Optimizing Drug Targeting of Alginate Beads Using Gabapentin as a Hydrophilic Model Drug

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ABSTRACT

Recently, alginates have shown great importance as an efficient tool for targeting drug via oral route at specific site of absorption. Calcium-cross linked alginate beads were prepared in this study by dripping the sodium alginate gel droplets into a cross linker solution of calcium chloride and then dried overnight at ambient temperature. The effect 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 was investigated. Alginate beads were modified by incorporating sodium lauryl sulfate (SLS) or ethylcellulose solid dispersion. Beads containing mixtures of drug and ethylcellulose-drug solid dispersion were shown to be the optimum considering mucoadhesion properties and sustaining gabapentin release. Mucoadhesion capabilities of alginate beads were shown to be decreasing upon adding SLS (30% and 23.33% in fasting- and fed-simulated conditions, respectively). Drug release was so fast (92.46% and 99.64% of drug was released in

fasting- and fed-simulated conditions). The incorporation of solid dispersion has led to well accepted mucoadhesion (74.44% after 8 hrs) as well as release properties (93.35% of drug released after 10 hours).

Key Words: Alginate; Targeting; Gabapentin; Sodium lauryl sulfate; Ethylcellulose; Solid dispersion.

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 (Chitnis and Ohman, 1990). 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(Aslani and Kennedy, 1996). The ratio of G to M residues was found to affect the release of drugs from calcium-cross-linked alginate systems (Batyrbekov et al., 2004).

Alginate systems were found to have a number of properties that are used to deliver DNA (Aggarwal et al., 1999), locally deliver enzymes (Barrias et al., 2005), immobilize enzymes (Zhu et al., 2005), oral immunization (Seo et al., 2002), and to act as adenovirus vector (Sailaja et al., 2002).

The mucoadhesive properties of alginate emphasized its use as an efficient tool to improve oral mucoadhesion for increasing bioavailability of drugs (Chickering et al., 1997) such as nicardepine HCl (Takka et al., 1998), gliclazide (Al-Kassas et al., 2007; and Prajapati et al., 2008), and diltiazem HCl (Das and Maurya, 2008) and to control systemic absorption of some narrow absorption window (NAW) drugs. Alginate was used to control and sustain the release of Gabapentinis an orally available γ-aminobutyric acid analog which is used to control partial seizures in combination with other antiseizure drugs (Michael et al., 2003). It is one of the NAW drugs that is actively absorbed from upper duodenal region via L-amino acid transporters (Stewart et al., 1993).

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

MATERIALS AND METHODS

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.

METHODS

Preparation of calcium alginate mucoadhesive beads

Different batches of calcium alginate mucoadhesive beads were prepared by ionotropic gelation. The amounts of sodium alginate, concentration of calcium chloride solution and quantity of

gabapentin used and the formulation parameters of the beads are listed in table 1. The gel solution of sodium alginate was made by dissolving the proper amount of sodium alginate in deionized water and stirring till a clear gel solution is formed. Gabapentin was dispersed evenly in deionized water and then added to the gel. After adding drug suspension to the gel, with gentle and consistent mixing for about 5 minutes takes place. The gel is then introduced into calcium chloride solution by dropping from the pump (model M362, Sage Instruments, Orion Research Inc., Massachusetts, USA). Formed beads are then strained, washed twice by deionized water and then left for drying in room temperature over night.

Determination of percent gabapentin load

Specific weight of beads is taken and crushed. The crushed beads are then placed in a vial and a proper amount of deionized water is added to it. The aliquot containing the drug was then analyzed for gabapentin content using the method published by Zour et al., (Zour et al., 1993). 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 percent drug load is given by the formula:

Drug load percent = (Wt_{Dg} / Wt_{Bd}) x100

where, Wt_{Dg} is the amount of drug loaded in beads, detected after extraction and Wt_{Bd} is the weight of beads

Determination of encapsulation efficiency

The process of determining encapsulation efficiency was done utilizing extraction of the drug from beads as previously mentioned (Reis et al., 2007). Then the drug content was determined and the encapsulation efficiency of the drug was given by the formula:

Percent encapsulation efficiency (EE) = (Wt_{De} / Wt_{Th}) x 100

where, Wt_{De} is the amount of drug loaded in beads, detected after extraction and Wt_{Th} is the amount of the drug assumed to be present theoretically in the weight of beads used.

Determination of swelling index

Swelling index of beads was determined according to previous study (Pongjanyakul, and Puttipipatkhachorn, 2007). A weight 100 m of beads was taken and placed in a vessel. 14 mL of deionized water were added to them. After predetermined time intervals, all beads are withdrawn from the vessel, carefully and quickly dried and then weighed. The swelling index is 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 water.

The test was performed using the same procedure mentioned above but with replacing deionized water with 0.1 N HCl solution and 0.01 N HCl solution containing 0.2% of NaCl and 0.25% SLS to detect the drug in the release media resembling gastric fluid without enzymes in fasting state and in fed state, respectively (Dorożyński et al., 2006).

Determination of mucoadhesive properties

The mucoadhesive property of the beads was evaluated employing the method described by Lehr et al. (Lehr et al., 1990) with modification. As shown in figure 1, a piece of the pig's intestine is trimmed and fixed tightly to the slide. A weight of 50 g is put on it for 30 seconds, then the load is removed and the slide containing the intestinal piece loaded with the beads is hanged on the disintegration apparatus. The microscopic slide is then hanged in disintegration testing apparatus. The media resembling gastric fluid without enzymes in fasting- and fed-simulated state at 37 ± 0.5 °C were used. Thirty beads are put randomly on the mucosal surface of the pig's intestine piece. At each time point, the number of beads remaining adhering to the mucosal surface of the hanged piece of pig's intestine is counted and the number is expressed as percentage of the total number of the beads loaded on the intestinal piece.

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 (Dorożyński et al., 2006).

The release of gabapentin from alginate beads was done using the procedure published in previous study (Pasparakis and Bouropoulos, 2006). An accurately weighed amount of the beads was placed in the vials each containing 15 mL of the dissolution media and warmed up in a shaking water bath at 37 ± 0.5°C. The speed of shaking was adjusted to be about 50 rpm. Samples of the dissolution media were withdrawn from each vial and replaced by equivalent amount of fresh dissolution media pre-heated to 37 ± 0.5°C. Samples withdrawn are analyzed using HPLC method previously mentioned above.

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 less than 0.05 was considered statistically significant.

Results and discussion

Drug load and encapsulation efficiency (EE)

Figures 2 and 3 show the percent drug load and encapsulation efficiency (EE) of the prepared alginate formulae. It was shown that, regarding percent drug load, 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. It was mentioned by Silva et al., 2006 that increasing alginate concentration has lead to a consequent increase in EE (Silva et al., 2006). Das and Maurya mentioned the same results in previous study (Das et al., 2008). This is suggested to be a result of reduced amount of drug that is lost from beads during cross linking (Lee and Min, 1996; and Singhal et al., 2010). Encapsulation efficiency also depends 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. Comparing formulae F13, F5, F14 revealed that increasing drug/polymer ratio resulted in increasing percent drug load and decreasing EE. These results agreed with previous study (Belgamwar et al., 2009). This is 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).

Swelling index

Figures 4 and 5 show swelling ratio of the prepared alginate formulae after 30 min. and 120 minutes in fasting- and fed-simulated conditions. It was shown that swelling ratio of beads increases as alginate gel decreases, drug/polymer ratio increases, cross linker concentration decreases and/or time of cross linking decreases. These results agreed to previous study (Roy et al., 2009). 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 (Aslani and Kennedy, 1996) with smaller cavities which accommodate lesser 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 (Flory and Rehner, 1943). According to this theory, the swelling ratio of a network (Q) can be described by the following equation:

$$Q^{5/3} = \{ [(i/2V_N.S^{3/2}) + (1/2 - X_i)/V_i] / V_e/V_o \}$$

where i/V_N is the concentration of the fixed charges referred to unswollen network, S is the ionic concentration in the external solution, $(1/2 - X_i)/V_i$ is the affinity of matrix for water, and V_e/V_o 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 (Takka et al., 1998; and Segi et al., 1989).

Mucoadhesion properties

Figures 6 and 7 show mucoadhesion of the prepared alginate formulae after 1 and 8 hours in fasting and fed simulated conditions, respectively. It was shown that mucoadhesion of beads decreases as alginate gel 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 (Chickering et al., 1995). Increasing degree of cross linking results 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 hours 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 (figures 4 and 5).

Drug release profile

Table 2 shows the time at which prepared 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 attributed to the increased viscosity of alginate (Tonnesen and Karlsen, 2002) and/or increased degree of cross

linking (Rastogi et al., 2007). 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, the more the beads swell.

Seeking 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, 9 and 10 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 figure 11. Controlling drug release form alginate beads was attempted using SLS (Taha et al., 2008) and solid dispersion (Bajpai and Sharma, 2004). SLSF formula showed inferior properties as compared to all other formulae. It was shown that incorporating SLS 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 hours. The compositions of OF, SLSF, SDF and FSF formulae are shown by table 4.

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 (Khan and Rhodes, 1972). It was calculated according to the equation mentioned by Khan and Rhodes (Khan and Rhodes, 1972) 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 $DE_{0\text{-}60min}$ 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, SLSF, SDF and FSF formulae that incorporating SLS 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.

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 SLS 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 hours, only 91.217% ± 2.523 of gabapentin was released).

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Table 1. Compositions and variables of formulation of different formulas.

Formula code	Sodium alginate conc. (% W/V)	Cross-linker conc. (% W/V)	Cross-linking time (min)	Cross-linker solution volume : gel volume (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

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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 ± 7.56	89.38 ± 2.38	198.33 ± 12.76
F2	81.73 ± 2.08	180.2 ± 14.57	76.75 ± 2.3	162.9 ± 14.2
F3	63.67 ± 2.71	129.5 ± 3.35	50 ± 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	121.3 ± 3.77	49.3 ± 1.7	118.65 ± 6.54
F7	20.32 ± 0.52	49.38 ± 3.8	20.5 ± 1.2	44.88 ± 2.07
F8	33.82 ± 1.86	78.7 ± 3.66	30.6 ± 1.51	71.87 ± 3.43
F9	61.74 ± 2.38	121.35 ± 3.99	49.28 ± 2.32	98.58 ± 5.9
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	40.2 ± 1.62	122.09 ± 1.7	39.94 ± 1.82	103.5 ± 1.49
F14	27.13 ± 2.42	73.9 ± 2.21	51.67 ± 15.37	66.63 ± 3.2

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.

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

	Yield	Hardness	Particle Size	Drug Load	Encapsulation Efficiency	Swelling Ratios	Mucoadhesion 1st	Mucoadhesion 2nd	Release Rate
Conc. Of Alginate	-	+	+	+	+	-	-	+	-
Conc. Of $CaCl_2$	N	+	-	-	-	-	-	+	-
Time of Cross Linking	-	+	-	-	-	-	-	+	-
$V_{Dps} : V_{CLS}$	+	N	N	-	-	N	N	N	N
Drug:Polymer Ratio	-	N		+	-	±	±	±	+

- \- Inversely Related
- \+ Directly Related
- N 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 (mL)	Drug : polymer ratio	SLS (g)	Free drug (% of total drug amount)	Drug-EC Solid Dispersion (% of the total drug amount)
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

Table 5 Dissolution efficiency of optimized formulae.

	Fasting-Simulated Conditions				
	0.5 (hr)	**1 (hr)**	**2 (hrs)**	**3 (hrs)**	**4 (hrs)**
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 (hr)	**1 (hr)**	**2 (hrs)**	**3 (hrs)**	**4 (hrs)**
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

Figures

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

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

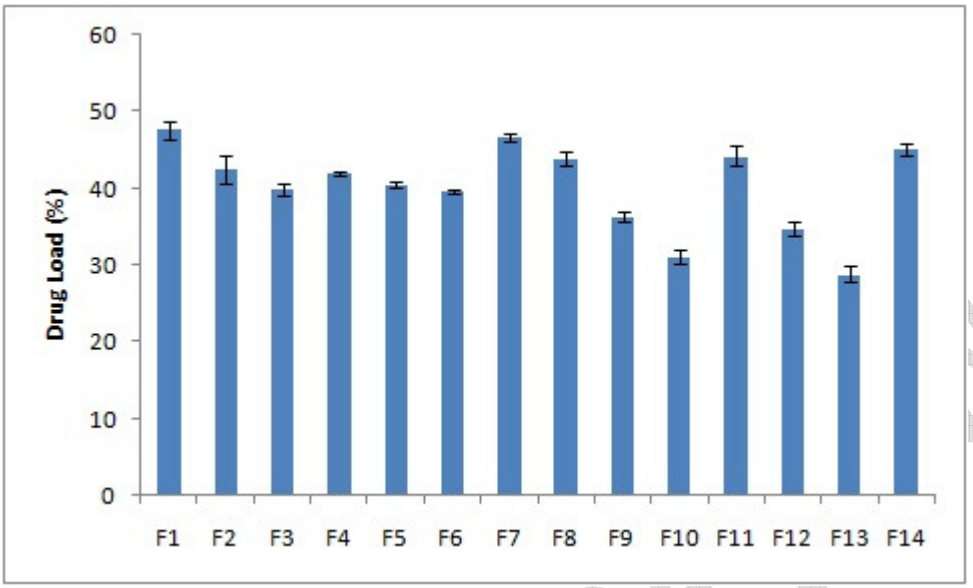

Fig. 3. Encapsulation efficiency of formulae F1 – F14. Each data point represents mean ± S.E. (n=3).

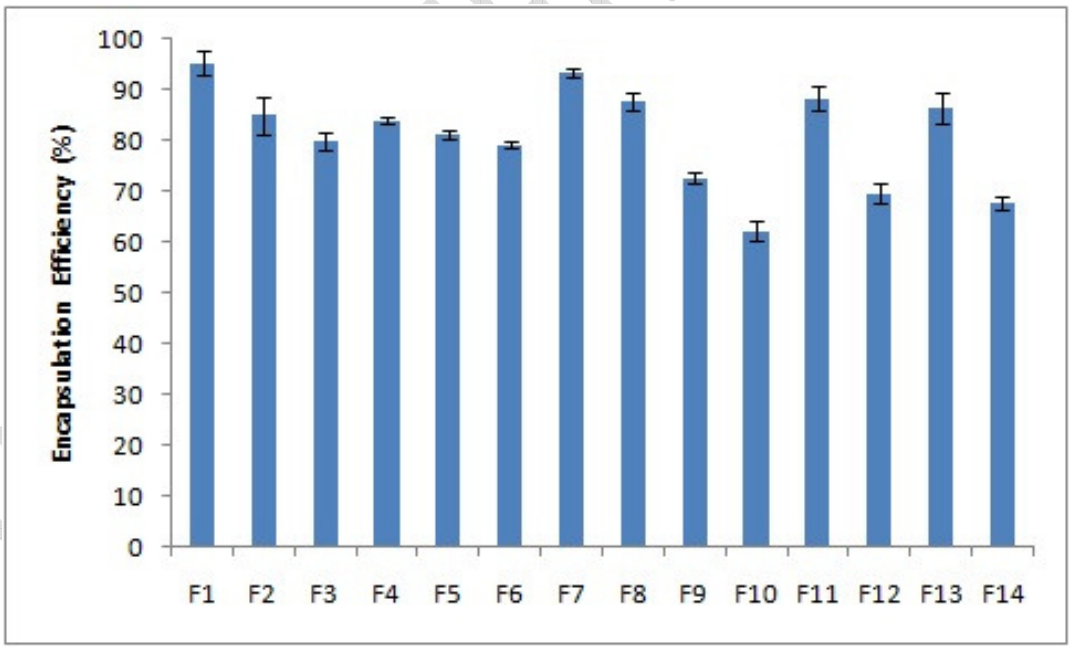

Fig. 4. Swelling ratio of formulae F1 – F14 after 30 and 120 minutes in fasting-simulated conditions. Each data represent mean ± S.E. (n=3).

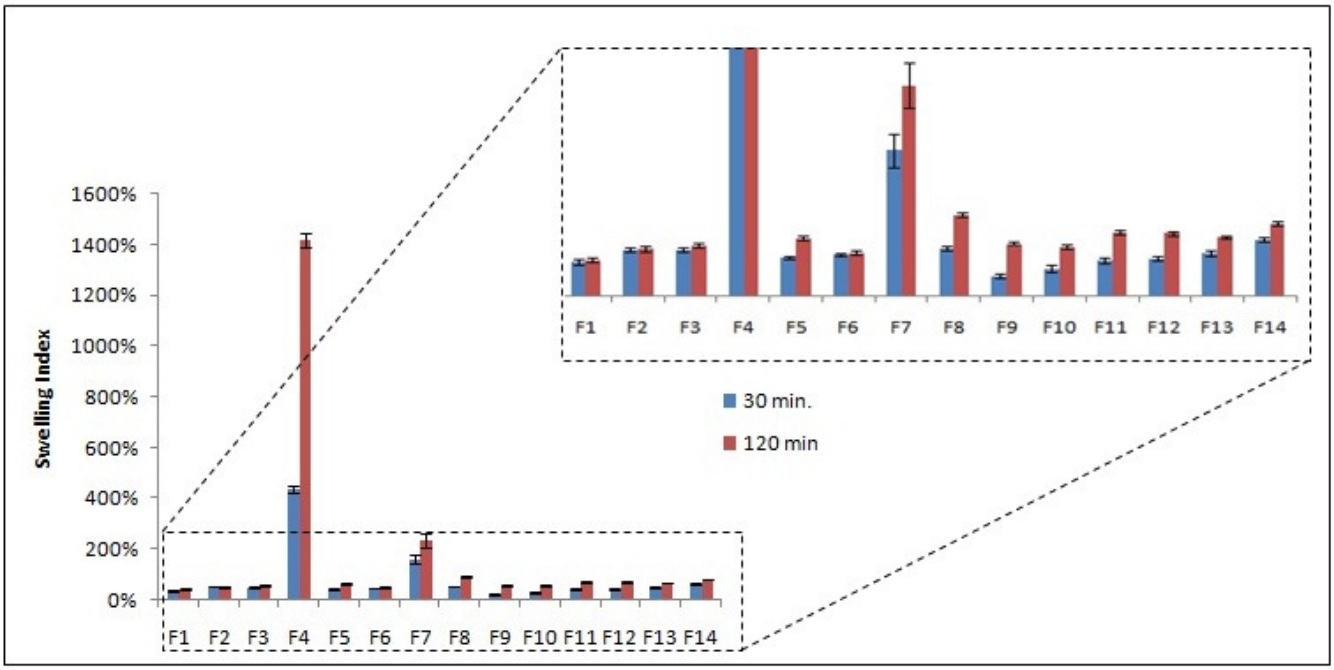

Fig. 5. Swelling ratio of formulae F1 – F14 after 30 and 120 minutes in fed- simulated conditions. Each data represent mean ± S.E. (n=3).

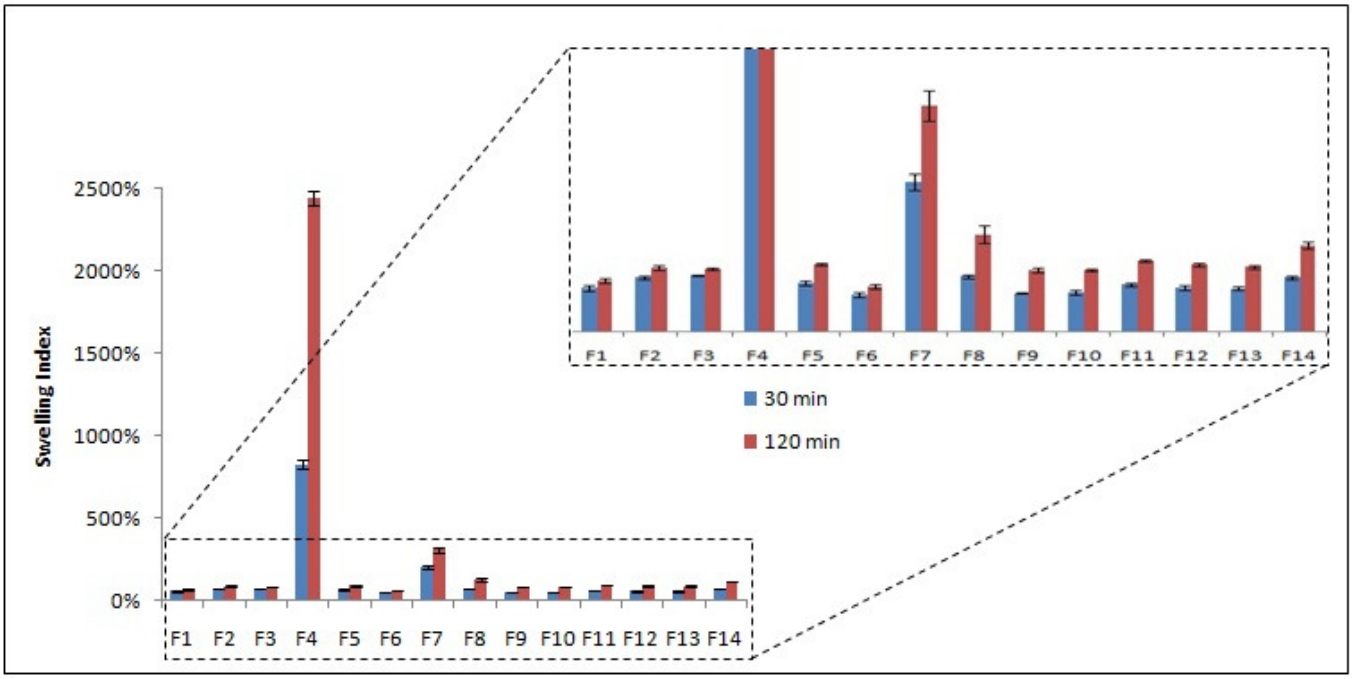

Fig. 6. Mucoadhesion of formulae F1 – F14 after 1 and 8 hours in fasting-simulated conditions. Each data represents mean ± S.E. (n=3).

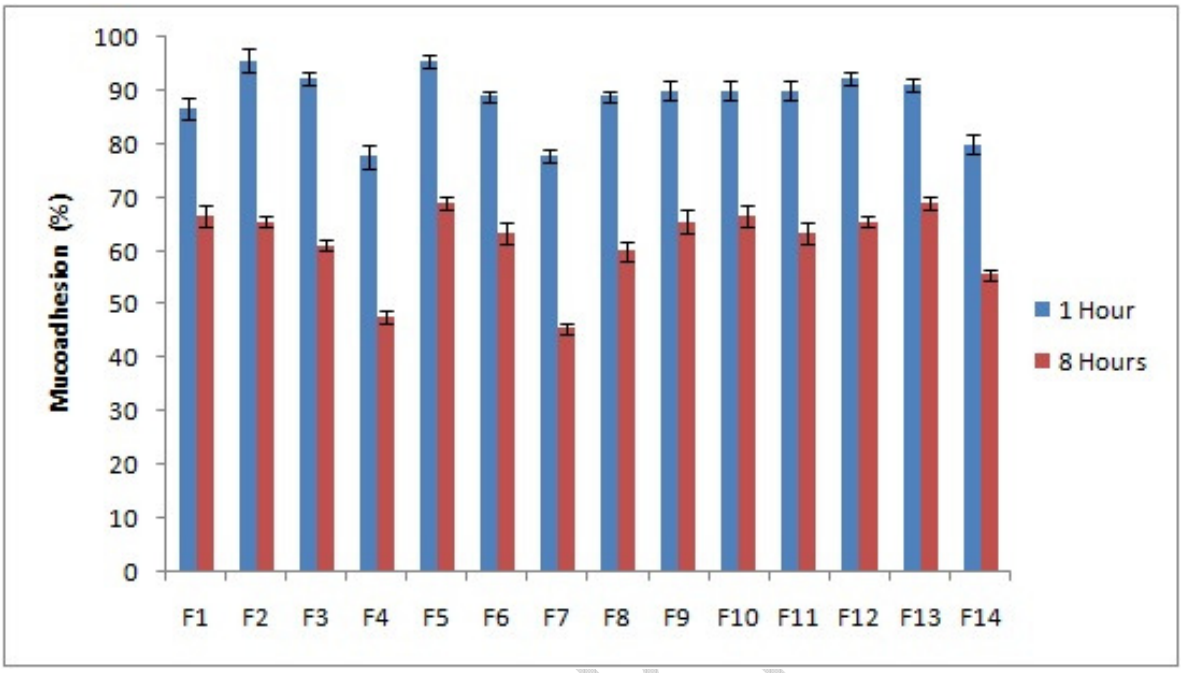

Fig. 7. Mucoadhesion of formulae F1 – F14 after 1 and 8 hours in fed-simulated conditions. Each data represents mean ± S.E. (n=3).

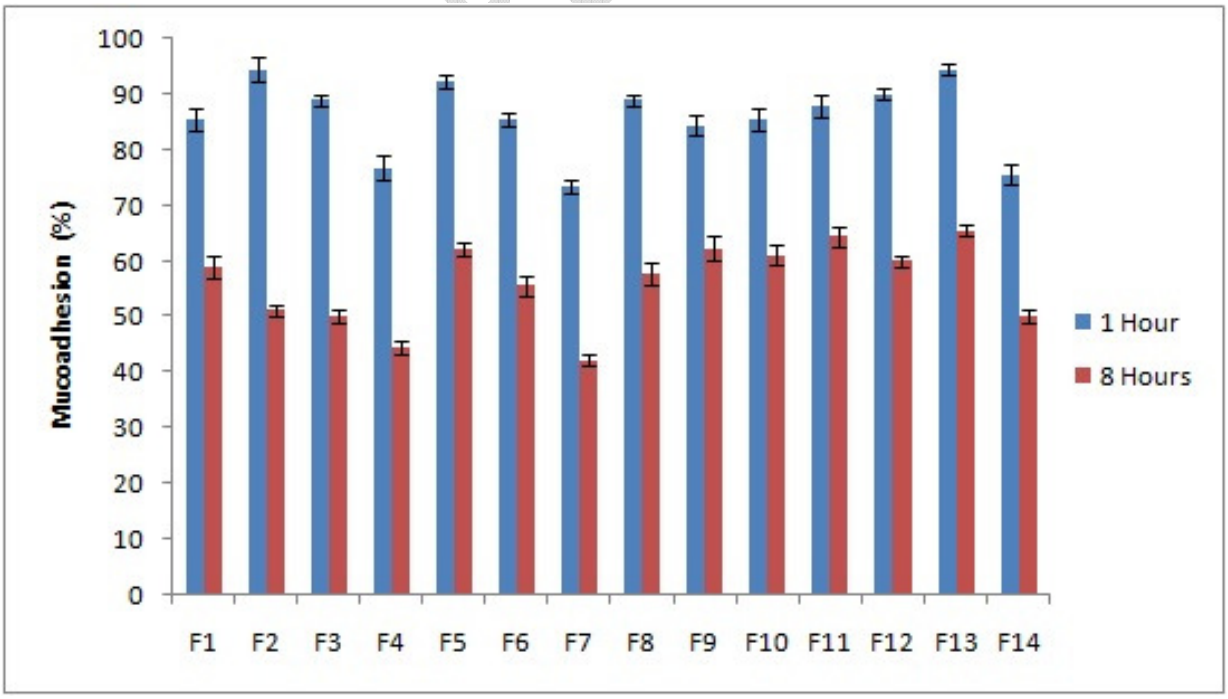

Fig. 8. Drug load and encapsulation efficiency of formulae OF, SDSF, SDF and FSF. Each data represents mean ± S.E. (n=3).

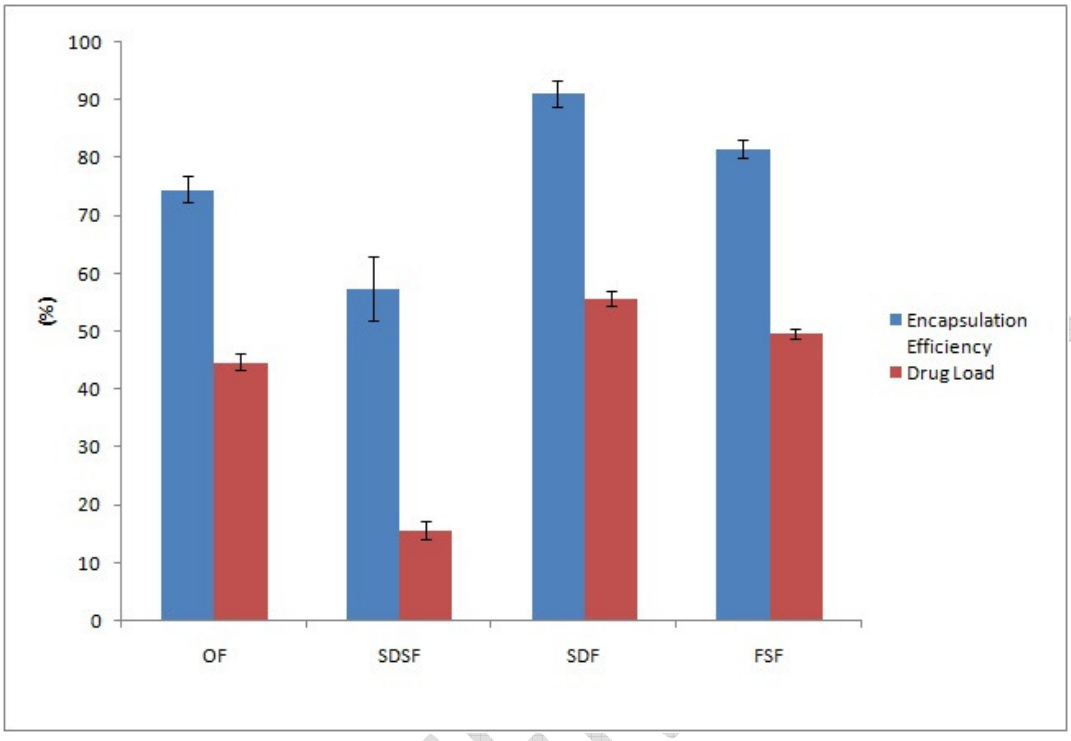

Fig. 9. Swelling ratio of formulae OF, SDSF, SDF and FSF after 30 and 120 minutes in fasting-simulated conditions. Each data represents mean ± S.E. (n=3).

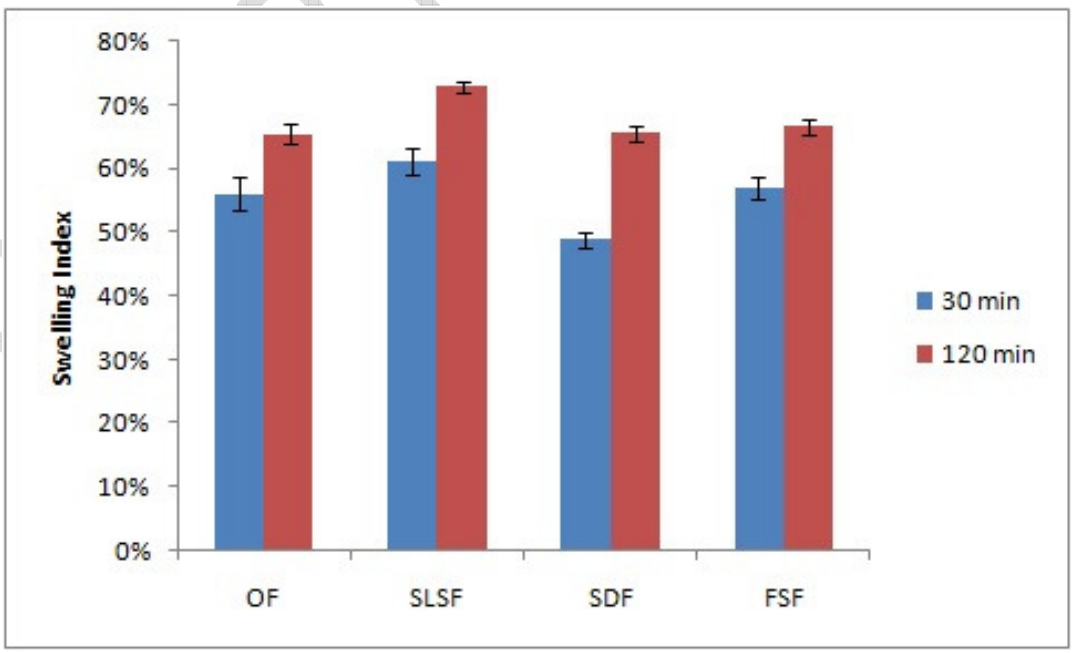

Fig. 10. Swelling ratio of formulae OF, SDSF, SDF and FSF after 30 and 120 minutes in fed-simulated conditions. Each data represents mean ± S.E. (n=3).

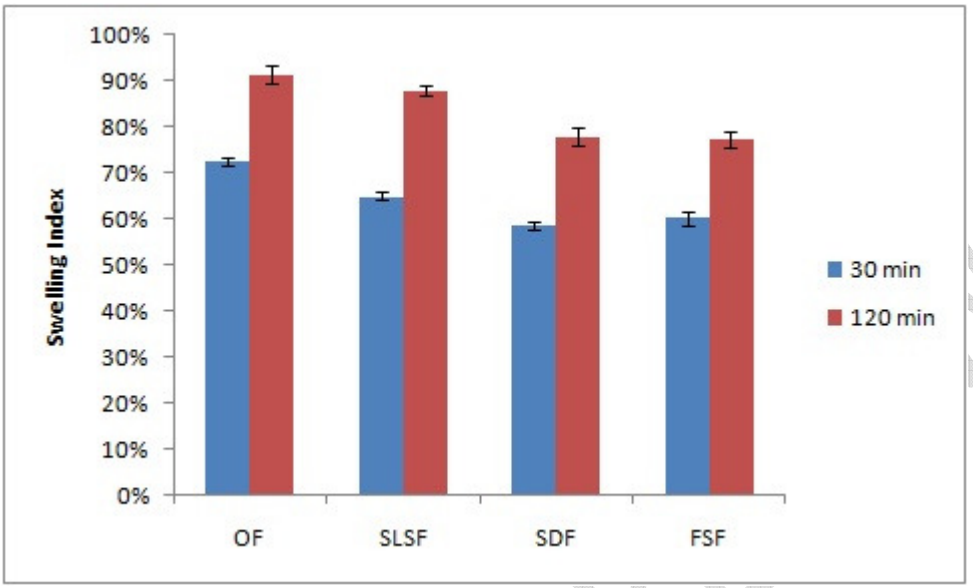

Fig. 11. Mucoadhesion of formulae OF, SDSF, SDF and FSF after 1 and 8 hours in fasting-simulated conditions. Each data represents mean ± S.E. (n=3).

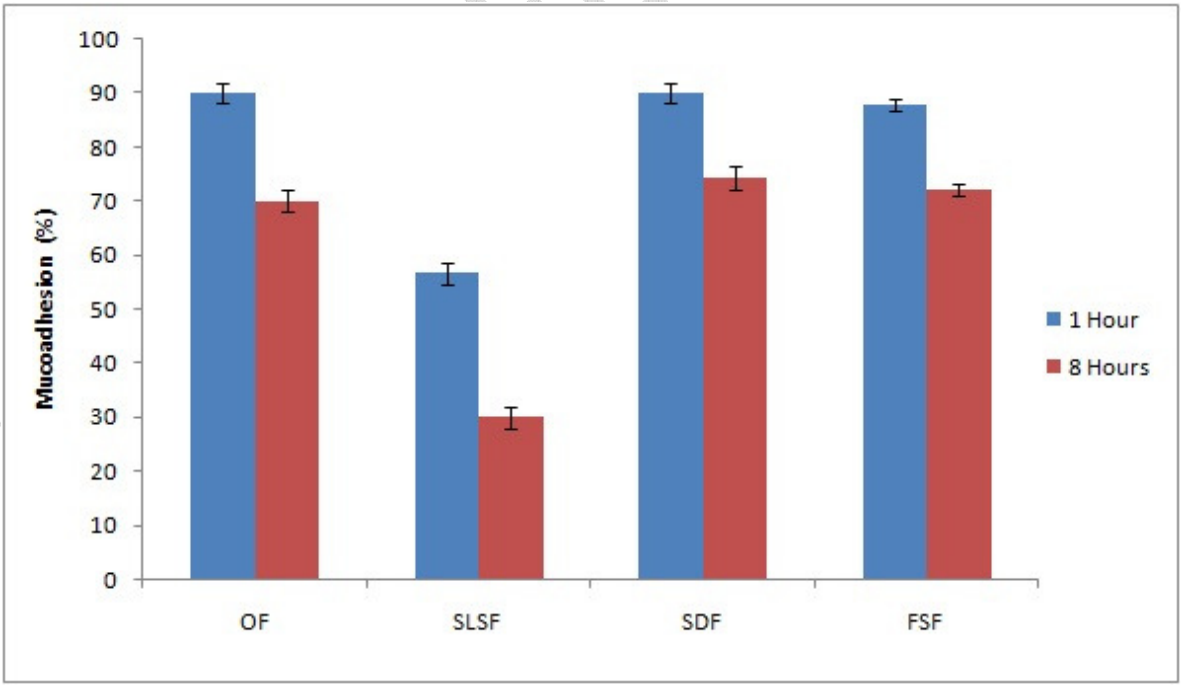

Fig. 12. Mucoadhesion of formulae OF, SDSF, SDF and FSF after 1 and 8 hours in fed-simulated conditions. Each data represents mean ± S.E. (n=3).

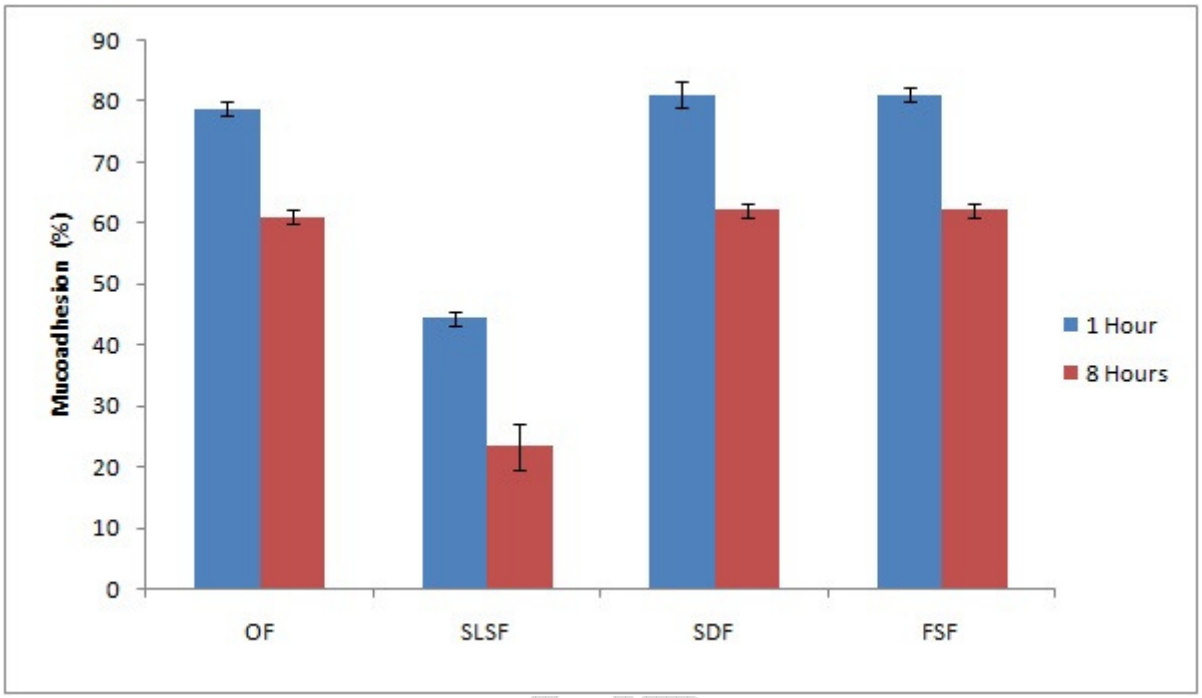

Fig. 13. Drug release profiles of formulae OF, SDSF, SDF and FSF in fasting-simulated conditions. Each data represents mean ± S.E. (n=3).

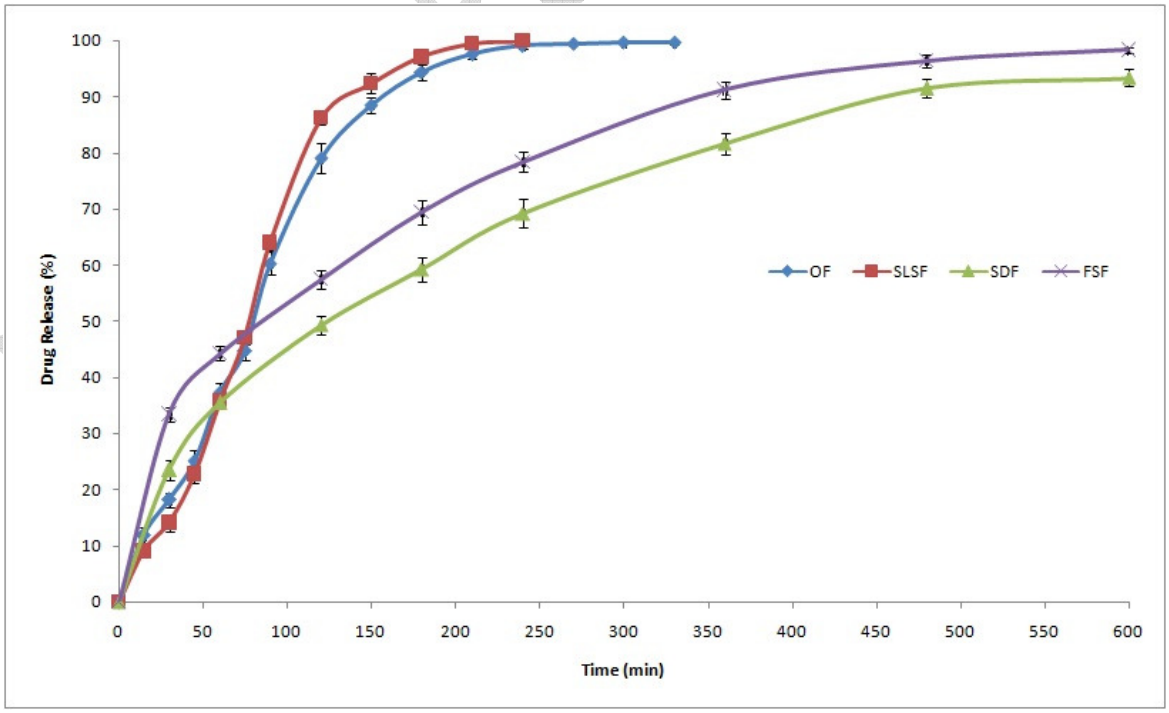

Fig. 14. Drug release profiles of formulae OF, SDSF, SDF and FSF in fed-simulated conditions. Each data represents mean ± S.E. (n=3).