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ORIGINAL ARTICLE

Formulation and Evaluation of Lamivudine Loaded Micro Particles by Novel Technique

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ABSTRACT
Present work is to formulate Lamivudine loaded microparticle by counterion induced aggregation method, employing simultaneous cold temperature and hyperosmotic solution treatment as a novel technique. Chitosan was chosen as polycation and smaller molecular electrolytes such as sodium citrate, sodium sulphate and were chosen as polyanions. The resulted aggregated microparticles were subjected to surface morphology, size distribution, in- vitro release and drug excipient interaction study. Results and discussion: Sodium citrate (SC) and sodium sulphate (SS) were able to form aggregates except as chitosan forms complexes and depends on pH and pKa of medium. Prepared aggregates were subjected to cold hyperosmotic dextrose solution to provide more mechanical strength. The percentage of entrapped drug was more in SC based microparticle as compared to SS. The SS and SC microparticles had average particle size of 1500 mm and 1300 mm respectively. Also, the SEM study showed SS particles were had smoother surface than SC.There was no such major interaction were found during FTIR and DSC study. In addition, stability study was per-formed and data showed no significant change in assay value for SS2. The microparticles prepared by above mentioned method had sufficient mechanical strength and were able to released drug.
Keywords: *Lamivudine, Micro Particles, Chitosan & Polycation.*

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INTRODUCTION

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. [1to3]. Controlled release drug delivery employs devices, such as polymer-based disks, rods, pellets encapsulates drug and releases at controlled rates for relatively long periods of time. One approach to produce sustained release of drugs is by the use of micro-particulate drug delivery systems. [4to6] in last decade several research works already reported based on microparticle. Several methods of preparing microparticulate drug delivery systems are available, e.g., Physical association, chemical crosslinking method, spheronization, spray granulation, coacervation and fluidized bed granulation etc.[7,8] The main disadvantages associated with those most techniques include high cost of manufacturing, need of specialized and high skill trained persons and equipment. Chemical crosslinking method involves formation of carboxylic acideamide bonding and Schiff base formation by some of -NH_2 and -OH chemical handlers such as glutaraldehyde, diglycidyl ether, diisocya-nate, diacrylate etc.[9,10] Whereas, physical association method involves crosslinking between small anionic molecules, such as citrates, sulphates, phosphates and large anionic macro- molecules such as, DNA, alginate, chondroitin sulphate, hy- aluronic acid, carboxymethyl cellulose, pectin, dextran sulphate and proteins with some

polycations.[11,12] However, mechanical stability is the major drawback associated with physical association method.[13,14] Meanwhile, chemical crosslinking method provides sufficient physical and mechanical stability but, the biocompatibilities of many cross-linkers are unknown, while others have been found to be relatively toxic and mechanism is not established.[15-17] Biodegradable polymers have attracted considerable attention as potential device for controlled drug delivery. Researchers have been carried on use of chitosan as network forming or gelling agent because of lesser cost, naturally occurring polysaccharide, biodegradability, non toxicity, provides protection against mucous from irritation.[18,19]. Chitosan is a linear polysaccharide composed of randomly distributed. b- (1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units. Due to the presence of nitrogen group in molecular structure, it shows cationicity and can produce counterion induced aggregate. [20]

Hence in the present study an attempt has been made to prepare microparticles by small anions such as, sodium citrate, sodium sulphate opposite charge polycation like chitosan. During the study simultaneous cold temperature and hyperosmotic solution treatment was adopted as novel method to provide sufficient mechanical strength and stability. Release study based on varying concentration of chitosan and polyanions were studied, as well as surface morphology, particle size, drug execipient interaction study was investigated.

MATERIAL AND METHODS

Lamivudine was obtained as gift sample from Cipla Ltd. (India). Sodium citrate, sodium sulphate and sodium phosphate were purchased from Loba Chemicals, India. All chemicals and solvents were used are of high analytical grade.

Preparation of drug loaded Microparticle

Microparticles loaded drug were prepared by counterion induced gelation/aggregation method. Required quantity of drug was dissolved in mixture of methanol and dichloromethane (1:1) to prepare drug solution. In another beaker required quantity of chitosan was dissolved in 1% v/v aspartic acid solution with or without barium sulfate ($BaSO_4$). Drug solution was dispersed into the polymer solution containing emulsifier Tween 80 and sonicated (Systronic) for to form emulsion. Then Emulsion was injected drop by drop with the help of needle into 20% w/v sodium citrate (SC)/sodium sulphate (SS) solution to form aggregates.[21] To provide mechanical stability, prepared aggregates were subjected to cold hyperosmotic solution of dextrose (2^0C/50 Osm/L). The drowned aggregates were collected and dried at 45^0C for 2 days. The collected microparticles were stored in a desiccator for further evaluation.

Table 1: Composition of Microparticles

Formulations	Drug: polymer (chitosan) (%)	Barium sulfate (mg)
SCF1	1:0.5	-
SCF2	1:0.75	-
SCF3	1:1	-
SCBF4	1:0.5	20
SCBF5	1:0.75	20
SCBF6	1:1	20
SS F7	1:0.5	-
SS F8	1:0.75	-
SS F9	1:1	-
SSB F10	1:0.5	20
SSB F11	1:0.75	20
SSB F12	1:1	20

Surface morphology study

Microparticle surface morphology was determined by Scanning Electron Microscope. Microparticles were coated with gold film using Ion Sputtering device under reduced pressure and mounted directly in sample holder.[22]

Particle size analysis

The microparticles were accurately weighed and sized using USP standard sieve set.The fraction of microparticles remaining on each sieve was collected and the mean particle size of the microparticles was recorded as the percentage of microparticles retained at each sieve and multiplied by the average particle size of the sieve used.[23] The results were evaluated by a frequency distribution curve, where the percentage of particles lying within a certain size range is plotted against the mean particle size.

Drug entrapment determination

Equivalent quantities (1gm) of microparticles were taken in a clean mortar and pestle. To that small amount of methanol was added and triturated. The triturated mixture was filtered by whatman filter paper (45m) and transferred to 100 ml volumetric flask; volume was adjusted with citrate buffer pH 3.0. Proper dilutions were made and analyzed by UV Visible spectrophotometer (1800, Shimadzu Co., Japan) at 270 nm. Encapsulation efficiency was calculated by following method.[24]

Drug entrapment efficiency **=** Practical drug content **/** Theoretical drug content X 100

***In-vitro* release study**

In-vitro dissolution study of prepared microparticles equivalent to 1gm of drug were carried out in USP dissolution rotating Paddle (USP XXIV Type-II, Electrolab, TDT008) for 30 h. Microparticles were transferred to 900 ml of dissolution fluid using rotation speed of 100 rpm and temperature of $37^0 \pm 0.5^0$ C. Dissolution was carried out with citrate buffer pH 3.0 for initial 2 h followed by next 28 h with phosphate buffer pH 7.4. Samples were withdrawn at predetermined time level and the aliquots were filtered by whatman filter paper (0.45 mm) and diluted appropriately with the release medium and absorbance was measured by UV Visible spectrophotometer (1800, Shimadzu Co., Japan) at the predetermined wavelength of each medium against a blank.[24]

FT-IR spectroscopy

The drug excipient interaction were studied using FTIR(Brooker, Japan). IR spectra for drug and powdered microparticles were recorded in a Fourier transform infrared spec-trophotometer. The spectra were scanned over 4000-500 cm^{-1} range. [25]

Differential scanning colorimetry (DSC) study

The DSC analysis of pure drug and drug-loaded microparticles were carried out using Shimadzu DSC 60. The analysis was performed at a rate 10^0C/ min 1 ranging from 20^0 C to 400^0 C temperatures [26].

RESULTS AND DISCUSSION

Lamivudine loaded microparticles prepared by counterion induced aggregation using SC, SS and SP. Since chitosan has pka value of 6.5 and bears positive charge under low pH only, associates with opposite charge anion and forms ionic complexes as aggregates.[28] Result showed microparticles were formed in SC and SS solution, except SP solution, as pH was found to be 3.72. Surface morphology and cross section were observed under SEM study as cited in Fig.1 (a), (b) and (c).

Microparticles were asymmetrical and their surfaces were irregular and un-even. Furthermore, it was noticed that sodium citrate (SC) microparticles had less smooth surfaces compared to sodium sulphate (SS) based. Physical appearance of SC based microparticles was slightly rough, whereas SS microparticles were Smoother as observed in Fig. 2(a), (b) and (c).

The fraction percent of weight distribution of different formulae of Lamivudine loaded microparticles were determined by sieve analysis. A frequency distribution curve was plotted between % weight retained and mean particle diameter. Results showed that maximum of 65% of particles were retained at mean diameter of 1400 mm and a least of 5% retained over mean diameter of 1800 mm for SC based formulations, whereas in SS based formulation the maximum of 70% microparticles retained at mean diameter of 1600 mm as showed in Fig. 3.

This method based on the actual amount of drug entrapped during formulation with respect to the initial amount of drug added. Percentage of drug entrapped was ranged from least 68.5 % to maximum of 101.5% for SC based formulation, whereas comparable less amount drugs entrapped in SS formulations. This could be the possible reason that, 20% sodium citrate exhibited PH value of 8.47 as compared to 20% sodium sulphate solution value of 6.82, hence formed more compact aggregate mass and entrapped more drug. Different drug: polymer ratio ranging from 1:1, 1:0.5 and 1:0.75 were selected and subjected to prepare ionic complexes by opposite charged anions. Complexes were subjected to simultaneous cold temperature and hypertonic treatment with or without $BaSO_4$ to provide more cationic property of chitosan dispersion. Lamivudine loaded microparticles were subjected to *in-vitro* dissolution study for 30 h. Initially on 0.5 h a minimum of 7% of drug were released from all formulation. As the dissolution proceeded, remarkable differences in release pattern were observed between them. In 10th hour of dissolution SC3 released minimum of 30.45% and maximum of 37.7% was observed for SC1. The pattern of release was followed for next 20 h. During 30 h maximum of 55.45% and minimum of 41.30% release was observed for SC1 and SC3 respectively. Whereas, formulations associated with $BaSO_4$ markedly controlled drug release than formulation without same as observed in SCB1-SCB3. A comparison was made between SC and SS based formulations. It was observed 10.99% and 10.57% drug were released in 0.5 h and followed upto 30 h as 60.24% and 54.38% for SS3 and SSB3 respectively as cited in Fig. 4(a) and (b). In brief, it can be concluded SC microparticles were able to prolong drug release more than SS microparticles

and this result may exhibit by pH dependent formation of microparticles. The values obtained from in-vitro dissolution studies were fitted to zero-order, first-order, and Higuchi release kinetics. The higher correlation coefficient (r2) was found with Higuchi's equation for all formulations and SS2 had greater r2 value of 0.986 compared to all. To confirm the exact mechanism of drug release, the data were fitted to Korsemeyere Peppas equation. Regression analysis was performed and "n" values were $0.52 < n < 0.55$. Hence it can be inferred that the release was based on Fickian diffusion. On the basis of the above results, SS2 was selected as a promising formulation for further studies. Peaks were recorded during FTIR study, there was no such interaction observed between drug and excipient as in Fig. 5(a), (b) and (c). FTIR study showed characteristic peak of NH stretching at 3352 cm^{-1} and 3290 cm^{-1} as primary and secondary amide respectively, whereas NH bending showed peak at 1645 cm^{-1}. A characteristic broad peak was observed at 1627 cm^{-1} for carbonyl (C=O stretching) and peaks at 3030 cm^{-1} and 2947 cm^{-1} were observed for CH=CH stretching and aliphatic CH_3 respectively. Differential scanning colorimetry studies were performed to assess the compatibility between drug and excipients by endothermal peak. Pure Lamivudine showed endo Thermal peak at 255.68^0 C and onset of peak was at 249.87^0 C.

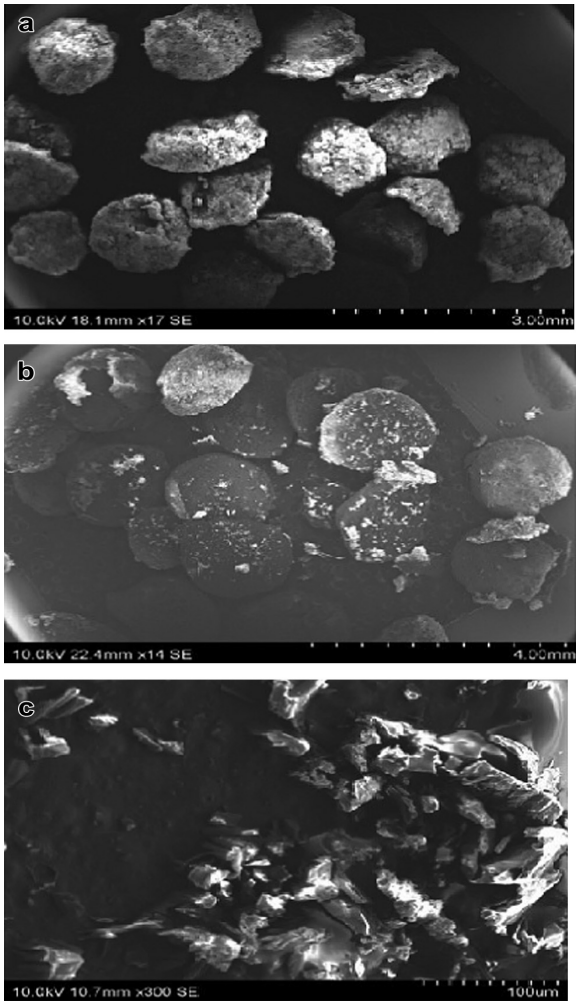

Figure 1(a): Surface morphology of SC microparticles. SC: Sodium citrate. (b): Surface morphology of SS microparticles. SS: Sodium sulphate. (c): Cross sectional observation of microparticles.

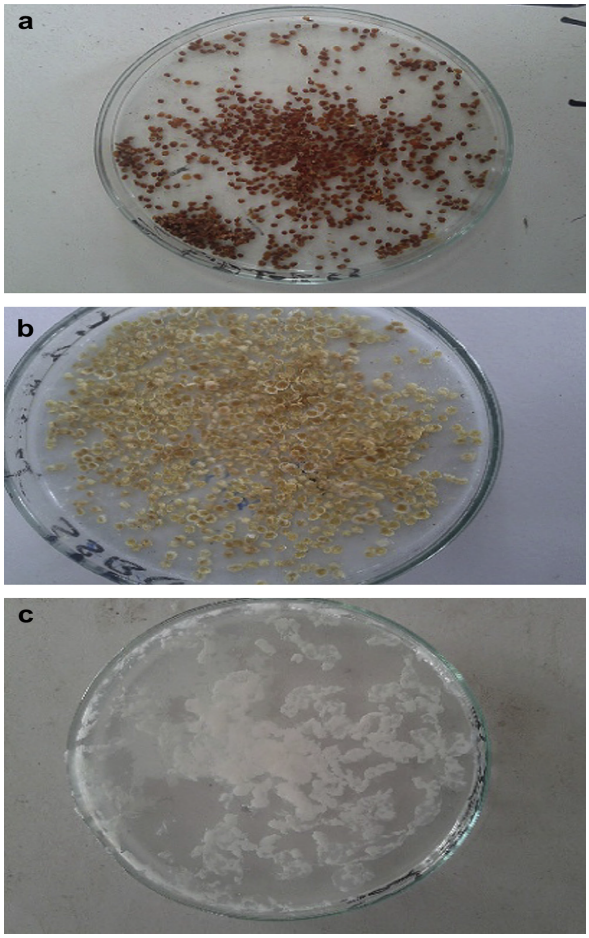

Fig. 2 (a): Physical appearance of SC microparticles. (b): Physical appearance of SS microparticles. (c): Physical appearance of SP aggregation

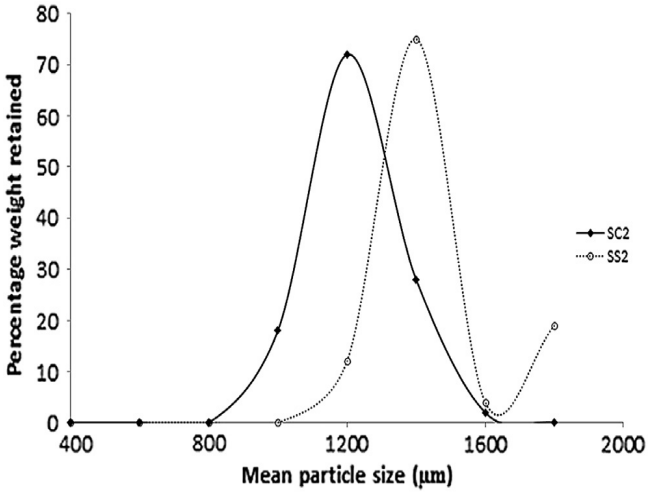

Fig 3: Particle size distribution curve

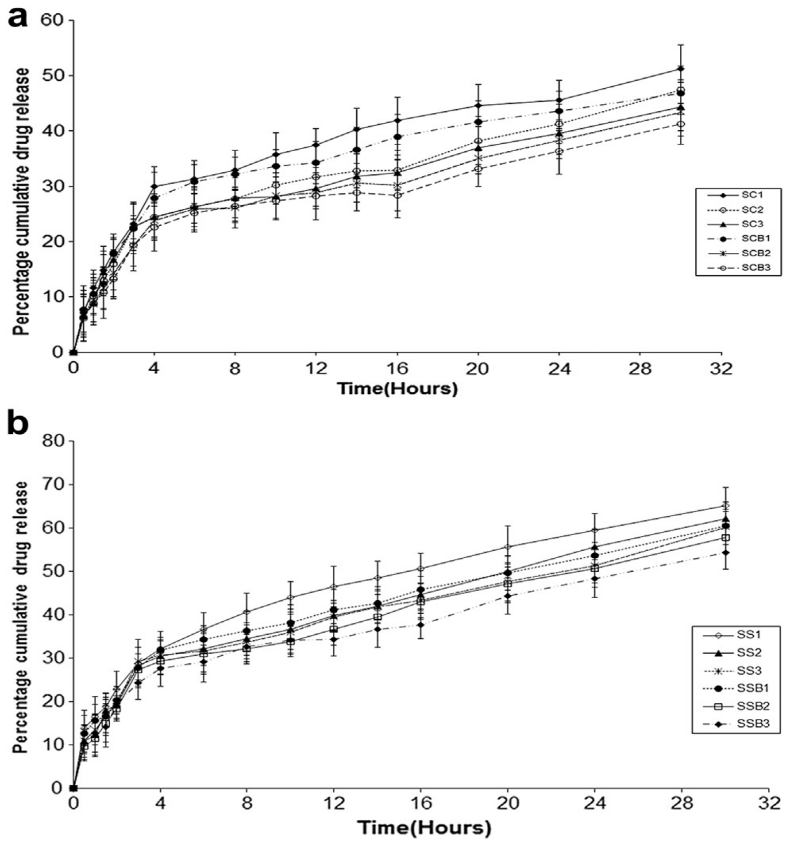

Fig. 4: (a) Dissolution release pattern for SC based microparticle.
(b): Dissolution release pattern for SS based microparticle.

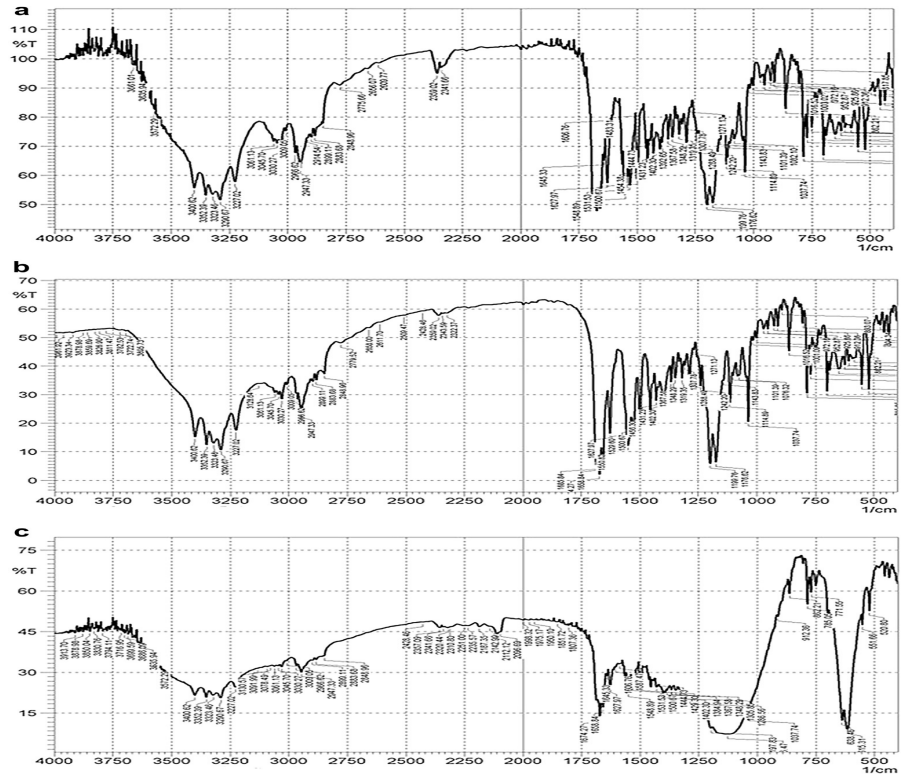

Fig. 5: (a) FTIR spectrum of pure drug.
(b): FTIR spectrum of chitosan.
(c): FTIR spectrum of SS2.

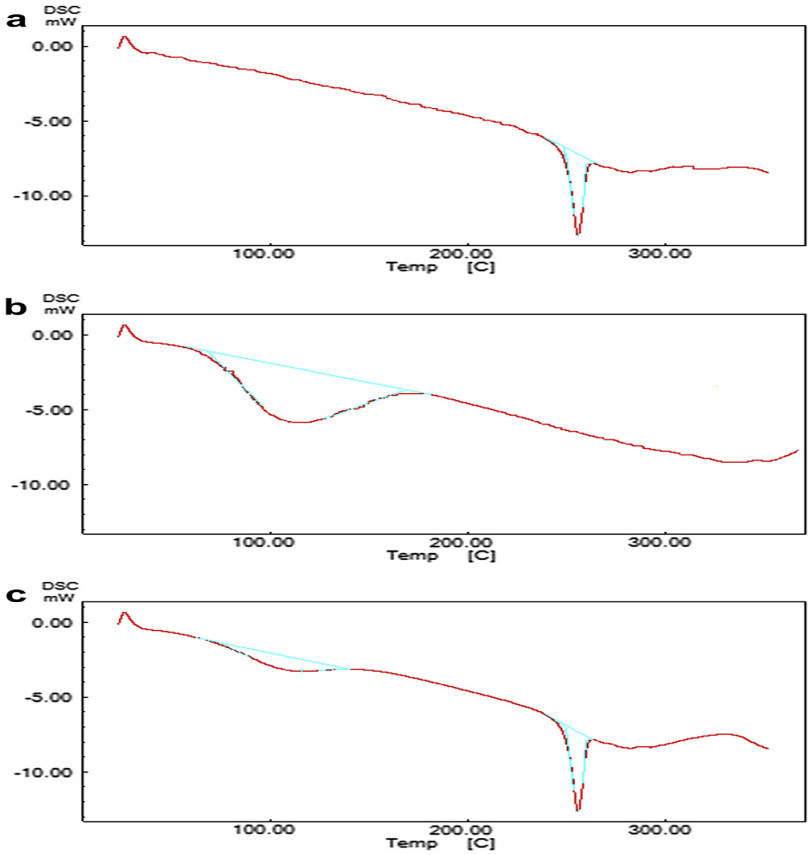

Fig. 6: (a) DSC thermogram of pure drug. Endothermal peak was observed at 255.68 C and onset of peak was at 249.87 C.
(b): DSC thermogram of chitosan. Endothermal peak was observed at 116.29 C.
(c): DSC thermogram of SS2.

Accelerated stability testing were carried out for SS2 based on the ICH guidelines considering 25±2°C & 60 ± 5% RH, 30±2°C & 65± 5% RH and 40 ± 2°C & 75± 5% RH, respectively. Microparticles equivalent to 1000 mg of pure drug placed in a humidity chamber. The samples were evaluated for drug assay at a regular interval of 3 months during the study of 24 months. There was no significance change in assay value as shown in Table 3. Thus, SS2 formulation batch confirmed its stability

Table 2: Stability study of Optimized batch (SS2).

Accelerated stability (40 ± 2°C & 75± 5% RH)				
Month	1	2	3	6
Drug Assay (%)	99.45 ±1.47	99.47±0.72	99.52±1.05	99.43±1.15

All values are represented as mean ±standard deviation

CONCLUSION

Lamivudine loaded chitosan microparticles were prepared by counter ion induced aggregation method. Simultaneous cold temperature and hyperosmotic solution treatment was adopted as a new method to provide mechanical strength. SC and SS solution were able to form aggregates, except SP solution. SEM study showed SC based microparticles were comparatively smaller and had more irregular surface than SS based. The dug release from selected microparticle was followed higuchi pattern and obeyed fickian diffusion. Furthermore the in-vivo and pharmacokinetic study have to carry out.

REFERENCES

1. https://go.drugbank.com/drugs/DB00709
2. https://medlineplus.gov/druginfo/meds/a696011.html
3. Singer JW, Bhatt R, Tulinsky J, et al. (2001). Water soluble poly-(L-glutamic acid)-Gly-camptothecin conjugates enhance camptothecin stability and efficacy in-vivo. J Control Rel., 74: 243- 247.
4. Berthold A, Cremer K, Kreuter J. (1996). Preparation and characterization of chitosan microspheres as drug carrier for prednisolone sodium phosphate as model for anti-inflammatory drugs. J Control Rel., 39:17 to e25.
5. Sinha VR, Singla AK, Wadhawan S, et al.(2004). Chitosan microspheres as a potential carrier for drugs. Int J Pharm., 274:1 to 33.
6. Rowley JA, Madlambayan G, Mooney DJ. (1999). Alginate hydrogels as synthetic extracellular matrix materials. Biomaterials, 20:45 to 53.
7. Tanihara M, Suzuki Y, Yamamoto E, Noguchi A, Mizushima Y. (2001). Sustained release of basic fibroblast growth factor and angiogenesis in a novel covalently crosslinked gel of heparin and alginate. J Biomed Mater Res., 56:216 to 221.
8. Hoare TR, Kohane DS. (2008). Hydrogels in drug delivery: progress and challenges. Polymer, 49:1993 to 2007.
9. Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R. (2004). Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur J Pharm Biopharm., 57:19 to 34.
10. Jameela SR, Jayakrishnan A. (1995). Glutaraldehyde crosslinked chitosan microspheres as a long acting biodegradable drug delivery vehicle: studies on the in vitro release of mitoxantrone and in vivo degradation of microspheres in rat muscle. Biomaterials, 16:769 to 775.
11. Genta I, Perugini P, Conti B, Pabanetto F. (1997). A multiple emulsion method to entrap a lipophilic compound into chitosan microspheres. Int J Pharm., 152:237 to 246.
12. Hamman JH. (2010). Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. Mar Drugs., 8:1305 to 1322.
13. Bhattarai N, Gunn J, Zhang M. (2010). Chitosan-based hydrogels for controlled, localized drug delivery. Adv Drug Deliv Rev., 62:83 to 99.
14. Mi FL, Shyu SS, Chen CT, Schoung JY. (1999). Porous chitosan microsphere for controlling the antigen release of Newcastle disease vaccine: preparation of antigen-adsorbed microsphere and in vitro release. Biomaterials, 20:1603 to 1612.
15. Lim LY, Wan LSC, Thai PY. (1997). Chitosan microspheres prepared by emulsification and ionotropic gelation. Drug Dev Ind Pharm.. 23:981 to 985.
16. Shu XZ, Zhu KJ. (2001). Chitosan/gelatin microspheres prepared by modified emulsification and ionotropic gelation. J Microencapsul., 18:237 to 245.
17. Kumar MN, Muzzarelli RA, Muzzarelli C, Sashiwa H, Domb AJ. (2004). Chitosan chemistry and pharmaceutical perspectives. Chem Rev., 104:6017 to 6084.
18. Shen EC, Wang C, Fu E, Chiang CY, Chen TT, Nieh S. (2008). Tetracycline release from tripolyphosphate chitosan cross-linked sponge: a preliminary in vitro study. J Periodontal Res., 43:642 to 648.
19. Denkbas EB, Ottenbrite RM. (2006). Perspectives on: chitosan drug delivery systems based on their geometries. J Bioact Compat Polym., 21:351 to 368.
20. Choi BY, Park HJ, Hwang SJ, Park JB. (2002). Preparation of alginate beads for floating drug delivery system: effects of CO2 gas forming agents. Int J Pharm., 239:81 to 91.
21. Das SK, Das NG. (1998). Preparation and in vitro dissolution profile of dual polymer (Eudragit RS 100 and RL 100) microparticles of diltiazem hydrochloride. J Microencapsul, 15:445.
22. Tomoda K, Kojima S, Kajimoto M, Watanabe D, Nakajima T, Makino K. (2005). Effects of pulmonary surfactant system on rifampicin release from rifampicin-loaded PLGA microspheres. Colloids Surf B., 45:1 to 6.
23. Ana JPS, Newton LP, Osvaldo F, John HC. (1996). Influence of formulation on the physicochemical properties of casein microparticles. Int J Pharm., 186:191 to 198.
24. El-Kamel AH, Sokar MS, Al Gamal SS, Naggar VF. (2011). Preparation and evaluation of ketoprofen floating oral delivery system. Int J Pharm., 220:13 to 21.
25. ele D, De Smet B, Beaulieu C, Huguet ML, Fournier A, Neufeld RJ. (1995). Production of alginate beads by emulsification: internal gelation. Physicochem Appl Microbiol Biotechnol, 43:644 to 650.
26. Shu XZ, Zhu KJ, Song W. (2001). Novel pH-sensitive citrate cross-linked chitosan film for drug controlled release. Int J Pharm, 212:19 to 28.