CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1188900

Available online at: http://www.iajps.com Research Article

PREPARATION, OPTIMIZATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF LAMIVUDINE

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Abstract:

Drug delivery systems [DDS] that can precisely control the release rates or target drugs to a specific body site have had an enormous impact on the health care system. Microspheres constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteristics. However, the success of these novel DDS is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the DDS with absorbing membranes.

Keywords: *DDS, lamivudine, polymers, mucoadhesive, microspheres*

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Please cite this article in press as N. Sandeepthi N.Jhancy laxmi bai., **Preparation, Optimization and Evaluation of Mucoadhesive Microspheres of Lamivudine,** Indo Am. J. P. Sci, 2018; 05(02).*

INTRODUCTION:

Mucoadhesion and mucoadhesive drug delivery systems

Mucoadhesive drug delivery systems are one of the novel drug delivery system, which utilize the property of bioadhesion of polymers that become adhesive on hydration[5]. These drug delivery systems can be used for targeting a drug to a particular region of the body for extended period of time[1].

Bioadhesion is an interfacial phenomenon in which two materials, atleast one of which is biological, are held together by means of interfacial forces[2]. The attachment could be between an artificial material and biological substrate, such as adhesion between a polymer and biological membrane. In case of polymer attached to the mucin layer of mucosal tissue, the term mucoadhesion is used**.** Mucosal adhesive materials have been investigated and identified[3]. These is generally hydrophilic macromolecules that contain numerous hydrogen bonds forming groups (e.g., hydroxyl and carboxyl groups) and will hydrate and swell when placed in contact with water. In most cases these materials require wetting to become adhesive. However, over hydration may result in the formation of slippery mucilage and a loss of the adhesive properties.

Mucoadhesive microspheres

Mucoadhesive microspheres include microparticle and microcapsules (having a core of the drug) of 1-1000µm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively[4]. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery, but coupling of mucoadhesive properties to microspheres as additional advantages, e.g. efficient absorption and enhanced bioavailability of the drugs due to high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of the drug to the absorption site achieved by anchoring plant lectins, bacterial adhesives and antibodies on the surface of the microspheres.

Mucoadhesive microspheres can be tailored to adhere any mucosal tissue including those found in eye, nasal cavity, urinary and gastrointestinal tract, thus offering the possibilities of localised as well as systemic controlled release of drugs. Microspheres prepared with mucoadhesive and biodegradable polymers undergo selective uptake by the M cells of peyer patches in gastrointestinal (GI) mucosa[5]. This uptake mechanism has been used for the delivery of protein and peptide drugs, antigens for vaccination and plasmid DNA for gene therapy.

Polymers used for mucoadhesive microspheres

Mucoadhesive polymers used in controlled drug delivery system

Polymers	Mucoadhesive property
Carboxy methyl cellulose	+++
Carbopol	+++
Tragacanth	+++
Polyacrylicacid	+++
Sodium alginate	+++
Hydroxy propyl methyl cellulose	++
Gum karaya	++
Gelatin	++
Guar gum	+
Polyethylene glycol	+
Hydroxyl propyl cellulose	+
Chitosan	

Note: +++ Excellent, ++ Fair, + Poor

Preparation of mucoadhesive microspheres

Solvent evaporation: It is the most extensively used method of microencapsulation, first described by Ogawa et al[6]. A buffered or plain aqueous solution of the drug (may contain viscosity building or stabilizing agent) is added to an organic phase consisting of the polymer solution in solvents like dichloromethane (or ethyl acetate or chloroform) with vigorous stirring to form the primary water in oil emulsion. This emulsion is then added to a large volume of water containing an emulsifier like PVA or PVP to form the multiple emulsions (w/o/w). The double emulsion, so formed is then subjected to stirring until most of the organic solvent evaporates, leaving solid microspheres. The microspheres can then be washed, centrifuged and lyophilise to obtain the free flowing and dried microspheres.

Hot melt microencapsulation:

This method was first used by Mathiowitz and Langer[7] to prepare microspheres of polyanhydrides copolymer of poly [bis (*p*-carboxy phenoxy) propane anhydride] with sebacic acid. In this method, the polymer is first melted and then mixed with solid

particles of the drug that have been sieved to less than 50µm. The mixture is suspended in a non-miscible solvent (like silicon oil), continuously stirred, and heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, e.g. polyanhydrides. Microspheres with diameter of 1-1000µm can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disadvantage of this method is moderate temperature to which the drug is exposed.

Solvent removal:
It is a non aqueous method of microencapsulation particularly suitable for water labile polymers such as the polyanhydrides. In this method, drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride[8]. This mixture is then suspended in silicone oil containing span 85 and methylene chloride. After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting microspheres can then be dried in vacuum.

Hydrogel microspheres:
Microspheres made of gel type polymers, such as alginates, are produced by dissolving the polymer in an aqueous solution, suspending the active ingredient in the mixture and extruding through a precision device, producing micro droplets which fall into a hardening bath that is slowly stirred. The hardening bath usually contains calcium chloride solution, whereby the divalent calcium ions cross linking the polymer formed gelled microspheres. The method involves an all aqueous system, which eliminates residual solvents in microspheres. Lim and Moss[9] developed this method for encapsulation of live cells, as it does not involve harsh conditions, which could kill cells. The surface of these microspheres can be further modified by coating them polycationic polymers, like polylysine after fabrication. The particle size of microspheres can be controlled by using various size extruders or by varying the polymer solution flow rates.

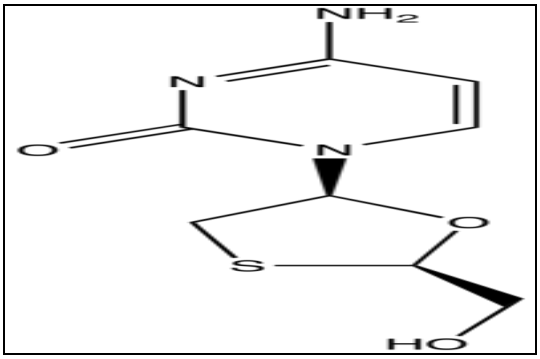
Spray drying: In this process, the drug may be dissolved or dispersed in the polymer solution and spray dried. The quality of spray dried microspheres can be improved by addition of plasticizers, e.g. citric acid, which promote polymer coalescence on the drug particles and hence promote the formation of spherical and smooth surfaced microspheres. The size of microspheres can be controlled by the rate of spraying, the feed rate of polymer drug solution, nozzle size, and the drying temperature[10].

Phase inversion microencapsulation:
The process involves addition of drug to a dilute solution of the polymer (usually 1-5%, w/v in methylene chloride). The mixture is poured into an unstirred bath of strong non solvent (petroleum ether) in a solvent ratio of 1:100, resulting in the spontaneous production of microspheres in the size range of 0.5-5.0µm can then be filtered, washed with petroleum ether and dried with air[11]. This simple and fast process of microencapsulation involves relatively little loss of polymer and drug.

Orifice ionic gelation technique[11,12]:
In this method sodium alginate and mucoadhesive polymer were dissolved in purified water to form homogeneous polymer solution. The active metabolite was added to the polymer solution and mixed thoroughly with stirrer to form viscous dispersion. The resulting dispersion was added manually dropwise into calcium chloride (10%) solution through a syringe no.18. The added droplets are retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce spherical rigid microspheres. The microcapsules will be collected by decantation and product thus separated washed repeatedly with water and dried at 45^0c for 12 hours.

Drug profile

Lamivudine [13,14,15]



Peak time (h)**:** 0.5-2
Peak concentrations (mcg/ml)**:** 1.5 ± 0.5

Mechanism of action:
Lamivudine (3TC), a synthetic nucleoside analogue with activity against HIV-1 and HBV. This deoxycytidine analogue is phosphorylated intracellularly and inhibits HIV reverse transcriptase as well as hepatitis B virus (HBV) DNA polymerase. Its incorporation into DNA results in chain termination. Most human DNA polymerases are not affected and systemic toxicity of 3TC is low. Point mutation in HIV-reverse transcriptase and HBV-DNA polymerase gives rise to rapid lamivudine resistance. Lamivudine usually is given with other antiretroviral agents, such as ZDV or D4T.3TC at a dose of 600 mg/day reduced HIV cells by 75%, and in combination with ZDV (Zidovudine), the reduction in viral load was 94%.3CT is rapidly absorbed through the GI tract.

METHOD
All other reagents used were of analytical grade. Distilled water was used throughout the study.
Preparation of calibration curve: Pipette out 0.2, 0.4, 0.6, 0.8, 1.0 ml of II stock solution (100 µg/ml) into a series of 10 ml volumetric flask and volume was adjusted to with pH 7.4 phosphate buffer solution to obtain 2, 4, 6, 8, and 10 µg/ml of solution. The absorbance of the resulting solutions was measured at 271 nm keeping pH 7.4 phosphate buffer as blank.Concentration versus optical density values are plotted and displayed in the figure 1 in the concentration range of 2-10µg/ml. The method obeyed Beer-Lamberts law and the solution was stable for 48h.

Table 1: **Calibration curve data for Lamivudine**

Concentration (µg/ml)	Mean absorbance*±SD
2	0.102 ± 0.005
4	0.199 ± 0.020
6	0.299 ± 0.011
8	0.402 ± 0.016
10	0.496 ± 0.008

***Average of three determinations**

Preparation of mucoadhesive microspheres
Orifice ionic gelation method[11,12]: Sodium alginate and mucoadhesive polymer chitosan were dissolved in purified water (10ml) separately. Then both the solutions were mixed to form homogeneous polymer solution. The drug was added to the polymer solution and mixed thoroughly with help of pestle and mortar to form viscous dispersion. The resulting dispersion was added dropwise into 10% w/v calcium chloride solution (100ml) through a syringe with needle (size no 21) with continuos stirring at 500 rpm. The added droplets were retained in the calcium chloride solution for 15 minutes to produce spherical rigid microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45^0C for 12 hours and stored in desiccators. Similarly sodium alginate myrrh microspheres and sodium alginate carbopol 934 microspheres prepared by dissolving required quantity of sodium alginate and mucoadhesive polymer in water. Then drug is added to polymeric solution and mixed thoroughly with help of pestle and mortar to form viscous dispersion. Then follow the procedure as mentioned above.

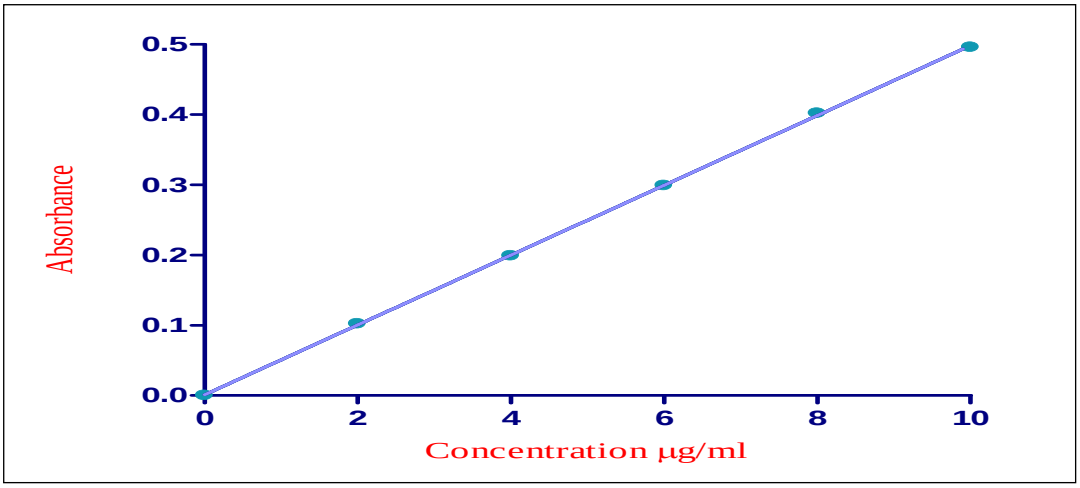


Fig. 1: Calibration curve for lamivudine in pH 7.4 phosphate buffer

Preparation of mucoadhesive microspheres (orifice ionic gelation method)

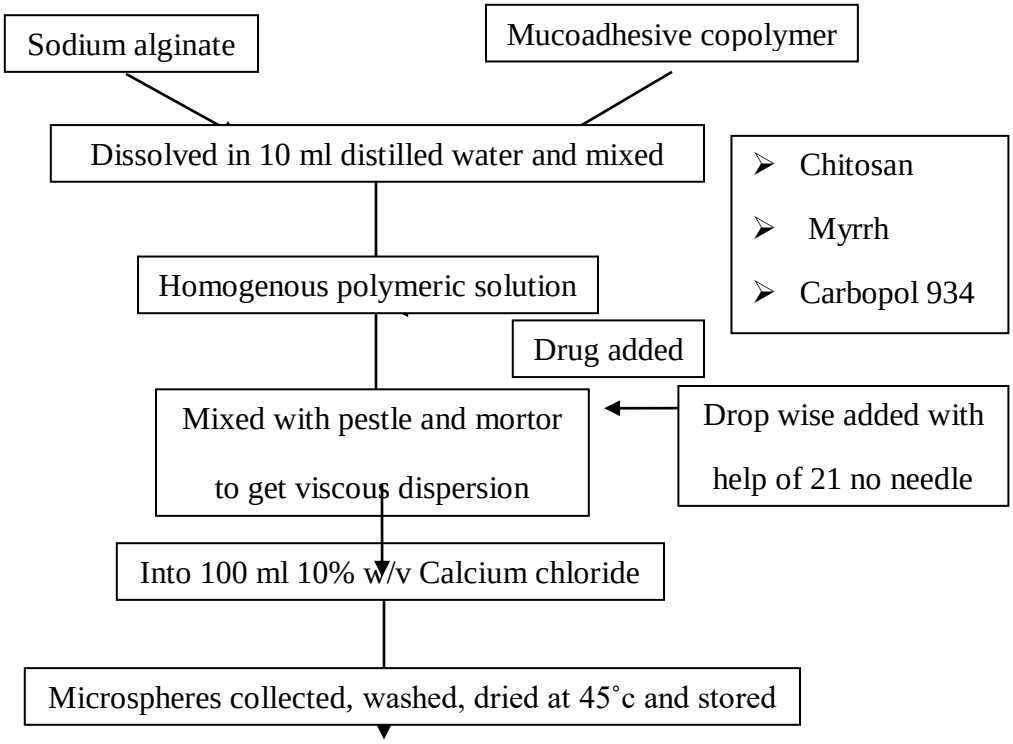


Table 2: Formulae for different sodium alginate mucoadhesive microspheres of Lamivudine

Batch size: 2G

Batches	Core: Coat	Lamivudine	Sodium Alginate	Chitosan
SS1	1:3(1:4)	500	300	1200
SS2	1:3(1:2)	500	500	1000
SS3	1:3(1:1)	500	750	750
SS4	1:3(2:1)	500	1000	500
SS5	1:3(4:1)	500	1200	300

RESULTS:

Table 3: Production yield of sodium alginate and chitosan formulations

Batches	**Production yield * ± SD**
SS-1	93.03 ± 0.02
SS-2	91.55 ± 0.05
SS-3	96.30 ± 0.08
SS-4	94.98 ± 0.02
SS-5	92.60 ± 0.05

Table 4: Percent drug content of sodium alginate and chitosan formulations

Batches	**Theoretical drug content(mg)**	**Practical drug content(mg)**	***% Drug content* ± SD**	**Coefficient of variation**
SS-1	50	49.10	98.21 ± 0.20	0.203
SS-2	50	48.97	97.68 ± 0.49	0.509
SS-3	50	49.00	98.01 ± 0.59	0.607
SS-4	50	49.66	99.33 ± 0.49	0.501
SS-5	50	49.56	99.13 ± 0.30	0.302

***Average of three determinations**

Table 5: Microencapsulation efficiency of sodium alginate and chitosan formulations

Batches	Microencapsulation efficiency* ±SD
SS-1	87.05 ± 0.11
SS-2	89.76 ± 0.89
SS-3	90.42 ± 1.53
SS-4	92.46 ± 0.40
SS-5	95.90 ± 0.75

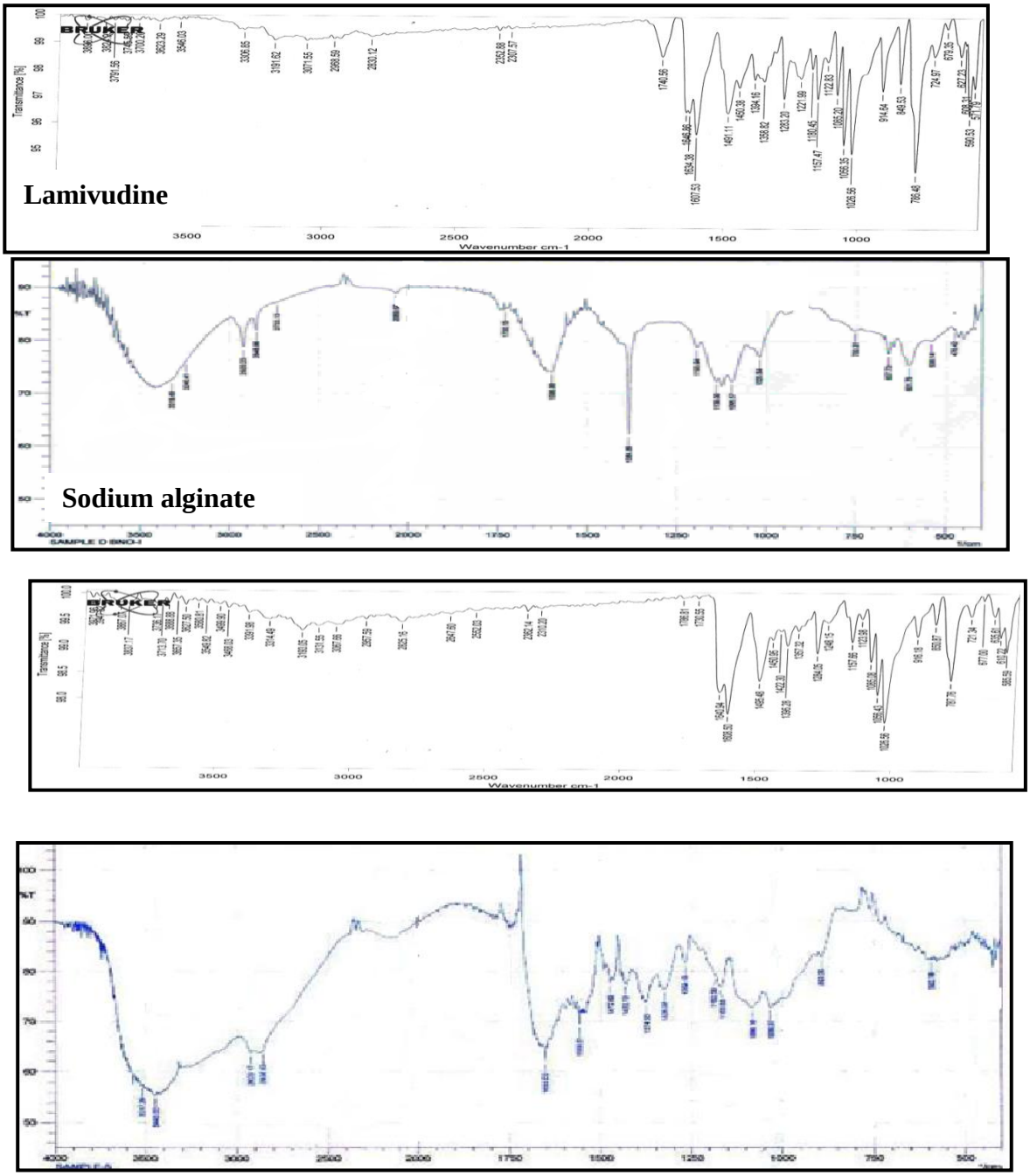


Fig.2: FTIR spectra of Lamivudine, Sodium alginate, Chitosan and SS-5

Table 6: Size analysis of sodium alginate and chitosan formulations

Formulation code			SS-1		SS-2		SS-3		SS-4		SS-5	
Size range		Arithmetic Mean size (μm) (Xi)	Percent* Retained (F_i)	Weight Size (X_iF_i)	Percent* Retained (F_i)	Weight Size (X_iF_i)	Percent* Retained (F_i)	Weight Size (X_iF_i)	Percent* Retained (F_i)	Weight Size (X_iF_i)	Percent* Retained (F_i)	Weight Size (X_iF_i)
Mesh	(μm)											
10/22	1700-710	1205	48.65	58630.3	50.02	60282.9	50.88	61313.6	53.18	64088.9	58.85	70920.6
22/44	710-355	532.5	51.34	27340.7	49.97	26610.4	49.11	26154.9	46.81	24928.5	41.14	21909.5
Average diameter (D_{av})			D_{av}=859.710		D_{av}=868.933		D_{av}=874.685		D_{av}=890.174		D_{av}=928.301	

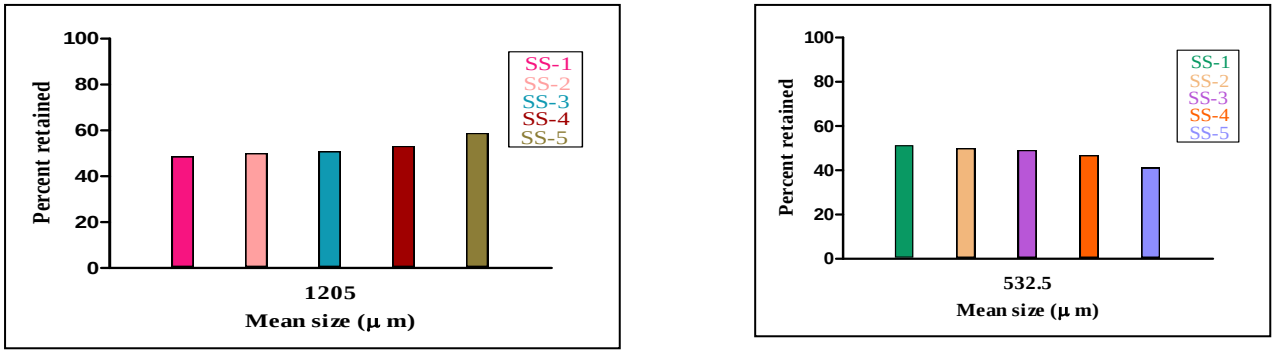
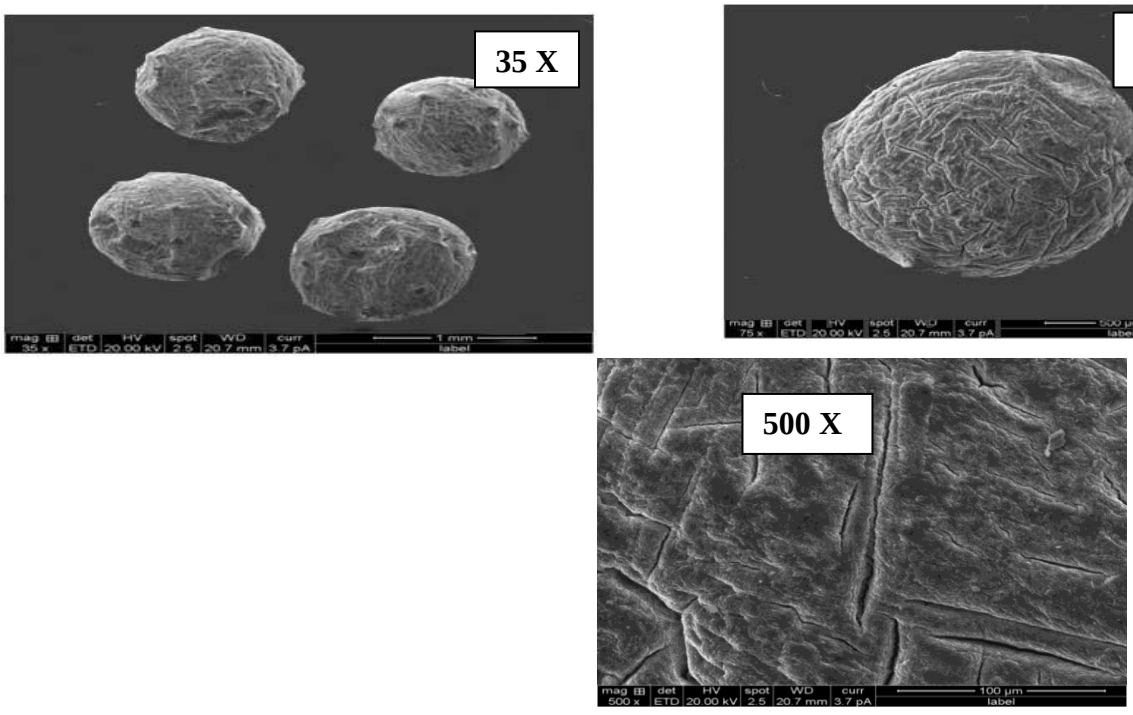


Fig. 3: Size distribution of sodium alginate and chitosan



Scanning electron micrographs of SS-5 formulation

Table 7: Swelling ratio of sodium alginate and chitosan formulations

Time (h)	SS-1		SS-2		SS-3		SS-4		SS-5	
	Weight of MC after swelling(mg)	Relative swelling	Weight of MC after swelling(mg)	Relative swelling	Weight of MC after swelling(mg)	Relative swelling	Weight of MC after swelling(mg)	Relative swelling	Weight of MC after swelling(mg)	Relative swelling
0	50	0	50	0	50	0	50	0	50	0
0.5	69	0.38	72	0.44	74	0.48	75	0.50	78	0.56
1	78	0.56	79	0.58	80	0.60	82	0.64	83	0.66
2	86	0.72	86	0.72	95	0.90	98	0.96	96	0.92
3	91	0.82	92	0.84	102	1.04	101	1.02	107	1.14
4	94	0.88	98	0.96	104	1.08	108	1.16	112	1.24
5	98	0.96	102	1.04	107	1.14	110	1.20	117	1.34
6	99	0.98	102	1.04	107	1.14	110	1.20	118	1.36

***Table 8: In vitro* wash off test of sodium alginate and chitosan formulations**

Batches	Percentage of microspheres adhering to tissue at different time interval (h)					
	0	**0.5**	**1.0**	**2.0**	**5.0**	**6.0**
SS1	50	92	81	75	60	60
SS-2	50	93	82	69	65	62
SS-3	50	93	86	77	68	66
SS-4	50	96	88	79	70	68
SS-5	50	96	87	78	72	70

Table 9: Dissolution data of sodium alginate and chitosan formulations

Cumulative percent drug release					
Time (h)	**SS-1**	**SS-2**	**SS-3**	**SS-4**	**SS-5**
0.25	7.21 ± 0.36	8.54 ± 0.91	9.14 ± 0.91	9.38 ± 0.36	12.64 ± 0.96
0.5	11.93 ± 0.36	13.01 ± 0.63	13.74 ± 0.72	16.03 ± 0.91	20.74 ± 0.91
0.75	15.80 ± 0.75	17.13 ± 0.21	18.82 ± 0.63	22.57 ± 1.27	28.97 ± 0.63
1	20.89 ± 0.55	21.01 ± 0.36	24.39 ± 0.55	28.75 ± 0.55	39.02 ± 0.55
2	25.74 ± 0.36	29.00 ± 0.73	30.21 ± 1.11	36.38 ± 0.91	52.94 ± 0.72
3	29.87 ± 0.75	33.62 ± 1.11	38.09 ± 0.96	44.51 ± 1.11	64.10 ± 0.91
4	35.21 ± 0.36	40.90 ± 0.21	46.46 ± 0.37	51.31 ± 0.91	73.22 ± 0.55
5	38.63 ± 0.42	46.49 ± 0.73	53.15 ± 0.56	57.77 ± 0.36	82.60 ± 0.42
6	42.05 ± 0.91	50.53 ± 0.36	59.84 ± 1.27	62.05 ± 0.42	87.63 ± 0.96
9	46.44 ± 0.91	58.91 ± 0.36	71.01 ± 0.56	75.40 ± 0.21	93.28 ± 0.75
12	50.11 ± 0.55	70.44 ± 0.21	73.50 ± 0.72	80.19 ± 0.55	95.44 ± 0.73

Table 10: Model fitting values for sodium alginate and chitosan formulations

	SS-1	SS-2	SS-3	SS-4	SS-5
Zero order	0.6835	0.8498	0.8329	0.7943	0.6619
1st order	0.8287	0.9666	0.9613	0.9685	0.9777
Matrix	0.9824	0.9985	0.9937	0.9945	0.9705
Peppas	0.9846	0.9966	0.9940	0.9887	0.9785
Hix. Crow	0.7877	0.9391	0.9313	0.9304	0.9186

Table 11: Parameters of Korsemeyer-Peppas equation for sodium alginate and chitosan formulations

	SS-1	SS-2	SS-3	SS-4	SS-5
n	0.4852	0.5329	0.5491	0.5355	0.5305
k	17.1925	19.2649	21.1935	23.9716	32.4740
Best fit	Peppas	Matrix	Peppas	Matrix	Peppas

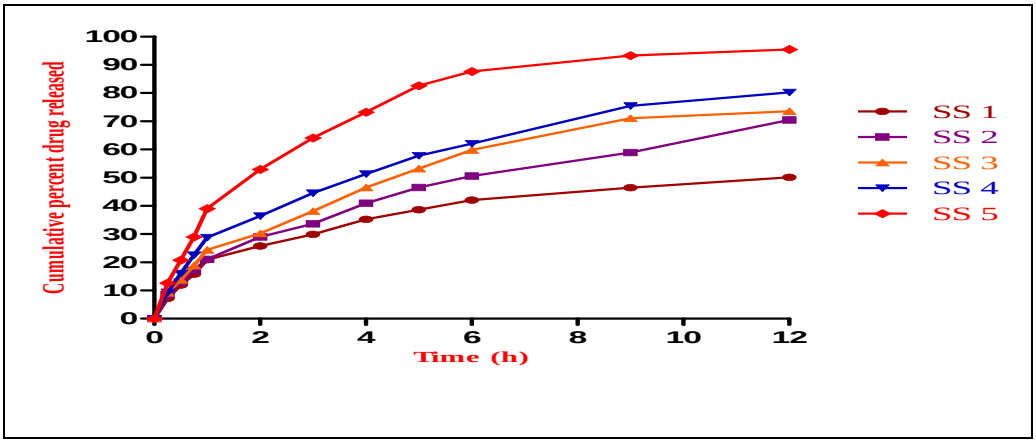


Fig. 4: Comparative dissolution profile of SS-1, SS-2, SS-3, SS-4 and SS-5

CONCLUSION:

The mucoadhesive microspheres of Lamivudine were conveniently prepared by orifice ionic gelation method using sodium alginate-mucoadhesive polymers (synthetic/natural) and mucilage isolated from the natural sources.

The production yields were in the range of **90.56 ± 0.07 to 97.58 ± 0.07** and the percentage drug content were in the range of **97.08 ± 0.89 to 99.33 ± 0.49** with low SD and CV value indicating uniform distribution of drug within the various batches of microspheres prepared with negligible loss during the formulation stage.

The percentage encapsulation efficiency was in the range of **83.75 ± 0.39** to **96.36 ± 0.63** and increased progressively with increase in the concentration of sodium alginate. This could be attributed due to formation of larger microspheres with increasing concentration of sodium alginate, thus entrapping more amount of drug.

The microspheres were distributed in the range of **783.618µm** to **945.718 µm**. The size of microspheres depends upon concentration of sodium alginate used in the formulation. The increase in size of microspheres was observed with increase in concentration of sodium alginate. This could be due to increase in viscosity of the polymeric dispersion, which eventually led formation of bigger particle during ionic gelation.

The characteristic lamivudine bands are seen in optimized formulation with drug carbonyl stretching vibration shifting to slightly lower wavelength ranging from 1739.67 cm^{-1} to 1730.55 cm^{-1}, shift to a

lower frequency as result of weakening of carbonyl radical double bond indicating mild to no interaction.

The scanning electron microscopy reveals that the microspheres were spherical, discrete with rough texture.

The swelling ratio depends upon concentration of polymer and type of mucoadhesive polymer used in the formulation. Swelling ratio shows direct relationship with sodium alginate concentration and increased with increasing concentration of sodium alginate.

The *in vitro* wash-off test results suggest that concentration and type of mucoadhesive polymer doesn't show much more difference in the mucoadhesive property.

In all the formulations the release rate was found to be optimum with few exceptional results. In SS-1 to SS-5 formulations prepared with chitosan, SS-5 shows good release. From the overall release rate studies, the mucoadhesive microspheres prepared with chitosan at higher concentrations of sodium alginate shows better release rate and said to be optimum formulation i.e., SS-5 shows maximum drug release of **95.44 ± 0.73** after 12 hours.

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