Optimization of Gabapentin Release and Targeting Absorption, Through Incorporation into Alginate Beads

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ABSTRACT

Aims: 1) To study the effect of some formulation variables on drug load, encapsulation efficiency, swelling ratio, mucoadhesion and drug release.
2) Optimize the mucoadhesion capabilities for targeting drug absorption and release-controlling capabilities of alginate beads.

Methodology: alginate beads were prepared by dripping sodium alginate gel into calcium chloride solution of and then dried overnight at ambient temperature. The effects of alginate concentration, cross linker concentration, cross linking time, volume of cross linking solution and drug/polymer ratio on drug load, encapsulation efficiency, swelling ratio, mucoadhesion and drug release were investigated. Formulae containing sodium lauryl sulfate (SLS), gabapentin-ethylcellulose solid dispersion, mixture of free drug and solid dispersion were prepared for modifying the drug release rate.

Results: Mucoadhesion of alginate beads were shown to be decreased upon adding SLS (30% after 8 hrs). Drug release was so fast (92.46% after 2 hrs). The incorporation of solid dispersion has led to well accepted mucoadhesion (74.44% after 8 hrs) as well as release properties (93.35% after 10 hrs) Beads containing mixtures of drug and ethylcellulose-drug solid dispersion showed acceptable mucoadhesion (74.44% after 8 hrs) and control of gabapentin release (93.35% after 10 hrs). Statistical analysis of variance between groups was performed using the one-way layout ANOVA with duplication. Significant differences in mean values were evaluated by Student's unpaired t test ($p < 0.05$).

Conclusion: A finally optimized formula was suggested by incorporating a combination of solid dispersion and free gabapentin in alginate system to achieve burst release of gabapentin and hence fast effect (33.417% was released during the first 30 minutes in fasting-simulated conditions) and controlled release (91.217% after 6 hrs).

Keywords: Alginate, Control release, Targeting, Gabapentin, Sodium lauryl sulfate, Ethylcellulose, Solid dispersion.

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1. INTRODUCTION

Alginic acid is a natural polysaccharide found in all species of brown algae. It exists as a linear polymer consisting of β-D-(1→4) mannuronic acid (M) and α-L-(1→4) guluronic acid (G) in varying proportions and sequential arrangement [1]. The homopolymer regions composed of M blocks and G blocks are interspersed with MG heteropolymeric regions. Alginic acid is a hydrophilic polymer that swells in the presence of water. Sodium alginate, which is the sodium salt of alginic acid, is soluble in water and can be cross-linked with divalent cations such as Ca^2+ and Zn^2+ and polyvalent ones to form an insoluble alginate. Calcium ion was found to bind selectively guluronic acid residues (GG) in a planar two-dimensional structure producing the so-called "egg box" structure [2]. The ratio of G to M residues was found to affect the release of drugs from calcium-cross-linked alginate systems [3].

Alginate systems were found to have a number of properties that are used to deliver DNA [4], locally deliver enzymes [5], immobilize enzymes [6], oral immunization [7], and to act as adenovirus vector [8].

The mucoadhesive properties of alginate emphasized its use as an efficient tool to improve oral mucoadhesion for increasing bioavailability of drugs [9] such as nicardepine HCl [10], gliclazide [11,12], and diltiazem HCl [13] and to control systemic absorption of some narrow absorption window (NAW) drugs.

Gabapentin is an orally available γ-aminobutyric acid analog which is used to control partial seizures in combination with other antiseizure drugs [14]. It is one of the NAW drugs since it is actively absorbed from upper duodenal region via L-amino acid transporters [15].

The aim of this study was to evaluate the effect of formulation variables on alginate beads properties and optimizing their drug targeting properties as well as release control profile using gabapentin as a hydrophilic model drug.

2. MATERIAL AND METHODS

2.1 Materials

Sodium alginate was purchased from Sigma Aldrich, St. Louis, USA. Gabapentin was a gift from Delta Pharm, 10th of Ramadan city, Egypt. Calcium chloride dihydrate from VWR Scientific, West Chester, PA, USA. Sodium lauryl sulphate (SLS) from Aldrich, Milwaukee, WI, USA. The other chemicals used were all of analytical and HPLC grade.

2.2 Methods

2.2.1 Preparation of calcium alginate mucoadhesive beads

Calcium alginate beads were prepared by ionotropic gelation. The amounts of sodium alginate, concentration of calcium chloride solution and quantity of gabapentin used and the formulation variables of the beads are listed in table 1. A gel solution of sodium alginate was made by hydrating the proper amount of sodium alginate in deionized water and stirring till a clear gel solution is formed. In separate vial, gabapentin was dispersed evenly in deionized water and then added to the gel. A gentle and consistent mixing for about 5 minutes. The formed gel containing the drug was then placed in a syringe pump (model M362, Sage Instruments, Orion Research Inc., Massachusetts, USA) then introduced into calcium chloride solution by dripping from a syringe pump. Beads were then strained, washed twice by deionized water and then left to dry at ambient temperature overnight.

Table 1. Compositions and Variables of Formulation of Different formulae.

FORMULA CODE	SODIUM ALGINATE CONC. (% W/V)	CROSS-LINKER CONC. (% W/V)	CROSS-LINKING TIME (MIN)	CROSS-LINKER VOL. : GEL VOL. (ML)	DRUG : POLYMER RATIO
F1	5	1	30	2:1	1:1
F2	2.5	1	30	2:1	1:1
F3	1.67	1	30	2:1	1:1
F4	1	0.5	30	2:1	1:1
F5	1	1	30	2:1	1:1
F6	1	2	30	2:1	1:1
F7	1	1	10	2:1	1:1
F8	1	1	20	2:1	1:1
F9	1	1	60	2:1	1:1
F10	1	1	120	2:1	1:1
F11	1	1	30	1:1	1:1
F12	1	1	30	3:1	1:1
F13	1	1	30	2:1	1:2
F14	1	1	30	2:1	2:1

2.2.2 Determination of drug load percentage

The process of determining percentage of drug loaded was done utilizing extraction of the drug from beads as mentioned by Reis and co-workers with little modification [16]. Specific weight of beads was taken and crushed. The crushed beads were then placed in a vial and a proper amount of deionized water was added to it. The vials containing crushed beads and water was shaken for 15 minutes for complete extraction of drug. The aliquot containing the drug was then analyzed for gabapentin using the method published by Zour et al. [17], The mobile phase was prepared in the ratio of 55:35:10 (water:methanol:acetonitrile). The flow was 1 mL/minute; the injected volume of all samples was 20 µL; and The UV detector was set to detect samples at 210 nm.
The percentage drug load was given by the formula:
Percentage Drug load = (Wt_{Dg} / Wt_{Bd}) x100
where, Wt_{Dg} is the amount of drug loaded in beads and Wt_{Bd} is the weight of beads

2.2.3 Determination of encapsulation efficiency

The content of gabapentin in certain weight of the beads was first determined by extraction then by HPLC quantification as previously mentioned (c.f. section 2.1.2 determination of percentage drug load). Encapsulation efficiency of the drug was given by the formula:
Percent encapsulation efficiency (EE) = (Wt_{Dg} / Wt_{Th}) x 100
Where, Wt_{Dg} is the amount of drug loaded in beads Wt_{Th} is the amount of the drug assumed to be present theoretically in the weight of beads used.

2.2.4 Determination of swelling index

Swelling index of beads was determined according to the method described by Pongjanyakul and Puttipipatkhachorn [18]. A weight of approximately 100 mg of beads was taken and placed in a vessel. 14 ml of testing medium were added to the beads. After predetermined time intervals, all beads were withdrawn from the vessel, carefully and quickly dried and then weighed. The swelling index was then calculated using the following formula:
Swelling index (S.I.) = $[(W_t-W_o)/W_o]$ x100
Where, W_t is the weight of beads determined at time t and W_o is the weight of beads determined before immersion of beads in testing medium.
Two testing media were used in this test, 0.1 N HCl solution; and 0.01 N HCl solution containing 0.2% of NaCl and 0.25% SLS to simulate gastric fluid without enzymes in fasting state and in fed state, respectively [19].

2.2.5 Determination of mucoadhesive properties

The mucoadhesive properties of the beads were evaluated employing the method described by Lehr et al. [20] with modification. The apparatus used was disintegration tester.

2.1.5.1 Tissue Preparation:

A pig's intestine excised freshly within the first hour of slaughtering was cut longitudinally and evacuated from its contents. The empty and flattened intestine was then washed carefully with water and divided into several segments. Tissue segments were then put in zip bags and are kept frozen at -15 °c. When needed, tissue segment(s) was/were taken out of the freezer and kept in the refrigerator 24 hrs prior to performing the mucoadhesive properties testing.

2.1.5.2 Apparatus Preparation

A piece of the pig's intestine was cut to be as long as a microscopic slide. This piece was then made to be fixed tightly to the microscopic slide using paper clips, the microscopic slide was designed to be hanged in a disintegration apparatus and during the test it was set to go up and down in the test solution.

The water bath of the disintegration apparatus was filled with testing solution and the temperature was adjusted to be 37°c. The volume of the solution in the water bath was adjusted so that at highest point of movement of the apparatus, slide didn't get out of the testing solution and at lowest point, it didn't touch the bottom. This was done to make the movement of the test solution in relation to the slide smooth and not turbulent.

As in testing the swelling index of the beads, two test media were used in this experiment, 0.1 N HCl solution; and 0.01 N HCl solution containing 0.2% of NaCl and 0.25% SLS to simulate gastric fluid without enzymes in fasting state and in fed state, respectively [19].

2.1.5.3 Performing Test:

The mucosal surface of the intestinal piece was irrigated with some of the test media to simulate the real conditions. 30 beads were then put randomly on the mucosal surface of the pig's intestine piece. A weight of 50 grams was put on the beads for 30 seconds, then the load was removed and the slide containing the intestinal piece loaded with the beads was hanged on the disintegration apparatus as shown in figure 1.

The apparatus was turned on and the piece of pig's intestine, bearing the beads, was allowed to go in and out of the test media freely.

At each time point, the number of beads remaining adhering to the mucosal surface of the hanged piece of pig's intestine was counted and the number is expressed as a percentage of the total number of the beads loaded on the intestinal piece.

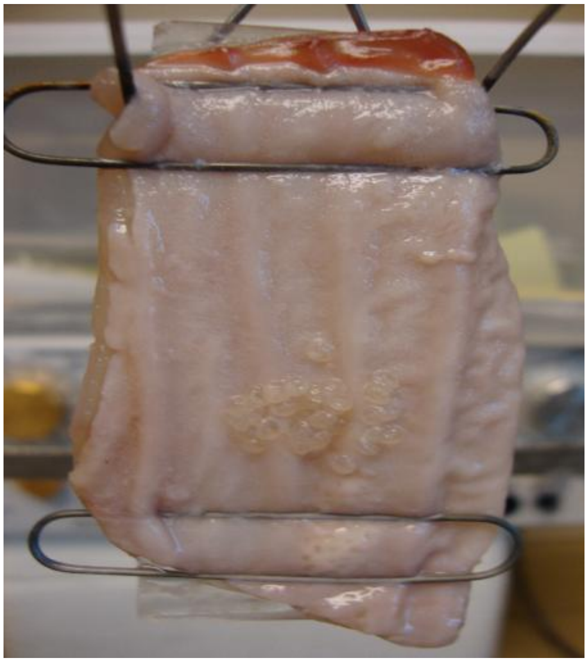

Fig. 1. Mucoadhesion testing showing pig's intestine fixed to a slide and beads adhering to it.

2.2.6 Determination of in-vitro release profile

In-vitro drug release study was performed in a simulated acidic environment in fasting and fed conditions of the stomach [19].
The release of gabapentin from alginate beads was done using the procedure published by Pasparakis and Bouropoulos [21]. An accurately weighed amount of the beads was placed in vials each containing 15 mL of dissolution media pre-warmed up in a shaking water bath at 37±0.5°C. The speed of shaking was adjusted to be 50 rpm. Samples of the dissolution media were withdrawn from each vial and replaced by equivalent amount of fresh dissolution media pre-warmed to 37±0.5°C. Samples withdrawn were analyzed using HPLC method previously mentioned above [17].

2.2.7 Statistical analysis

Data are presented as means±SE. For group comparisons, the one-way layout ANOVA with duplication was applied. Significant differences in mean values were evaluated by Student's unpaired t test. A p value of <0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Drug load and encapsulation efficiency (EE)

Figures 2 and 3 show the percentage drug load and encapsulation efficiency (EE) of the prepared alginate formulae. It was shown that, regarding drug loading capacity, increasing gel concentration, increasing drug/polymer ratio resulted in increasing percent drug load. Decreasing concentration of cross linker, decreasing time of cross linking and/or reducing volume of cross linking solution also resulted in increasing percent drug load. This agreed to results mentioned by Silva and co-workers showing that increasing alginate concentration lead to a consequent increase in EE [22]. Das and Maurya mentioned the same results in previous study [13]. This might be attributed to reduced amount of drug that is lost from beads during cross linking [23,24]. Encapsulation efficiency also depended on the amount of drug lost during cross linking, therefore, the effect of the gel concentration, concentration of cross linker, time of cross linking, volume of cross linking solution on EE would resemble that on drug load. However, regarding drug/polymer ratio, the amount of drug lost during cross linking is not the only determining factor. A comparison between formulae F13, F5, F14 revealed that increasing drug/polymer ratio resulted in increasing percent drug load and decreasing EE. These results agreed to results published by Belgamwar et al. [25]. This is suggested to be attributed to the fact that increasing drug/polymer ratio result in increasing the amount of drug in the beads (drug load) and at the same time increasing the amount of drug lost during cross linking (thus reducing the amount of drug existing in beads as compared to the originally incorporated amount, i.e., reducing EE).

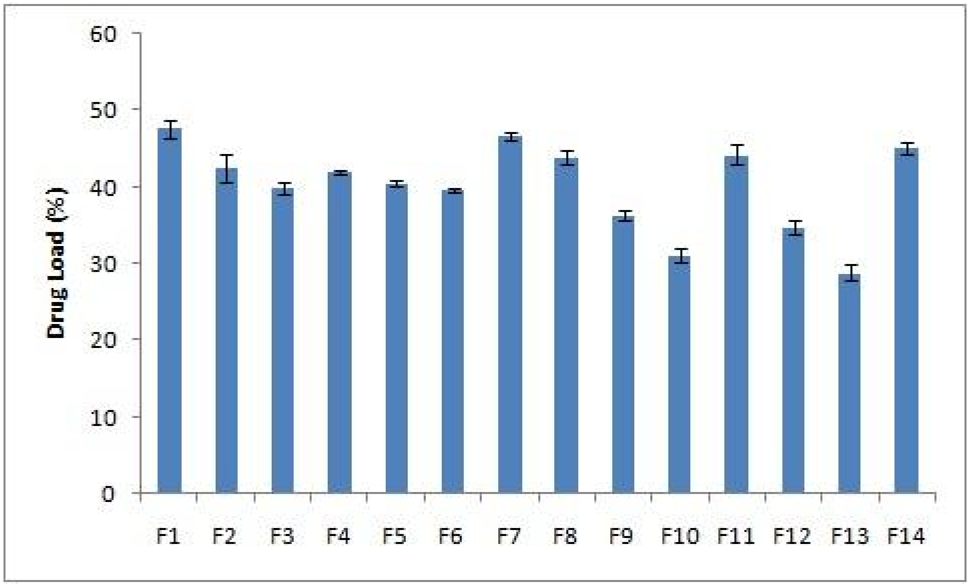

Fig. 2. Percentage drug load of formulae F1 – F14. Each data point represents mean ± S.E. (n=3).

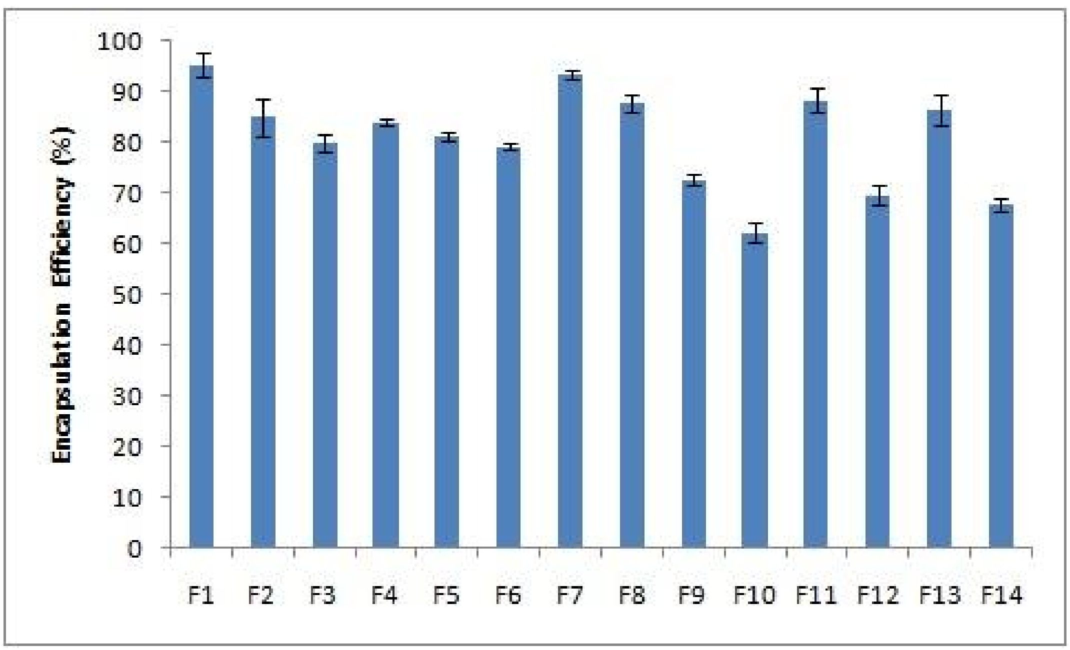

Fig. 3. Encapsulation efficiency of formulae F1 – F14. Each data point represents mean ± S.E. (n=3).

3.2 Swelling index

Figures 4 and 5 show swelling index of the prepared alginate formulae after 30 minutes and 120 minutes in fasting and fed-simulated conditions. It was shown that swelling ratio of beads increases as alginate gel concentration decreases, drug/polymer ratio increases, cross linker concentration decreases and/or time of cross linking decreases. These results agreed to a previous study done by Roy et al. [26]. It was also shown by Ramesh Babu and co-workers that increasing the concentration of cross linker solution has led to a decrease in the water uptake by sodium alginate–methylcellulose blend microspheres [27]. This observation may be attributed to the fact that increasing calcium ions concentration in the cross linking solution leads to formation of the “egg-box” structure of calcium alginate [2] with smaller cavities which accommodate less amount of water and hence decreasing water retained by alginate and SI of beads. This can be also explained on the basis of Flory’s theory of swelling [28]. According to this theory, the swelling ratio of a network (Q) can be described by the following equation:

Q5/3 = { [(i/2VN.S3/2) + (1/2 – Xi)/Vi] / Ve/Vo}

where i/VN is the concentration of the fixed charges referred to unswollen network, S is the ionic concentration in the external solution, (1/2 – Xi)/Vi is the affinity of matrix for water, and Ve/Vo is the cross link density of network.

Volume of cross linking solution had no effect on the swelling of alginate beads. Swelling of beads in fed-simulated conditions was shown to be higher than in fasting-simulated ones, which was also reported in many cases [10,29].

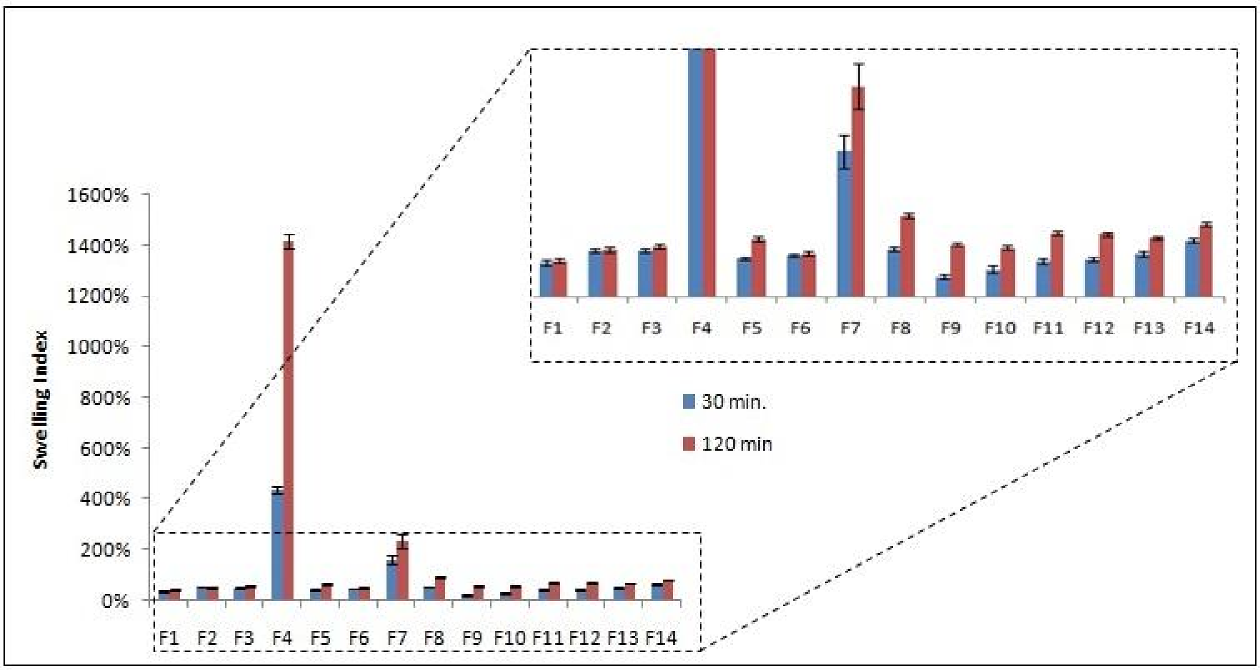

Fig. 4. Swelling indices of formulae F1 – F14 after 30 and 120 minutes in fasting-simulated conditions. Each data represent mean ± S.E. (n=3).

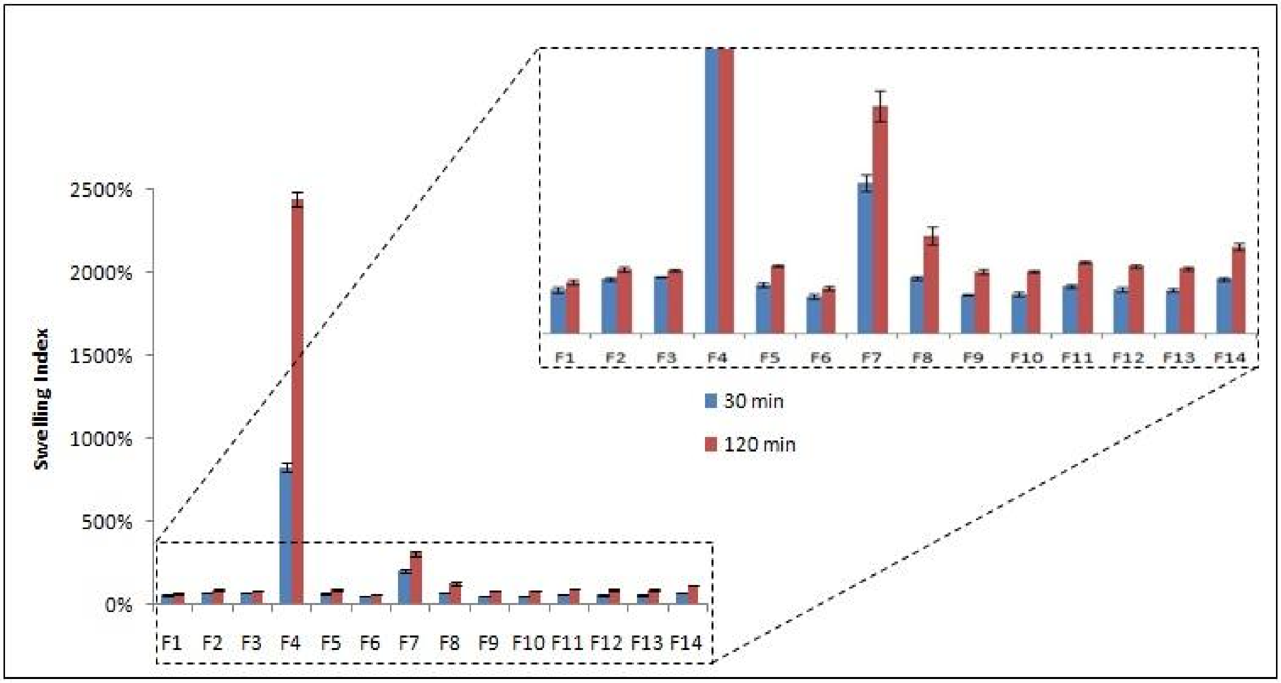

Fig. 5. Swelling indices of formulae F1 – F14 after 30 and 120 minutes in fed-simulated conditions. Each data represent mean ± S.E. (n=3).

3.3 Mucoadhesion properties

Figures 6 and 7 show mucoadhesion of the prepared alginate formulae after 1 and 8 hrs in fasting and fed-simulated conditions, respectively. It was shown that mucoadhesion of beads decreases as alginate gel concentration decreases, drug/polymer ratio increases, cross linker concentration decreases and/or time of cross linking decreases. It has been reported by Chickering and Mathiowitz that surface charge density plays an important role in mucoadhesion. They also reported that polyanionic polymers, such as alginate, are more efficient than polycationic or nonionic polymers in mucoadhesion [30]. Increasing degree of cross linking resulted in reducing the surface negative charge on the alginate beads resulting in decreasing efficiency of mucoadhesion. It was shown also that volume of cross linking solution had no effect on the swelling of alginate beads. Formula F4 (corresponding to cross linker concentration of 0.5 %) and formula F7 (corresponding to cross linking time of 10 minutes) showed a way less mucoadhesion after 8 hrs as compared to other formulae. This is attributed to the increase in weight of beads prepared according to these formulae to a high extent as compared to other formulae. This is shown in SI study (c.f. figures 4 and 5).

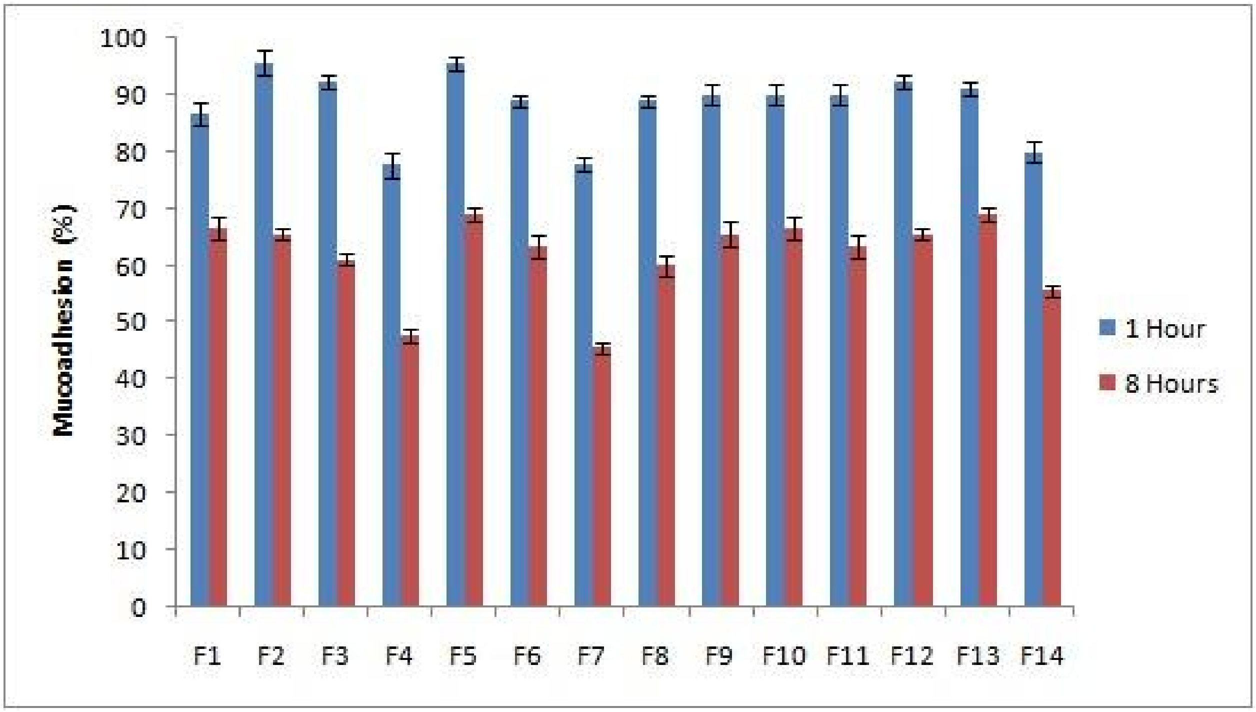

Fig. 6. Mucoadhesion of formulae F1 – F14 after 1 and 8 hrs in fasting-simulated conditions. Each data represents mean ± S.E. (n=3).

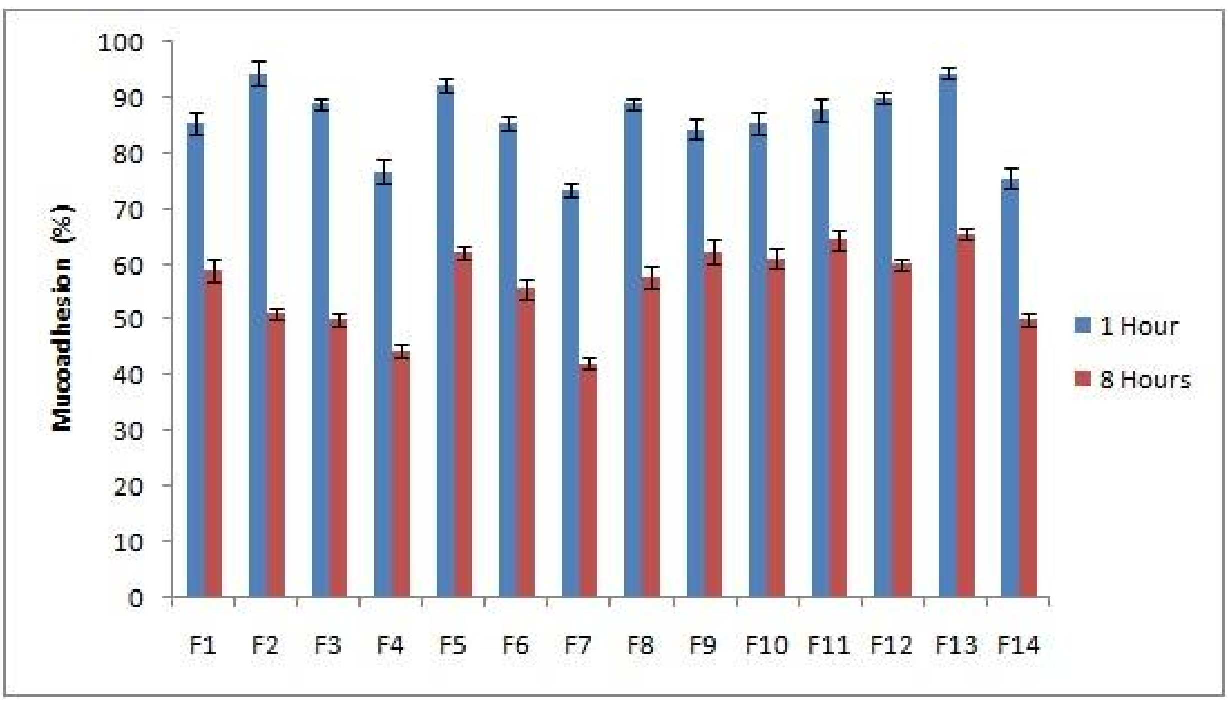

Fig. 7. Mucoadhesion of formulae F1 – F14 after 1 and 8 hrs in fed-simulated conditions. Each data represents mean ± S.E. (n=3).

3.4 Drug release profile

Table 2 shows the time at which alginate formulae released 50% and 90% of their drug content. It was shown that the rate of drug release from alginate system was retarded as the concentration of alginate gel was increased; the drug/polymer ratio was reduced, cross linker concentration was increased and/or cross linking time was increased. This is suggested to be attributed to the increased viscosity of alginate [31] and/or increased degree of cross linking [32]. Rokhade and co-workers studied polymer network microspheres and reported that increasing drug/polymer ratio resulted in faster drug release from the microspheres [33]. It was shown also that release in fed-simulated conditions was faster than that in fasting-simulated ones. Formulae showing high swelling index showed also a fast release of the drug and vice versa. This is attributed to the fact that swelling index of beads is indicative for the interaction between beads and media. The more the interaction between beads and media is, the more the beads swell.

Table 2. T_{50} and T_{90} of drug release from alginate formulae

	Fasting Conditions		Fed Conditions	
	T_{50}* (min)	**T_{90}** (min)**	**T_{50}* (min)**	**T_{90}** (min)**
F1	98.63 ± 2.38	211.00 ± 7.56	89.38 ± 2.38	198.33 ± 12.76
F2	81.73 ± 2.08	180.20 ± 14.57	76.75 ± 2.30	162.90 ± 14.20
F3	63.67 ± 2.71	129.50 ± 3.35	50.00 ± 1.85	102.41 ± 6.68
F4	17.63 ± 0.57	37.41 ± 1.89	16.91 ± 0.85	33.28 ± 1.22
F5	42.47 ± 1.81	100.18 ± 4.04	35.01 ± 1.73	85.02 ± 2.71
F6	66.48 ± 2.31	121.30 ± 3.77	49.30 ± 1.70	118.65 ± 6.54
F7	20.32 ± 0.52	49.38 ± 3.80	20.50 ± 1.80	44.88 ± 2.07
F8	33.82 ± 1.86	78.70 ± 3.66	30.60 ± 1.51	71.87 ± 3.43
F9	61.74 ± 2.38	121.35 ± 3.99	49.28 ± 2.32	98.58 ± 5.90
F10	65.62 ± 1.61	117.95 ± 4.51	53.73 ± 3.36	108.03 ± 2.89
F11	45.59 ± 0.95	86.03 ± 2.13	35.75 ± 1.37	79.48 ± 3.05
F12	51.95 ± 1.56	92.73 ± 3.78	31.87 ± 1.96	78.68 ± 2.57
F13	40.20 ± 1.62	122.09 ± 1.70	39.94 ± 1.82	103.50 ± 1.49
F14	27.13 ± 2.42	73.90 ± 2.21	51.67 ± 15.37	66.63 ± 3.20

** T_{50} is the time at which 50% of the drug was released from the beads*
*** T_{90} is the time at which 90% of the drug was released from the beads*

3.5 Seeking for an optimal formulation

Table 3 shows a summary of the studied factors and their effect on the properties of alginate beads.An optimized formula (OF) was suggested so that the effects of formulation factors can be compensated. It was shown from figures 8-12 that the percent drug load, EE, SI and mucoadhesion of OF formula were accepted for targeting and delivering gabapentin to the upper duodenal region. However, OF formula showed fast release that is not suitable for sustaining the release of the drug as shown in figures 13,14. Controlling drug release form alginate beads was attempted using SDS [33] and solid dispersion [34]. The compositions of OF, SDSF, SDF and FSF formulae are shown by table 4. SDSF formula showed inferior properties as compared to all other formulae. It was shown that incorporating SDS into gel beads has facilitated the release of drug during both cross linking process and drug release study. This resulted in reduction of the percent drug load and encapsulation efficiency; and improper sustained release drug delivery system profile. The use of solid dispersion for sustain the release of the drug had no effect on the targeting properties of alginate beads but sustained the release of the drug to a great degree. To obtain a very fast release and a sustained one, the drug incorporated into beads was divided into two portions, the first portion (1/3 of the total amount) is free drug to produce a fast release and the second portion (2/3 of the total amount) was solid dispersion to sustain the release of the drug. The release of this system, as shown in figure 10, exhibited a fast release (almost 33% during the first half an hour) and sustained release during the rest of the 10 hrs.

Table 3. summary of the studied factors and their effect on the properties of alginate system.

	Drug Load	**Encapsulation Efficiency**	**Swelling Index**	**Mucoadhesion**		**Release Rate**
				1st	**2nd**	
Conc. Of Alginate	+ *	+	- **	-	+	-
Conc. Of CaCl2	-	-	-	-	+	-
Time of Cross Linking	-	-	-	-	+	-
VDps : VCLS	-	-	N †	N	N	N
Drug:Polymer Ratio	+	-	± ‡	±	±	+

* Inversely Related
** Directly Related
† Not Related
‡ Increase to certain Limit or beyond Certain Limit

Table 4. Compositons and Formulation Variables of Modified Alginate Formulae

Formula Code	Sodium Alginate Gel Concentration (% W/V)	Cross Linking Solution Concentration (% W/V)	Cross Linking Time (min)	Cross-Linking solution Volume : Gel Volume	Drug : polymer Ratio	SDS (g)	Free Drug (% of the Total Amount of Drug Added)	Drug-EC Solid Dispersion (% of the Total Amount of Drug Added)
OF *	2	1	30	1:1	3:2	-	-	-
SDSF **	2	1	30	1 : 1	3 : 2	3	100	0
SDF †	2	1	30	1 : 1	3 : 2	-	0	100
FSF ‡	2	1	30	1 : 1	3 : 2	-	33.33	66.67

* Optimized formula
** SDSF sodium dodecyl sulfate formula
† solid dispersion formula
‡ finally suggested formula

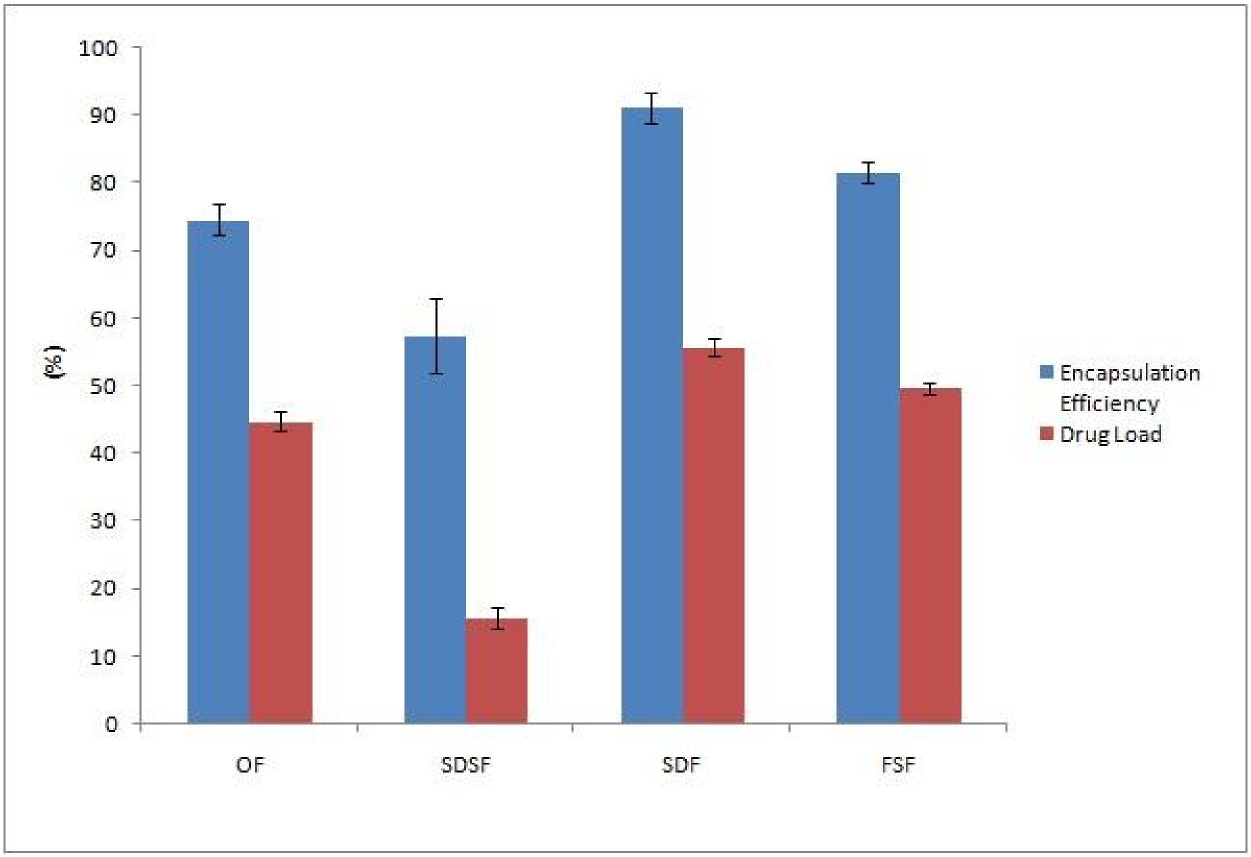

Fig. 8. Drug load and encapsulation efficiency of formulae OF, SDSF, SDF and FSF. Each data represents mean ± S.E. (n=3).

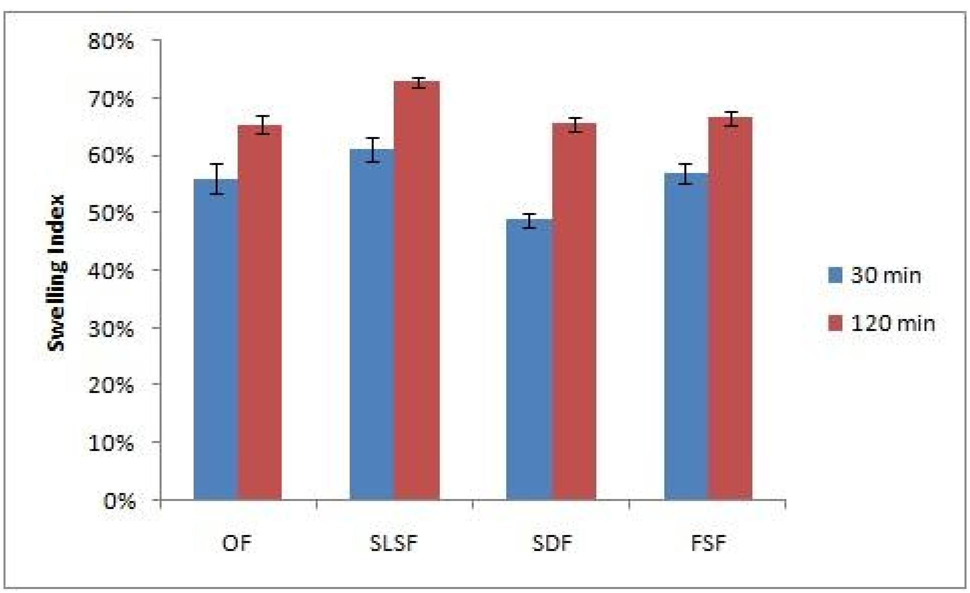

Fig. 9. Swelling ratio of formulae OF, SDSF, SDF and FSF after 30 and 120 minutes in fasting-simulated conditions. Each data represents mean ± S.E. (n=3).

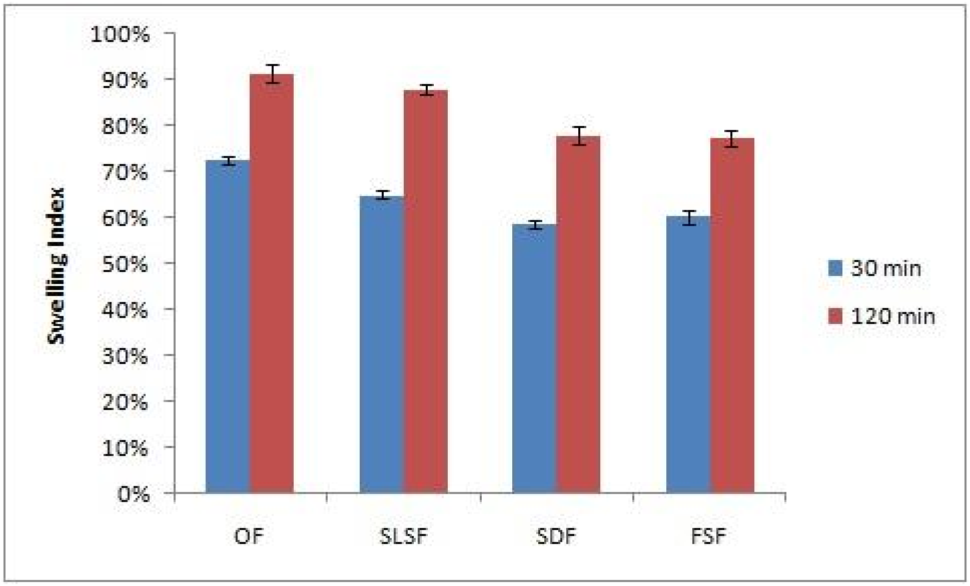

Fig. 10. Swelling ratio of formulae OF, SDSF, SDF and FSF after 30 and 120 minutes in fed-simulated conditions. Each data represents mean ± S.E. (n=3).

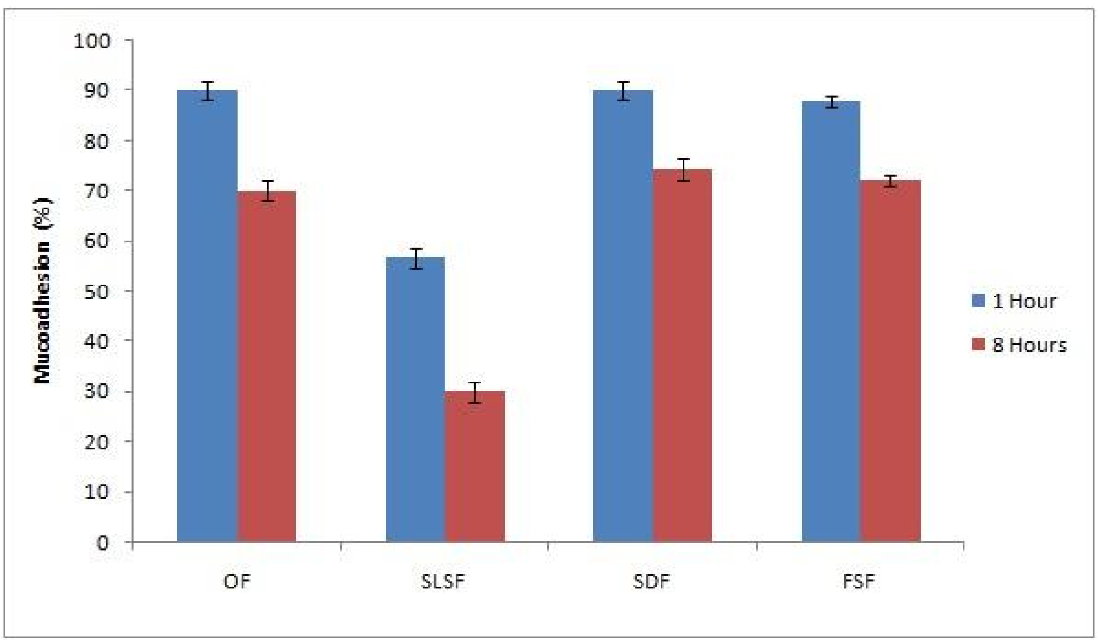

Fig. 11. Mucoadhesion of formulae OF, SDSF, SDF and FSF after 1 and 8 hrs in fasting-simulated conditions. Each data represents mean ± S.E. (n=3).

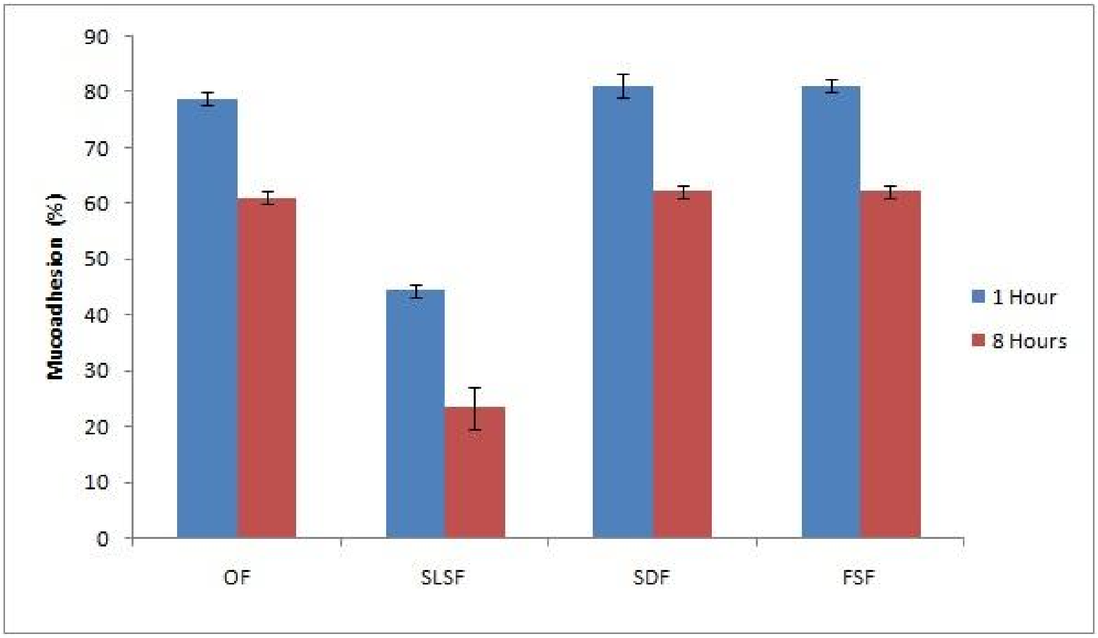

Fig. 12. Mucoadhesion of formulae OF, SDSF, SDF and FSF after 1 and 8 hrs in fed-simulated conditions. Each data represents mean ± S.E. (n=3).

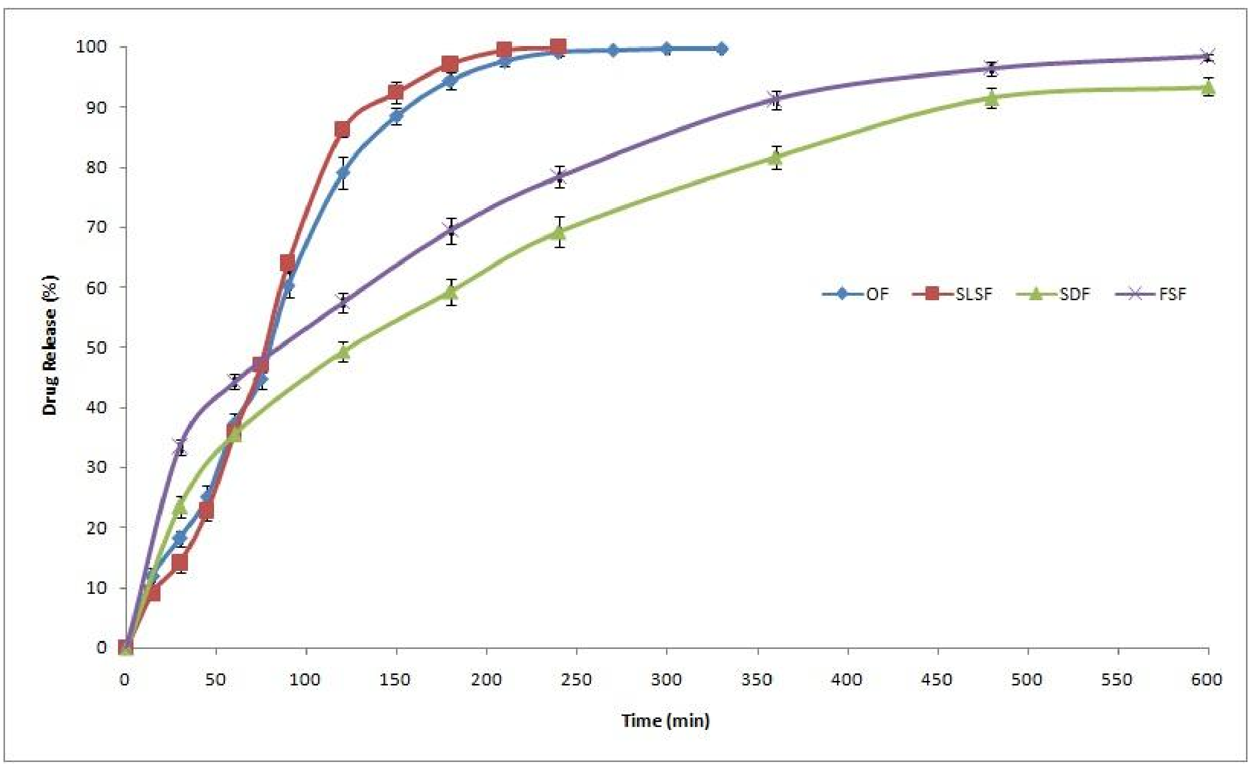

Fig. 13. Drug release profiles of formulae OF, SDSF, SDF and FSF in fasting-simulated conditions. Each data represents mean ± S.E. (n=3).

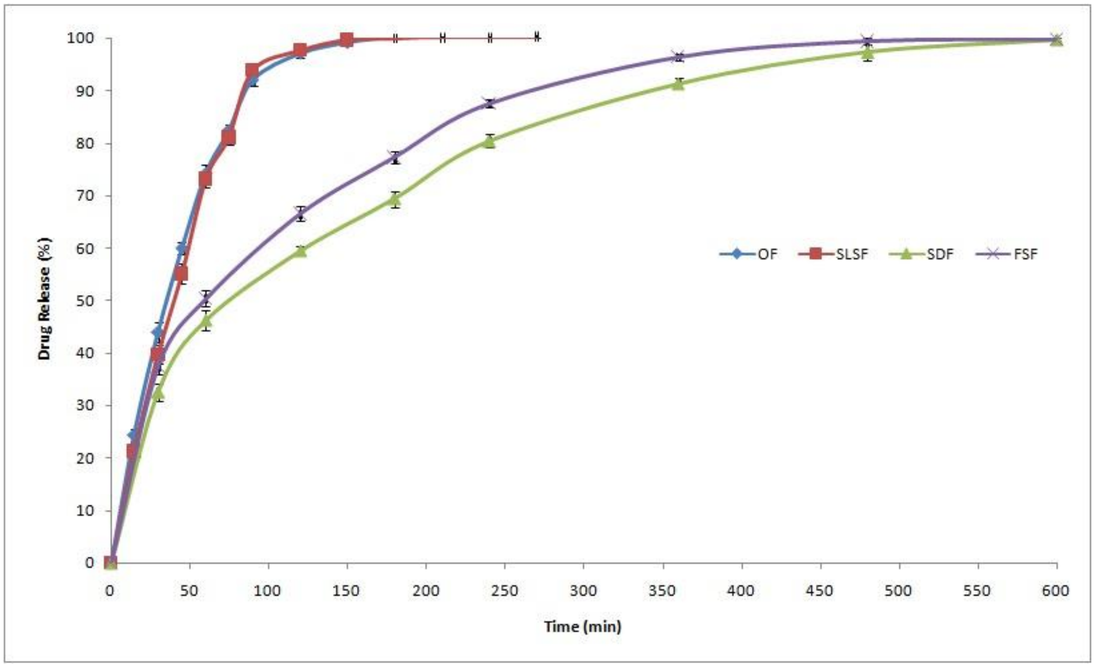

Fig. 14. Drug release profiles of formulae OF, SDSF, SDF and FSF in fed-simulated conditions. Each data represents mean ± S.E. (n=3).

The dissolution efficiency (D.E.), which is a suitable comparative parameter for the quantification of dissolution data, was utilized to assess the effect of alginate modification on the dissolution rate of the drug [35]. It was calculated according to the equation mentioned by Khan and Rhodes [35] as follows,

Dissolution Efficiency (D.E.) = $\frac{\int_0^t y.dt}{y_{100}.t}$

Dissolution efficiencies of optimized formulae are given by table 5. The DE0-60min for OF, SLSF, SDF and FSF formulae were shown to be 265.68, 258.54, 7.06 and 8.48, respectively. It was shown from the values of DE of OF, SDSF, SDF and FSF formulae that incorporating SDS into alginate beads had insignificant effect on retarding drug release. However, the use of EC solid dispersion retarded the release of gabapentin from alginate beads significantly.

Table 5. Dissolution efficiency of modified formulae.

	Fasting-Simulated Conditions				
	0.5 h	**1 h**	**2 h**	**3 h**	**4 h**
OF	135.28	256.68	1047.02	1200.02	1241.19
SDSF	114.71	258.64	1117.11	1228.84	1249.87
SDF	2.45	7.06	9.04	10.71	25.15
FSF	3.23	8.48	10.57	12.31	28.26
	Fed-Simulated Conditions				
	0.5 h	**1 h**	**2 h**	**3 h**	**4 h**

OF	324.77	489.75	1227.81	1252.57	1254.92
SDSF	296.03	481.47	1233.39	1250.96	1252.11
SDF	3.28	8.81	10.74	12.48	28.64
FSF	3.66	9.74	11.98	13.74	30.67

4. CONCLUSION

The optimized formula, OF formula, has shown acceptable drug load, encapsulation efficiency, swelling index and mucoadhesion but not sustained gabapentin release profile ,i.e. alginate system is not capable of fulfilling requirements of producing gabapentin sustained release dosage form (spatial placement and temporal delivery) by just adjusting formulation variables.

Incorporating SDS released gabapentin even faster than OF formula. It also reduced targeting capabilities of alginate system as indicated by fast detachment of beads from intestine piece during mucoadhesion testing.

Incorporating solid dispersion of EC with gabapentin in alginate beads instead of free drug retarded the release of gabapentin from alginate beads successfully. Ethylcellulose - gabapentin solid dispersion also increased the drug load and EE with minor positive impact on the mucoadhesion capabilities of alginate beads.

A finally optimized formula has been suggested by incorporating a combination of solid dispersion and free gabapentin in the ratio of 1:2 in alginate system to achieve burst release of gabapentin and hence fast effect (33.417% ± 2.087 of gabapentin was released during the first 30 minutes in fasting-simulated conditions) and sustained release and hence maintained effect (after 6 hrs, only 91.217% ± 2.523 of gabapentin was released).

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AUTHORS' CONTRIBUTIONS

“All authors read and approved the final manuscript.”

REFERENCES

1. Chitnis, CE, Ohman, DE. Cloning of Pseudomonas aeruginosa algG, which controls alginate structure. J. Bacteriol. 1990;172(6):2894-2900.
2. Aslani, P, Kennedy, RA. Effect of gelation condition and dissolution media on the release of paracetamol from alginate gel beads. J. Microencapsul. 1996;13:601–614.
3. Batyrbekov, EO, Iskakov, RM, Boldyrev, DY, Yu, VK, Dzhumagulova, ZKh, Praliev, KD, Zhubanov, BA, Berlin, KD. Controlled delivery of analgetics from calcium alginate beads. Materials Res. Society Symposium Proceeding, PA, USA. 2004;EXS-1:I4.4.1-I4.4.4.
4. Aggarwal, N, HogenEsch, H, Guo, P, North, A, Suckow, M, Mittal, SK. Biodegradable alginate microspheres as a delivery system for naked DNA. Can. J. Vet. Res. 1999;63:148-152.

5. Barrias, CC, Lamghari, M, Granja, PL, Sá Miranda, MC, Barbosa, MA. Biological evaluation of calcium alginate microspheres as a vehicle for the localized delivery of a therapeutic enzyme. J. Biomed. Material Res. 2005;74A(4):545-552.
6. Zhu, H, Srivastava, R, Brown, JQ, McShane, MJ. Combined physical and chemical immobilization of glucose oxidase in alginate microspheres improves stability of encapsulation and activity. Bioconjugate Chem. 2005;16:1451-1458.
7. Seo, JY, Seung, Y, Ahn, BY, Kwon, IC, Chung, H, Jeong, SY. Cross-protective immunity of mice induced by oral immunization with pneumococcal surface adhesion A encapsulated in microspheres. Infect. Immun. 2002;70(3):1143-1149.
8. Sailaja, G, HogenEsch, H, North, A, Hays, J, Mittal, SK. Encapsulation of recombinant adenovirus into alginate microspheres circumvents vector specific immune response. Gene Ther. 2002;9(24):1722–1729.
9. Chickering, DE, Jacob, JS, Desai, TA, Harrison, M, Harris, WP, Morrell, CN, Chaturvedi, P, Mathiowitz, E. Bioadhesive microspheres: III. An in vivo transit and bioavailability study of drug-loaded alginate and poly(fumaric-co-sebacic anhydride) microspheres. J. Control. Release. 1997;48:35-46.
10. Takka, S, Ocak, ÖH, AcartÜrk, F. Formulation and investigation of nicardipine HCl–alginate gel beads with factorial design-based studies. Eur. J. Pharm. Sci. 1998;6:241–246.
11. Al-Kassas, RS, Al-Gohary, OMN, Al-Faadhel, MM. Controlling of systemic absorption of gliclazide through incorporation into alginate beads. Int. J. Pharm. 2007;341:230-237.
12. Prajapati, SK, Tripathi, P, Ubaidulla, U, Anand, V. Design and development of gliclazide mucoadhesive microcapsules: in vitro and in vivo evaluation. AAPS PharmSciTech 2008;9(1):224-230.
13. Das, MK, Maurya, DP. Evaluation of diltiazem hydrochloride-loaded mucoadhesive microspheres prepared by emulsification and internal gelation teschnique. Acta Poloniae Pharmaceutica - Drug Res. 2008;65(2):249-259.
14. Michael, J, McLean, MD, Gidal, BE. Gabapentin dosing in the treatment of epilepsy. Clinical Therapeutics 2003;25(5):1382-1406.
15. Stewart, BH, Kugler, AR, Thompson, PR, Bokbrader, HN. A Saturable transport mechanism in the intestinal absorption of gabapentin is the underlying cause of the lack of proportionality between increasing dose and drug levels in plasma. Pharm. Res. 1993;10(2):276-281.
16. Reis, CP, Ribeiro, AJ, Neufeld, RJ, Veiga, F. Alginate microparticles as novel carrier for oral insulin delivery. Biotech. Bioeng. 2007;96(5):977-989.
17. Zour, E, Lodfi, SA, Nusbett, RU, Silbering, SB, Shaturvedi, PR. Stability studies of gabapentin in aqueous solutions. Pharm. Res. 1992;9(5):595-600.
18. Pongjanyakul, T, Puttipipatkhachorn, S. Xanthan-alginate composite gel beads: Molecular interactions and in vitro characterization. Int. J. Pharm. 2007;331:61-71.
19. Dorożyński, P, Kulinowski, P, Jachowicz, R, Jasiński, A. Development of a system for simultaneous dissolution studies and magnetic resonance imaging of water transport in hydrodynamically balanced systems: A technical Note. AAPS PharmSciTech 2006;8(1), Article 15, E1-E4.
20. Lehr, CM, Bouwstra, JA, Tukker, JJ, Junginger, HE. Intestinal transit of bioadhesive microspheres in an in situ loop in the rat. A comparative study with copolymers and blends based on poly(acrylic acid). J. Control. Release 1990;13(1):51-62.
21. Pasparakis, G, Bouropoulos, N. Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate–chitosan beads. Int. J. Pharm. 2006;323:34–42.
22. Silva, CM, Ribeiro, AJ, Figueiredo, IV, Gonçalves, AR, Veiga, F. Alginate microspheres prepared by internal gelation: Development and effect on insulin stability. Int. J. Pharm. 2006;311:1-10.

23. Lee, BJ, Min, GH. Oral controlled release of melatonine using polymer-reinforced and coated alginate beads. Int. J. Pharm. 1996;144:37-46.
24. Singhal, P, Kumar, K, Pandey, M, Saraf, SA. Evaluation of acyclovir loaded oil entrapped calcium alginate beads prepared by ionotropic gelation method. Int. J. Chem. Tech. Res. 2010;2(4):2076-2085.
25. Belgamwar, V, Shah, V, Saurana, SJ. Formulation and evaluation of oral mucoadhesive multiparticulate system containing metoprolol tartarate: An in vitro – ex vivo characterization. Curr. Drug Deliv. 2009;6:113-121
26. Roy, A, Bajpai, J, Bajpai, AK. Development of calcium alginate-gelatin based microspheres for controlled release of endosulfan as a model pesticide. Indian J. Chem. Tech. 2009;17:388-395.
27. Babu, VR, Sairam, M, Hosamani, KM, Aminabhavi, TM. Preparation of sodium alginate-methylcellulose blend microspheres for controlled release of nifedipine. Carb. Pol. 2007;69:241–250.
28. Flory, PJ, Rehner Jr., J. Statistical mechanics of cross-linked polymer networks. II. Swelling. J. Chem. Phys. 1943;11:521-526.
29. Segi, N, Yotsuyanagi, T, Ikeda, K. Interaction of calcium-induced alginate gel beads with propranolol. Chem. Pharm. Bull. 1989; 37(11):3092–3095. Cited after: Takka, S, Ocak, ÖH, Acartürk, F. Formulation and investigation of nicardipine HCl–alginate gel beads with factorial design-based studies. Eur. J. Pharm. Sci. 1998;6:241-246.
30. Chickering, DE, Mathiowitz, E. Bioadhesive microspheres: I. A novel electrobalance-based method to study adhesive interactions between individual microspheres and intestinal mucosa J. Control. Release 1995;34:251–261.
31. Tonnesen, HH, and Karlsen, J. Alginate in Drug Delivery Systems. Drug Dev. Ind. Pharm. 2002;28:621–630.
32. Rokhade, AP, Agnihotri, SA, Patil, SA, Mallikarjuna, NN, Kulkarni, PV, Aminabhavi, TM. Semi-interpenetrating polymer network microspheres of gelatin and sodium carboxymethyl cellulose for controlled release of ketorolac tromethamine. Carb. Pol. 2006;65:243–252.