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RESEARCH PAPER

EFFECTIVE MANAGEMENT OF H. PYLORI INFECTION VIA MULTIDRUG LOADED GASTRORETENTIVE FILMS

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Abstract

The objective of the present work was to develop innovative gastro retentive formulation based on mucoadhesive patch systems using the solvent casting technique, allowing a final structure with improved cohesion by mucoadhesive swelling and which releases drugs in the stomach.(6). Chitosan used as a natural polymer, and PAA is a mucoadhesive polymer. The mucoadhesive gastro retentive films bearing amoxicillin trihydrate, metronidazole and famotidine were prepared using solvent casting method (1). This method provides good drug content uniformity. Various formulation variables i.e. percentage of glycerol plasticizer, concentration of chitosan and chitosan : PAA ratio, which affects the GR films preparation and its characterization were optimized on the basis of their effect on film weight, thickness, folding endurance and tensile strength. The various variables were optimized by varying one variable and keeping other variable constant(1).

Keywords

Gastroretention, Mucoadhesion, Diffusion, Drug release

INTRODUCTION

Oral delivery of drugs is the most preferred administration route due to ease of administration. Drug bioavailability of pharmaceutical oral dosage forms is influenced by various factors. One important factor is the gastric residence time (GRT) of these dosage forms. Indeed, gastric retention has received significant interest in the past few decades as most of the conventional oral delivery systems have shown some limitations related to fast gastric emptying time(4). A gastro retentive dosage form (GRDF) can overcome this problem and is particularly useful for drugs that are primarily absorbed in the duodenum and upper jejunum segments(2).
Food effects and the complex motility of the stomach play a major role in gastric retention behavior. Several approaches of non-effervescent and effervescent formulation technologies have been used and patented in order to increase gastric residence time of the GRDF(3). The objective of the present work was to develop innovative gastro retentive formulation based on mucoadhesive patch systems using a standard manufacturing process (the solvent casting technique have been chosen), allowing a final structure with improved cohesion by mucoadhesive swelling and which releases drugs in the stomach. In present work mucoadhesive film of amoxycillinetrihydrate, metronidazole and femotidine was prepared to prolong the residence time at the site of application (or) absorption and to facilitate the intimate contact with the underlying absorption surface to improve and enhance the bioavailability. The mucoadhesive films of amoxycillinetrihydrate,metronidazole and femotidine were prepared by using chitosan and polyacrylic polymer(5)

MATERIAL AND METHOD

Method

Chitosan solution was prepared by dispersing chitosan in 2% (v/v) aqueous acetic acid solution and stirring overnight. Specified amount of amoxicillin trihydrate (10mg), metronidazole (5mg) and famotidine (0.15mg) were added to chitosan solution and stirred for 15 min with a magnetic stirrer. Then, glycerol was added to it.
Film-forming solutions were magnetically stirred for 3 h. Cast onto a clean petri-dishes and dried at 35°C for about 48 h. dried films were conditioned in a desiccator containing a saturated solution of sodium bromide (58% RH) at 25°C.

Characterization of Mucoadhesive Gastro Retentive Films:

- **Film Weight:**

For evaluation of film weight three films of each formulation were taken and weighed individually. The average weight were calculated and reported.

- **Thickness:**

For evaluation of film thickness three films of each formulation were taken and the film thickness was measured using micrometer screw gauge at three different places and the mean thickness of films were calculated and reported.

- **Folding Endurance:**

Folding Endurance of the films was determined by repeatedly folding one film at the same place till it broke or folding up to 300 times manually which was considered satisfactory to reveal good film property. The no. of times, the film could be folded at the same place without breaking gave the value of folding endurance. The mean value of three films was calculated.

Table 1: Physical Evaluation of the Different GR Film Formulations

Chitosan (% W/V)	PAA (% W/V)	Glycerol (%)	Film weight (mg)	Thickness (mm)	Tensile strength (Kg mm^{-2})
C1=2.0	P1=2.0	G1=20	185±1.02	0.73± 0.05	2.16 ±0.48
		G2=30	**187 ±0.41**	**0.74 ±0.07**	**8.11 ±0.93**
		G3=40	194 ±1.64	0.79 ±0.02	3.67±0.13
	P2=1.0	G1=20	144 ±0.74	0.56 ±0.02	2.03±0.17
		G2=30	**148±0.12**	**0.58±0.08**	**14.83±0.34**
		G3=40	147±0.56	0.57±0.01	9.38±0.21
	P3=0.5	G1=20	113±1.53	0.42±0.06	3.68±0.20
		G2=30	**117±0.39**	**0.44±0.03**	**18.07±0.55**
		G3=40	120±0.30	0.45±0.08	14.11±0.71
C2=1.0	P1=2.0	G1=20	134±1.06	0.51±0.04	0.78±0.39
		G2=30	**140±0.43**	**0.55±0.07**	**4.11±0.82**
		G3=40	142±0.18	0.56±0.01	1.93 ±0.05
	P2=1.0	G1=20	92±0.39	0.35 ± 0.03	0.83± 0.71
		G2=30	**93±0.81**	**0.36± 0.09**	**4.79±0.43**
		G3=40	95±0.69	0.38 ±0.05	2.04± 0.50
	P3=0.5	G1=20	73±0.50	0.25±0.07	1.09±0.83
		G2=30	**77±1.32**	**0.28±0.10**	**5.53±0.66**
		G3=40	79±0.91	0.29±0.04	2.87±0.75
C3=0.5	P1=2.0	G1=20	119±0.82	0.45± 0.09	0.39 ± 0.04
		G2=30	**121±0.13**	**0.46±0.02**	**1.83±0.18**
		G3=40	124±1.67	0.51±0.10	0.64±0.37
	P2=1.0	G1=20	72±0.42	0.24±0.07	0.56±0.21
		G2=30	**76 ±1.16**	**0.27 ±0.01**	**2.71±0.31**
		G3=40	79±0.63	0.29±0.06	0.98±0.78
	P3=0.5	G1=20	41±0.19	0.16±0.07	0.72±0.14
		G2=30	**45±1.25**	**0.19±0.10**	**3.56±0.57**
		G3=40	47±0.94	0.20±0.03	1.81±0.69
C1=2.0		G1=20	88±0.83	0.32±0.08	4.03±0.82
		G2=30	**91±0.19**	**0.34±0.04**	**22.86±0.53**
		G3=40	93±1.56	0.36±0.03	15.42±0.20
C2=1.0		G1=20	39±0.66	0.15±0.01	1.82±0.28
		G2=30	**42±0.30**	**0.17±0.04**	**6.17±0.95**
		G3=40	45±0.57	0.19±0.02	3.43±0.74
C3=0.5		G1=20	18±0.37	0.08±0.03	0.75±0.43
		G2=30	**21±0.95**	**0.09±0.07**	**4.03±0.75**
		G3=40	23 ±1.03	0.10±0.05	1.89± 0.18

The percentage (%) of plasticizer is given in relation to the total dry weight of the polymers. N=3; ±SD

- **Percent Swelling**

After determination of the original film weight and diameter, three films of each formulation were allowed to swell on the surface of agar plate kept in an incubator maintained at 37± 0.2°C. Increase in weight of the film (n=3) was determined at every 1 h interval for 6 h.

The Percent swelling (%S) was calculated using the following equation:

PERCENT SWELLING (% S) = (Xt–Xo/Xo) ×100,

Where,

$X_t$is the weight of the swollen film after time t,

X_0 is the initial film weight at zero time.

The mean value of three reading were calculated and reported

Table 2: Percent Swelling of Different GR Films Formulations

Time (h)	Formulation code											
	C1P1G2	C1P2G2	C1P3G2	C2P1G2	C2P2G2	C2P3G2	C3P1G2	C3P2G2	C3P3G2	C1G2	C2G2	C3G2
1	43.08 3.12	48.36 1.58	41.01 1.82	35.22 2.95	36.13 1.03	40.71 2.63	28.11 2.12	31.49 3.49	33.17 0.28	13.28 0.72	10.97 0.49	8.37 0.29
2	54.23 0.63	55.03 2.57	49.32 1.51	41.63 1.92	43.61 2.29	47.69 3.16	32.85 1.28	35.281.83	38.631.32	17.52 0.60	13.19 0.77	11.73 0.82
3	60.68 0.44	63.52 2.78	58.15 2.69	50.17 2.06	54.33 1.83	56.83 2.94	39.40 2.06	42.39 0.48	47.51 2.59	21.31 0.51	17.34 1.03	14.84 0.16
4	66.43 2.30	68.13 2.29	63.42 0.37	56.34 3.10	59.17 2.35	61.41 3.02	44.08 1.88	49.03 1.71	53.48 1.37	25.81 0.19	20.81 0.41	17.63 0.94

5	72.34 0.66	76.47 1.34	69.23 2.62	62.08 0.81	67.02 0.16	66.56 2.70	51.94 1.02	55.73 2.05	59.32 1.79	28.17 1.21	23.37 0.86	20.16 0.38
6	79.73 1.25	82.21 2.41	77.14 3.02	68.14 3.83	71.49 4.52	74.03 2.07	57.37 1.54	62.14 2.76	65.60 4.19	32.52 0.98	28.92 1.83	24.55 0.76

N=3; ±SD

Percent Swelling of Different GR Films Formulations:

- **Percent moisture sorption**

Three films (2x2 cmsize) of each formulation were dried in the oven at 30± 2°C. After drying, the weight of each film was measured. The films were successively transferred to desiccators over saturated salt solutions of sodium nitrite (75 ±5% RH) at 25°C. After each 1, 3, 5 days, the conditioned films were weighted and noted the weight and placed back to desiccators.

Table 3: Percent Moisture Sorption of Different GR Films Formulations

Time (Day)	Formulation code											
	C1P1 G2	C1P2 G2	C1P3 G2	C2P1 G2	C2P2 G2	C2P3 G2	C3P1 G2	C3P2 G2	C3P3 G2	C1G2	C2G2	C3G2
1	7.03 0.18	7.32 0.92	7.94 0.25	5.82 0.69	6.14 0.50	6.65 0.98	4.83 0.12	5.12 0.35	5.46 0.22	8.69 0.92	6.97 0.81	5.64 0.16
3	9.29 0.72	9.18 0.16	9.63 0.22	7.14 0.38	8.05 0.20	8.38 0.16	6.17 0.46	6.55 0.61	6.85 0.19	10.43 0.10	8.87 0.33	6.99 0.47
5	11.62 0.55	11.90 1.28	12.71 0.87	9.53 0.41	10.18 1.82	10.89 0.49	**7.31** **0.71**	8.03 1.26	8.96 1.01	**13.11** **1.30**	11.14 0.97	9.07 0.83

N=3; ±SD

Drug Content Uniformity

Three films (2x2 cm size) of each formulation were taken in separate 100 mL volumetric flasks; 100 mL of acetate buffer (pH 5.0) was added and continuously stirred for 24 h using magnetic bar and magnetic stirrer. The solutions were filtered and filtrate was diluted and absorbance's were taken at 228 nm, 320 nm and 266 nm using UV Spectrophotometer and determined the drug content. The average of drug contents of three films was taken as final reading and reported.

Table 4: Drugs Content Uniformity of Different GR Films Formulations

S. No.	Formulation code	%Amoxicillin Trihydrate	%Metronidazole	% Famotidine
1	C1P1 G2	84.04 2.67	88.93 3.44	89.51 2.63
2	C1P2 G2	89.72 1.83	91.46 3.81	82.91 1.32
3	C1P3 G2	92.38 3.41	85.33 2.57	86.34 1.85
4	C2P1 G2	86.14 3.73	82.70 3.65	90.89 3.02
5	C2P2 G2	83.55 2.51	89.29 3.97	84.22 2.18
6	C2P3 G2	91.02 3.29	85.56 2.85	85.87 1.43
7	C3P1 G2	82.25 2.83	80.41 1.97	87.20 1.02
8	C3P2 G2	88.61 3.79	83.82 2.06	86.59 1.49
9	C3P3 G2	85.12 3.18	86.47 3.78	89.62 2.57
10	C1G2	90.47 4.21	84.76 2.64	91.08 3.74
11	C2G2	87.18 3.93	87.10 3.37	83.73 2.79
12	C3G2	85.51 3.52	82.37 2.6	88.16 2.37

N=3; SD

- **Surface pH**

The method was used to determine the surface pH of the films. A combined glass electrode was used for this purpose. The films were allowed to swell by keeping them in contact with 1 mL 0.1 M HCl or enzyme free simulated gastric fluid (pH 1.2 ± 0.1) for 2 h at room temperature and pH was noted down by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1 min. The mean value of three films were calculated[7]

- **In vitro Residence Time**

The In vitro residence time was determined using IP disintegration apparatus. The disintegration vessel of test apparatus was filled with 800 mL 0.1 M HCl or simulated gastric fluid (SGF, pH 1.2) maintained at 37 2°C. The segment of rat stomach mucosa, 3 cm length, was glutted to the surface of a glass slab, vertically attached to the apparatus. Three films of each formulation were hydrated from one surface using 0.1 M HCl or simulated gastric fluid (SGF, pH 1.2) and then the hydrated surface was brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down in such a way that the film was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time required for complete erosion or detachment of the film from the mucosal surface was recorded (n=3).

Table 5: Surface pH and In vitro Residence Time of Different GR Films Formulations

Parameters	Formulation code											
	C1P1 G2	C1P2 G2	C1P3 G2	C2P1 G2	C2P2 G2	C2P3 G2	C3P1 G2	C3P2 G2	C3P3 G2	C1G2	C2G2	C3G2
Surface pH	1.21 0.03	1.19 0.04	1.14 0.08	1.17 0.02	1.39 0.02	1.22 0.01	1.20 0.03	1.29 0.06	1.24 0.05	1.12 0.04	1.34 0.05	1.18 0.01
In vitro Residence Time (h)	8.52 0.36	7.77 0.18	6.93 0.51	6.36 0.83	5.24 0.13	4.69 0.72	4.08 0.25	3.40 0.38	2.94 0.92	6.01 0.20	3.98 0.12	2.54 0.69

N=3; ±SD

RESULTS AND DISCUSSION

In 20 % glycerol,The films weight values range from 18±0.37 to 185±1.02 and films thickness values range from 0.08±0.03 to 0.73±0.05 and are referred to films C3G1 and C1P1G1, respectively.

In 30 % glycerol,The films weight values range from 21±0.95 to 187±0.41 and films thickness values range from 0.09±0.07 to 0.74±0.07 and are referred to films C3G1 and C1P1G1, respectively.

In 40% glycerol,The films weight values range from 23±1.03 to 194±1.64and films thickness values range from 0.10±0.07 to 0.79±0.07 and are referred to films C3G1 and C1P1G1, respectively.

Percent swelling was found to be high for C1P2G2 film (82.21±2.41), due to regular porous nature of the PIC and a stronger electrostatic interaction between both polymeric chains would facilitate a better matrix-solvent interaction, allowing an efficient and rapid swelling and low for C3G2 film (24.55±0.76) due to a greater number of bonds in the network structure that retarded the drugs diffusion.Percent swelling was found to be slightly lower forC1P1G2 film (79.73±1.25) than C1P2G2 film (82.21±2.41), owing to the fact that this film was less porous and the existence of a higher amount of PAA and chitosan chains within the interpolymer complex obstructed the uptake of solvent in the network.

Percent water sorption was found to be high for C1G2 film (13.11±1.39), and low for C3P1G2 film (7.31±0.71). Water absorption capacity of films increased with increased concentration of chitosan and decreased concentration of PAA.

The surface pH of the film was found to be in the range of 1.39±0.02 to 1.12±0.04 for formulation C1P1G2 to C3G2. This film pH is closed to the SGF pH 1.2. Hence, these films may not cause any irritation to the gastric mucosa after its application..

The In vitro residence time of the film C1P1G2 to C3G2 on the mucosal membrane was observed and it was noted that formulation C1P1G2 film remained on the mucosal membrane for more time (8.52±0.36) as compared to other formulations. It could be due to the presence of maximum concentration of chitosan and PAA (2:2).

Drugs content uniformity was found to be in the range of 82.25±2.83 to 92.38±3.41 (amoxicillin trihydrate), 80.41±1.97 to 91.46±3.81 (metronidazole) and 82.91±1.31 to 91.08± 3.74 (famotidine) for formulation C1P1G2 to C3G2.

The greater the PAA content in the IPC, the faster the release rate of drugs were achieved; therefore the C3P1G2 film with the highest chitosan-PAA ratio (0.5:2.0) attained 87.91±1.83 of amoxicillin, 96.09±3.70 of metronidazole and 92.1±3.07 of famotidine release. This may be attributed to the fact that PAA presented a greater amount of free carboxylic pendent groups that would lead to a major expansion of the polymeric chains, increasing the rate of movement of the erosion front.

The rapid swelling and drugs release demonstrated by formulation C1P2G2 might be beneficial for site-specific amoxicillin, metronidazole and famotidine delivery in the stomach, because of the limitations of the gastric emptying time.

Drugs permeation across the stomach mucosal membrane for C1P2G2 and C1G2GR films was assessed and maximum drug permeation was observed with C1P2G2 films as it releases maximum amount of drugs while films C1G2 shown minimum drug permeation as it releases minimum amount of drugs and less bioadhesion, and low swellability due to absence of PAA and IPC.

The mucoadhesive studies indicated that the formulation C1P2G2 films showed good mucoadhesive property, which is desirable for site-specific delivery to stomach. Formulation C1P2G2 containing chitosan and PAA in the ratio of 2:1 showed optimum adhering of film at the end of 8 h compared to C1G2 film formulation.

CONCLUSION

In the light of the above-mentioned considerations, it is clear that the developed mucoadhesive films incorporated with amoxicillin, metronidazole and famotidine in this study were effectively retained in GIT and kept retained drug stable at acidic pH. This indicates the potential use of mucoadhesive films in treating H. pyloriinfections.Percent swelling was found to be high for C1P2G2 film (82.21 ± 2.41), due to regular porous nature of the PIC and a stronger electrostatic interaction between both polymeric chains would facilitate a better matrix-solvent interaction, allowing an efficient and rapid swelling and low for C3G2 film (24.55 ± 0.76) due to a greater number of bonds in the network structure that retarded the drugs diffusion.

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REFERENCES

1. Silva, C. L., Pereira, J. C., Ramalho, A., Pais, A. C. C., Sousa, J. S. "Films based on chitosan polyelectrolyte complexes for skin drug delivery: Development and characterization". J. of membrane science. 2008; 320: 268-279.
2. Arora, S., Ali, J., Ahuja, A., Khar, R. K., and Baboota, S. Floating Drug Delivery Systems: A Review .AAPS Pharm. Sci. Tech. 2005; 6(3): 1-10.
3. M. Praveen Kumar, D. Dachinamoorthi, Devanna, K.B.Chandrasekhar and T.RamanjireddyGastroretentive Delivery OF Mucoadhesive Films Containing Pioglitazone.
4. Kagan, L., Hoffman, A., 2008. Systems for region selective drug delivery in gastrointestinaltract: biopharmaceutical considerations. Expert Opin. Drug Deliv.5, 681–692 .
5. Klausner, A., Eyal, S., Lavy, E., Friedman, M., Hoffman, A., 2003. Novel levodopa gastroretentive dosage form: in-vivo evaluation in dogs. J. Control. Release 88, 117–126.
6. Talwar, N., Sen, H., Staniforth, J., 2000.WO Patent no.WO 0015198.
7. Bottenberg, P., Cleymaet, R., Muynek, C. D., Remon, J. P., Coomans D., Slop, D. "Development and testing of bioadhesive, fluoride containing slow-release tablets for oral use". J. Pharm. Pharmacol.1991;43: 457–464.