different batches of microspheres was carried out in pH 7.4 phosphate buffer. In order to keep the total surface area of the microspheres constant and thus to get comparable results, the release studies were carried out using the same size fractions of microspheres containing equivalent amount of lamivudine from different batches of microspheres. The microspheres were prepared by emulsification-ionic internal gelation technique using BaCO3 as crosslinking agent. The microspheres cross-linked with Ba2+ showed a delay in disintegration and consequently a slow release of drug was obtained. It can be explained with the fact that the large size of barium ions (1.74 Å) produced hard and nonporous microspheres. Therefore, the exchange of Ba2+ ions in the microspheres with Na+ ions of the phosphate buffer and their removal as insoluble barium phosphate was interfered and attributed as delayed swelling of the microspheres and slow release. The release profiles are illustrated in Figure 6. It was observed that the amount of drug release decrease with an increase in the concentration of gum sterculia in the different batches of formulations. It can be attributed to an increase in the densities of the polymer matrix resulting in larger microspheres and this in turn increase the diffusion path length, which the drug molecules have to be traverse. It was observed that the microspheres had swollen and eroded in the phosphate buffer. Slow erosion of barium

cross-linked alginate-sterculia microspheres could occur through slight degradation of alginate backbone into smaller fragments but this process was thought to be delayed due to the presence of gum sterculia along with alginate.

Figure 5. *In Vitro* release profile (Zero order) of lamivudine loaded microspheres from different batches



Release Kinetics

The *in vitro* drug release profiles of lamivudine were applied on various kinetic models in order to evaluate the mechanism of drug release. The different kinetic models evaluated were zero order, first order and Higuchi. After linearization of the results obtained in the dissolution test, the best fit with higher correlation coefficients (R^2) was shown in Higuchi, zero order, and followed by first order equations as given in the table-4. High correlation was observed for Higuchi rather than zero and first-order models, indicating that the drug release from microspheres was diffusion controlled. The data obtained were also put in Korsemeyer-Peppas model in order to find out *n* value, which describes the drug release mechanism. The *n* values of microspheres of different batches were ranged between 0.53-0.598 (0.43-0.89), indicating that the mechanism of the drug release was non-Fickian type. This kind of release is the

characteristics of swelling-controlled system in which the rate of solvent uptake into a polymer is largely determined by the rate of swelling and relaxation of the polymer chains. It is assumed that the drug molecules diffuse out through a dissolving gel like layer formed around the drug during the dissolving process.

Table 4. *In vitro* release kinetic parameters of different batches of microspheres

FORMULATIONS	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSEMEYER PEPPAS MODEL
	R^2	K_0 (%/h)	R^2	K (h^{-1})	R^2	K_h (%/$h^{1/2}$)	*n*
LK1	0.946	5.79	0.956	0.172	**0.979**	24.88	**0.541**
LK2	0.963	5.722	0.944	0.161	**0.974**	24.30	**0.556**
LK3	0.956	4.827	0.929	0.161	**0.973**	23.11	**0.57**
LK4	0.959	3.932	0.929	0.131	**0.975**	20.74	**0.536**
LK5	0.966	3.716	0.947	0.103	**0.978**	19.56	**0.53**
LK6	0.974	3.581	0.97	0.082	**0.98**	18.8	**0.598**

CONCLUSION:

The attempt to prepare controlled release biodegradable microspheres of lamivudine using gum sterculia as microencapsulating agent was successful. The method employed was an industrially feasible one, as it involves gelling and emulsification which can be controlled precisely. Since the gum is from natural origin, it is non-toxic, biodegradable and comparatively cheaper than other synthetic biodegradable polymers. Further studies in the area of novel drug delivery systems can be carried out by taking this gum as a natural biodegradable polymer in future.

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