Original Article

Composite alginate hydrogel microparticulate delivery system of zidovudine hydrochloride based on counter ion induced aggregation

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Abstract

Aim: The present study deals with preparation of zidovudine loaded microparticle by counter ion induced aggregation method. During this study effect of polyacrylates and hypromellose polymers on release study were investigated. **Materials and Methods:** The ion induced aggregated alginate based microparticles were characterized for surface morphology, particle size analysis, drug entrapment study, in-vitro study, Fourier-transform infrared (FTIR) spectroscopy, and differential scanning calorimetry (DSC) study. **Results and Discussion:** The result showed Eudragit RL-100 (ERL) based formulations had smoother surface as well as their mean particle sizes were found greater compared with Eudragit RS-100 (ERS) microparticles. Furthermore, drug entrapments were found to be more in ERL formulae as compared with ERS. RL3 released 101.05% drug over a period of 8th h and followed Higuchi profile and Fickian diffusion. Moreover, data obtained illustrated that, higher amount of quaternary ammonium group, alkali value, and glass transition temperature may be possible reason for improving permeability of ERL based formulations. It was also noticed, hyroxypropyl methylcellulose (HPMC) K4M premium grade polymer sustained drug release more than HPMC K15M. In addition, drug-excipient interaction study was carried out by FTIR and DSC study.

Key words: Eudragit RL-100, Higuchi profile, hypromellose, hyroxypropyl methylcellulose K4M premium, microparticle, zidovudine

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Introduction

Zidovudine hydrochloride (azidothymidine) is a class of nucleoside reverse transcriptase inhibitor, has been used for successive treatment for HIV/AIDS infection. It works by selectively inhibiting the viral reverse transcriptase, an enzyme, so that viral replication process inhibited and leads to patient clinical and immunological responses.[1,2] This virustatic drug has lower bioavailability of 75% due to considerable first-pass metabolism and lower half-life of 0.5-3 h, thus necessities administration of frequent dosing to maintain a therapeutic level. However, major side-effects like neutropenia and anemia are commonly associated with frequent administration.[3,4] Thus, great attention has been paid for designing sustained/controlled delivery system to overcome the possible disadvantages or undesirable effects. Controlled release drug delivery employs devices, such as polymer-based disks, rods, pellets, or particulate - that 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 microparticulate drug delivery systems.[5-7] In the last decade, several research works already reported based on microparticle.

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Several methods of preparing microparticulate drug delivery systems are available, e.g., spheronization, spray granulation, coacervation, and fluidized bed granulation, etc., The main disadvantages associated with those techniques include high cost of manufacturing, need of specialized and high skill trained persons and equipment. Counterion induced aggregation technique involves ionic cross-linking between polyanionic electrolyte biopolymers such as alginates, carboxymethylcellulose, chitosan, etc., and counter ions like polycations (Ba^{2+} or Ca^{2+}) to produce cross-linked aggregate.[8-10] Biodegradable polymers have attracted considerable attention as potential device for controlled drug delivery.[11] Researches have been carried on the use of sodium alginate as network forming or gelling agent because of lesser cost, naturally occurring polysaccharide, biodegradability, non-toxicity, provides protection against mucous from irritation and high swelling capacity on contact with gastric fluid. In order to provide stability and protection against the external environment several researchers reported concomitant use of hypromellose or hyroxypropyl methylcellulose (HPMC)[12,13] and pH independent polymers like polyacrylates (Eudragit RS and RL [ERS and ERL]).[14,15] Recently, biocompatible polysaccharides-based microparticles for intranasal protein delivery were successfully developed.[16] Lyophilized polysaccharide based lysozyme microparticles were also prepared and tested for stability.[17]

Hence, in the present study an attempt has been made to prepare hydrogel based microparticles by HPMC, ERS/ERL and Sodium alginate as polymeric matrix device. Release study based on the effect of concentration of variable polymers were studied, as well as surface morphology, particle size, drug-excipient interaction study were investigated.

Materials and Methods

Materials

Zidovudine hydrochloride and sodium alginate were purchased from sigma chemical (NJ, USA). Hypromellose K4M premium and K15M were obtained from Ashland Aqualon Functional Ingredients (Wilmington, DE, USA). ERL-100/RL-100 were obtained from Emzor exports Pvt. Ltd. (Ahmedabad, India). Barium choride (BaCl2) and calcium chloride (CaCl2) were obtained from Ranbaxy Fine Chemicals Ltd., 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 [Table 1]. Required quantities of sodium alginates were dissolved in 20 ml of demineralized water to form homogenous polymer solution. When sodium alginate was uniformly mixed then specified quantity of HPMC K4M and HPMC K15M were added and homogenized (PandaPLUS 2000, GEA Nitro soave, Italy) for 30 min to prepare polymer dispersion. Meanwhile, in another beaker 300 mg of zidovudine was dissolved in 12 ml of methanol and mixed to get clear solution. To the above mentioned, required quantity of polyacrylates (ERL-100/ERS-100) were added and uniformly homogenized. The drug mixtures were added to polymer dispersion and stirring (Ika 2581000, Germany) was continued for 15 min to form homogenous dispersion. Finally, the dispersions were poured to 100 ml of 10% BaCl2/CaCl2 solution through 24G needle to form hydrogels.[18] The obtained hydrogels were allowed to stirred for further 15 min, filtered, collected and dried at 40°C for 2 days. The collected microparticles were stored in a desiccator for further evaluation.

Surface morphology analysis

Microparticle surface morphology was determined by scanning electron microscope (SEM, Philips-XL 20). Microparticles were coated with gold film using Ion-Sputtering device under reduced pressure and mounted directly in the sample holder.[19] To study the internal morphology microparticles were subjected to liquid nitrogen and cut with a sharp razor blade.

Particle size analysis

The microparticles were accurately weighed and sized using US pharmacopoeia (USP) standard sieve set in the range of

Table 1: Formulation composition of drug loaded microparticle

Formulation	Zidovudine (mg)	Sodium alginate (%)	HPMC K4M premium (mg)	HPMC K15M (mg)	Eudragit RS-100 (%)	Eudragit RL-100 (%)	$BaCl_2$ (%)	$CaCl_2$ (%)
RS1	300	1.50	0	100	3	0	0	10
RS2	300	2.50	25	75	3	0	0	10
RS3	300	3.50	75	25	3	0	0	10
RS4	300	3.50	50	50	3	0	0	10
RL1	300	1.50	100	0	0	3	10	0
RL2	300	2.50	75	25	0	3	10	0
RL3	300	3.50	25	75	0	3	10	0
RL4	300	3.50	50	50	0	3	10	0

HPMC: Hyroxypropyl methylcellulose; BaCl2: Barium choride; CaCl2: Calcium chloride

200-1400 μm (Rx-86-1, Cole-Parmer Instrument Co., USA). 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.[20] 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 (300 mg) of microparticles were taken in a clean mortar and pestle. To that 10 ml of methanol was added and triturated. The triturated mixture was filtered by Whatman filter paper (45 μ) and transferred to 100 ml volumetric flask; volume was adjusted with phosphate buffer pH 7.4. Proper dilutions were made and analyzed by ultraviolet (UV) Visible spectrophotometer (1601, Shimadzu Co., Japan) at 266 nm. Encapsulation efficiency was calculated by the following method.

Drug entrapment efficiency = Practical drug content/ theoretical drug content × 100. (1)

In-vitro release study

In-vitro dissolution study of prepared microparticles equivalent to 300 mg of the drug were carried out in USP dissolution rotating basket (USP XXIV Type-I, ERWEKA dissolution tester DT 620) for 8 h. Microparticles were filled in capsule No-1 and transferred to 900 ml of dissolution fluid using basket rotation speed of 75 rpm and temperature of 37 ± 0.5°C. Dissolution was carried out with 0.1 N HCl for initial 2 h followed by 6 h with phosphate buffer pH 7.4. Samples were withdrawn at a predetermined time level (0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h). The aliquots were filtered by Whatman filter paper (0.45 μm) and diluted appropriately with the release medium and absorbance was measured by UV Visible spectrophotometer (1601, Shimadzu Co., Japan) at the predetermined λmax of each medium against a blank.[21,22]

Fourier-transform infrared spectroscopy

The drug-excipient interaction were studied using Fourier-transform infrared (FTIR) (FTIR 8400S, Schimazu). IR spectra for drug and powdered micropaticles were recorded in a FTIR spectrophotometer with KBr pellets. The spectra were scanned over 4000–500/cm range.[23]

Differential scanning calorimetry study

The differential scanning calorimetry (DSC) analysis of pure drug and drug-loaded microparticles were carried out using Shimadzu DSC 60. The analysis was performed at a rate 10°C/min ranging from 20°C to 300°C temperature.[24]

Results and Discussion

Surface morphology and particle size analysis

Zidovudine loaded microparticles prepared by counter ion induced aggregation and their surface morphology and cross section (not displayed in figure) were observed under SEM study as cited in Figure 1. Microparticles were asymmetrical and their surfaces were irregular and uneven. Furthermore, it was noticed that ERS-100 microparticles had more roughs and ridges as compared to ERL-100 based. Cross-sectional area showed large opening and channels.

The fraction percent of weight distribution of different formulae of zidovudine 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 50% of particles were retained at mean diameter of 800 μm and a least of 11% retained over mean diameter of 400 μm for ERS based formulation, whereas in ERL based formulation the maximum of 48% microparticles retained at mean diameter of 1200 μm [Figure 2]. To confirm the above stated information further SEM study were done for RS3 and RL3 as displayed in Figure 3.

Drug entrapment determination

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

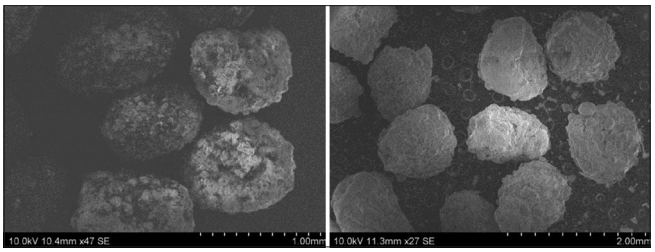

Figure 1: Surface morphology of drug loaded ERS and ERL microparticle. ERS is Eudragit RS-100 based formulation. ERL is Eudragit RL-100 based formulation

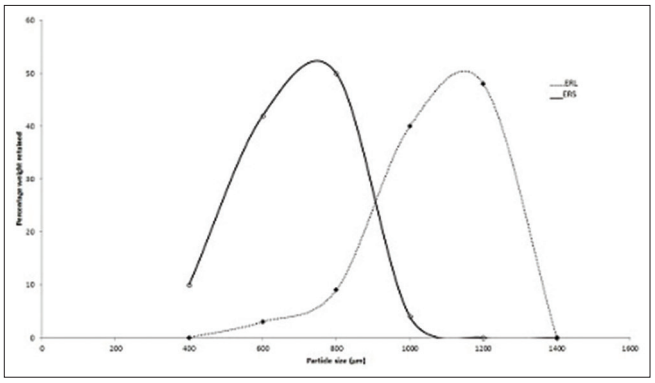

Figure 2: Particle size distribution curve. ERS and ERL stands for Eudragit RS-100 and Eudragit RL-100, respectively

21.93 to 79.21. It was observed that ERL based formulation had higher drug entrapped when compared to ERS based as cited in Table 2. This could be the possible reason that ERL based formulation had a smooth surface when compared to ERS formulations, thus entrapped more drug as observed in figures.

In-vitro release study

Different two grades of hypromellose such as HPMC K4M premium, HPMC K15M and polyacrylates (ERS-100 and ERL-100) and natural polymer like sodium alginate ranging from 1.5% to 3.5% were used to formulate zidovudine loaded microparticles and those formulations were subjected to in-vitro drug dissolution studies [Table 3]. Formulation RS1-RS4 contained ERS and 10% CaCl2, whereas RL1-RL4 contained only ERL and 10% BaCl2. Result showed that during initial 15 min, 19.42-28.24% of the drug was released for RS1-RS4 and 21.64-40.4% for RL1-RL4. Several articles reported an increase in the amount of sodium alginate can progressively retard drug release.[25] However, during this study it was shown that hypromellose had a major influence on release. A comparison was done between RS1 and RL1 as they contained only HPMC K15M and HPMC K4MP. It was observed that for the time period of 0.5 h RL1 released only 22.66%, meanwhile 25.46% drug was released by formulation RS1 and such type release pattern was maintained for further 3 h. Similar pattern can be observed in case of between formulation RS3 and RS4 as they contained equal quantity of sodium alginate. Result showed RS3 released 22.84% at the end of 2 h and 63.28% up to 8 h, whereas 34.18% and 68.35% drug release were observed by RS4 at the end of 2 and 8 h respectively. As both above mentioned formulation contained 3.5% of sodium alginate but differed in hypromellose grades, hence it can be proved that HPMC K4M Premium grade have more retarding efficiency than HPMC K15M [Figure 4].

Another remarkable difference was observed between ERS and ERL based microparticles. E RL formulations were able to release comparatively greater 75.96%, 83.84%, 101.05%, and 89.03% for RL1, RL2, RL3, and RL4, respectively at

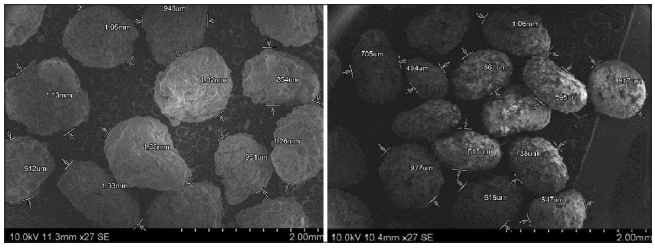

Figure 3: Particle size analysis of Eudragit RL (ERL) and Eudragit RS (ERS) microparticles. Accelerating voltage = 10 kV Mag = ×27 working distance = 11.3 mm (ERL). Accelerating voltage = 10 kV Mag = ×27 Working distance = 10.4 mm (ERS)

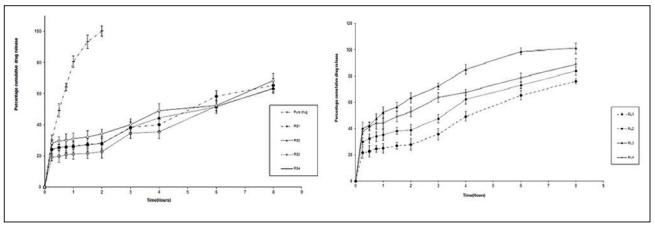

Figure 4: In-vitro release pattern of RS1-RS4 and RL1-RL4. RS1-RS4 are Eudragit RS-100 based formulations. RL1–RL4 are Eudragit RL-100 based formulations

Table 2: Characterization of microparticles

Characterization parameter	Formulation							
	RS1	RS2	RS3	RS4	RL1	RL2	RL3	RL4
Drug entrapment efficiency (%)	23.68±2.14	21.93±1.38	35.09±2.36	51.84±0.97	49.47±2.43	72.10±1.72	79.21±1.76	78.05±1.74
Mean particle size (μm)	798±5.05	801±7.59	807±9.47	799±10.36	1205±4.98	1336±5.26	1381±3.49	1138±8.57

Data are represented as mean±SD. SD: Standard deviation

Table 3: Percentage cumulative of drug release from microparticles

Time (h)	Percentage cumulative of drug release								
	Pure drug	RS1	RS2	RS3	RS4	RL1	RL2	RL3	RL4
0	0	0	0	0	0	0	0	0	0
0.25	30.37±3.12	24.06±3.23	24.61±3.18	19.42±2.45	28.24±1.34	21.64±4.23	30.11±2.64	40.4±4.24	37.66±2.35
0.5	49.23±4.24	25.46±4.68	25.57±2.11	19.71±3.59	29.68±2.57	22.66±4.12	32.44±3.56	42.15±2.56	41.46±3.18
0.75	64.21±2.45	25.77±3.84	26.36±4.29	20.81±2.41	29.98±4.28	24.56±2.36	33.95±3.68	47.46±3.73	43.92±4.29
1	80.58±3.62	26.4±2.58	26.12±3.45	21.32±3.26	31.2±3.49	25.05±3.45	35.34±4.27	52.19±4.12	43.99±3.78
1.5	93.39±4.3	27.4±3.56	27.14±4.28	21.81±3.4	32.27±3.84	26.87±2.56	38.37±2.67	56.38±3.72	49.19±4.23
2	100.2±3.22	28.15±4.38	28.1±3.58	22.84±4.19	34.18±2.97	27.77±4.38	39.07±4.18	63.4±3.71	52.62±3.76
3		38.46±3.28	38.12±4.05	34.69±3.27	40.23±3.58	35.68±4.12	47.62±3.27	72.17±2.35	63.76±3.56
4		40.21±4.23	44.53±3.67	35.48±4.28	49.24±4.19	49.28±3.56	62.28±3.49	85.12±3.68	67.52±2.18
6		58.29±3.68	51.58±3.1	51.97±3.28	52.48±3.27	65.27±3.67	72.98±2.35	98.82±2.37	78.75±3.28
8		65.35±4.36	63.67±3.67	63.28±2.17	68.35±4.65	75.96±2.12	83.84±3.13	101.05±4.01	89.03±4.2

Data are represented as mean±SD. SD: Standard deviation

the end of 8 h than ERS microparticles. The ERS and ERL formulations contained copolymer of acrylic and methacrylic acid esters with quaternary ammonium group. The ratio of trimethylammonioethyl methacrylate chloride is more in ERL and ammonium group is responsible for permeability and forms channel, so that surrounding fluid diffuse out the drug particle to outside.[26] Though ERL contains methacrylate chloride, which is insoluble; the higher alkali value and glass transition temperature might be other possible reasons for higher permeability of ERL than ERS. Among all formulation RL3 released 101.05% of drug in 8th h. 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 RL3 had greater r2 value of 0.949 compared to all. To confirm the exact mechanism of drug release, data were fitted to Korsemeyer-Peppas equation. Regression analysis was performed and "n" values were 0.38< n < 0.498. Hence, it can be inferred that the release was based on Fickian diffusion. On the basis of the above results, RL3 was selected as a promising formulation for further studies.

Drug-excipient interaction by Fourier-transform infrared and differential scanning calorimetry

Fourier-transform infrared study showed characteristic broad peaks of carbonyl (C=O stretching) at 1666/cm and 2083/cm for azido group for pure zidovudine. When peaks were recorded separately with excipients as physical mixture, there were no such changes in peaks were observed, but a slight change in peak was observed at 1599/cm especially for carbonyl group, which could be the reason for the solvents used during formulation. Moreover, a weak absorption peak was observed at 1413/cm for aromatic C=C stretching. Free -CH3 group showed peak at 2926/cm and 3333/cm, 3458/cm peaks were observed for -OH stretching and -NH stretching respectively [Figure 5].

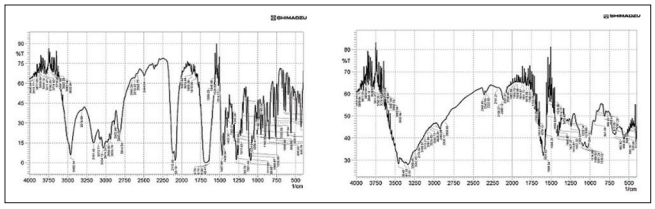

Figure 5: Fourier-transform infrared spectra of pure zidovudine and RL3. RL3 is best Eudragit RL-100 based formulation

Differential scanning colorimetry studies were performed to assess the interaction between drug and excipients. Pure zidovudine showed characteristic endothermic peak at 128.8°C in thermogram. Whereas RL3, Drug + ERL, drug + HPMC K4M and Drug + HPMC K15M (not displayed in figure) showed endothermal peaks at 124.0°C, 127.01°C, 127.8°C, and 123.0°C, respectively [Figure 6]. The results indicated lower the value of melting endothermal peak of samples as compared with pure drug. Hence, it can be concluded slight interaction between drug and excipients and may be explained as lowering of crystallinity of drug in formulation. Another endothermic peak was observed at 166.92°C for RL3, which may be the presence of impurity.

Stability study

Long-term, intermediate, and accelerated stability testing were carried out for RL3 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 300 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 4. Thus, RL3 formulation batch confirmed its stability. Furthermore, the in-vivo and pharmacokinetic study have to carry out.

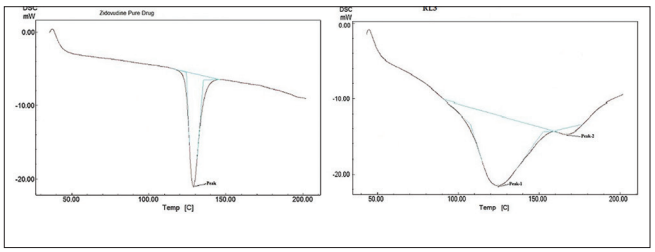

Figure 6: Differential scanning calorimetry thermogram of pure zidovudine and RL3. Peak: 128.85°C, onset of peak: 124.21°C, heat: −248.54 MJ (pure zidovudine). For peak-1 the values observed are, peak: 124.42°C, onset of peak: 107.55°C, heat: −619.80 MJ. For peak-2 as additional peak, the values observed are, peak: 166.92°C, onset of peak: 160.30°C, heat: −19.71 MJ (RL3)

Table 4: Stability study of best batch (RL3)

Long term stability study (25±2°C and 60±5% RH)						
Days (month)	3	6	9	12	18	24
Drug assay (%)	99.29±1.21	99.19±0.43	100.16±0.62	99.32±0.07	99.27±1.41	99.09±0.97
Intermediate stability (30±2°C and 65±5% RH)						
Days (month)	3	6	9	12	18	24
Drug assay (%)	99.57±2.35	99.01±0.12	99.28±2.06	98.27±1.72	98.37±0.52	97.29±1.09
Accelerated stability (40±2°C and 75±5% RH)						
Days (month)	1	2	3	6	-	-
Drug assay (%)	99.65±2.47	99.38±1.72	99.32±2.05	99.03±1.15	-	-

Data are represented as mean±SD. SD: Standard deviation

Conclusion

Hence, from the above mentioned work it can be concluded that, the suitable combination of hypromellose and polyacrylate polymers can effectively use to release drug for a longer period of time. It was also observed that ERL based formulation had more drug entrapment and prolonged for more time than ERS.

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