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DESIGN AND COMPARISON OF PERIODONTAL STRIPS OF GATIFLOXACIN FOR PERIODONTAL DISEASES

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ABSTRACT

Treatment of periodontitis aims at eradicating or controlling specific pathogens. Antibiotics are prime candidates for patient with recently diagnosed active periodontitis. In antimicrobial susceptibility, profile may cause periodontitis. The purpose of this study was to design different polymeric strips containing same concentration of drug, ability to control and compare drug release characteristics by cross-linking and to study various pharmacokinetic parameters like physicochemical, stability and mass balance studies.

Key wards: Periodontitis, Gatifloxacin, Cross-linking, Evaluation parameters.

INTRODUCTION

Periodontitis is an inflammatory response to the overgrowth of anaerobic organisms such as *spirochetes* and *bacteriods* and in some cases, *micro-aerophilic* organisms in the subgingival plaque. Among the organisms, *actinomycetemcomitans* and *prophyromonas* gingivalis has been considered as an aggressive periodontal pathogen because of its high association with periodontitis. If unchecked, it results in the destruction of the bone and soft tissues supporting the tooth, which causes tooth loss. Clinical signs such as bluish red thickened marginal gingiva, gingival bleeding and localized pain are suggestive of the presence of periodontal pockets[1,2].

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The major approach in the prevention and treatment of periodontitis is the removal of supra and subgingival plaque. This is not always successful in the conventional therapy, root planning, scaling and surgeries. Among the drug delivery system, the local administration of antibiotics into periodontal pockets has received considerable awareness and patient compliance as compared to systemic administration though it has several drawbacks associated with their use. Antimicrobial agents[3] are widely used incase of moderate to severe periodontal diseases to aid in eradication or suppression of plaque bacteria to an acceptable level. Chitosan, which is a hydrophilic biopolymer obtained by alkaline deacetylation of chitin, a major component of arthropod shells, is having favorable properties such as no toxicity, biocompatability and biodegradability. Moreover, chitosan itself possesses antimicrobial activity[4].

The objective of this study was to gain information about different polymers with same concentration of gatifloxacin. For this purpose, films were prepared. The pharmaceutical parameters like hardness, thickness, stability, *in vitro* drug release characteristics, and mass balance studies were performed on prepared films.

EXPERIMENTAL

Materials and methods

Ethylcellulose, hydroxypropylmethyl cellulose (K_4M), hydroxypropyl cellulose (HPC), potassium dihydrogen phosphate and polyvinylpyrrolidone (K-30) were obtained from Loba Chemicals Pvt. Ltd and Ozone Pharmaceuticals, Mumbai, respectively. Eudragit RL-100 and Eudragit RS-100 were from Degussa India Pvt. Ltd, Mumbai. Drug gatifloxacin was obtained from Micro Labs, Bangalore. Solvents chloroform, dichloromethane, acetic acid and plasticizer dibutyl phthalate were from Merck Limited, Mumbai. Chitosan polymer was obtained from Central Institute of Fisheries Technology, Matysapuri, Kochin. All other reagents were of special reagent grade.

Preparation of drug loaded chitosan strips

The polymer strips prepared by solution casting method by pouring the drug-polymer dispersion into the center of leveled glass moulds and allowed to dry at room temperature (30°C) for 24 hours. After drying, films were cut into strips of required size (7 × 2 mm), wrapped in aluminum foil separately and stored in a desiccator until further use[5].

Films containing zero percent (placebo), 10%, 20% and 30% w/w of the drugs to the weight of polymer were prepared as given in Table 1.

Table 1: Chitosan strips containing drugs–parameters for preparation.

	Strip code	Drug	% of drug
Uncross-linked	CP	-	0
	G10	GATI	10
	G20	GATI	20
	G30	GATI	30
Cross-linked films	G30-2 hrs	GATI	30
	G30-4 hrs	GATI	30

Where CP is plain strip, G is gatifloxacin

Preparation of drug loaded ethyl cellulose strips

Strips were prepared by dissolving ethylcellulose with co-polymer HPC or HPMC K_4M or PVP or Eudragit RL-100 or Eudragit RS-100 and alone with the chloroform and dichloromethane (1 : 1), dibutylphthalate (1% w/w of that of polymer) as a plasticizer using magnetic stirrer in a closed beaker to get uniform distribution of polymer into the strips. Into this, gatifloxacin by w/w percentage of polymer was added. After complete mixing, 10 mL solution was poured into the clean-leveled glass moulds[6]. The solvent was allowed to evaporate slowly by inverting a glass funnel with a cotton plug closed in the stem of the funnel at room temperature for 24 hr. After complete evaporation of solvent, cast strips were obtained, which were then cut into pieces of 7 × 2 mm, wrapped in an aluminum foil and stored in desiccators at relative humidity at room temperature in a dark place until for further evaluation study.

The optimum concentration of ethyl cellulose (18-22 cps) used for the preparation of strips were found to be between 2-10% w/w, because at this concentration, the strips were flexible, easily removable from the die and maintain controlled release of drug as indicated in Table 2.

Evaluation of polymeric strips containing gatifloxacin

The compactability studies[7, 8] were conducted with individual polymer strips along with drug. Various pharmaceutical properties such as weight variation, hardness, thickness, stability, drug release characteristics and mass balance studies were determined on these strips. The results are reported in Tables 3-5.

Table 2: Composition of polymers and drug gatifloxacin in different formulations

Ingredients	Strip code and composition in % w/v					
	EP_1	**HP_2**	**PP_3**	**ELP_4**	**ESP_5**	**HCP_6**
Ethylcellulose	3	3	3	3	3	3
HPMC K_4M	-	0.5	-	-	-	-
PVP K-30	-	-	0.5	-	-	-
Eudragit RL-100	-	-	-	0.5	-	-
Eudragit RS-100	-	-	-	-	0.5	
Hydroxy propyl cellulose (HPC)	-	-		-	-	0.5
Dibutyl phthalate (%w/w)	1	1	1	1	1	1
Gatifloxacin %	3.07	3.07	3.07	3.07	3.07	3.07

Based on dry powder weight. In each of the formulations, 10 mL of chloroform : dichloromethane (1 : 1) ratio was used.

For weight determination, twenty strips of the same size (7 × 2 mm) were weighed on an electronic balance and the average weight was calculated. Hardness measurement and tensile strength were conducted in the instrument, which is specially designed in our laboratory. Thickness was measured using micrometer screw gauge. Drug content estimation was conducted on the drug loaded strips of known weight (dimension 7 × 2 mm) dissolved in 10 mL of acetic acid 1% (v/v) and were crushed until the strips dissolved.

The stability of the entire drug loaded polymer strips were studied at different temperatures using the reported procedure. The strips (7 × 2 mm) were weighed in 3 sets (12 strips in each set). The strips were wrapped individually in aluminum foil and also in butter paper and placed in petri dishes. These containers were stored at ambient humid conditions, at room temperature (27 ± 2°C), oven temperature (40 ± 2°C) and in refrigerator (5-8°C) for a period of 10 weeks. The samples were analyzed for physical changes such as colour and

texture. The drug content was estimated at an interval of 2 weeks. The drug solutions were further scanned to observe any possible spectral changes. There were no spectral changes.

Table 3: Physical characteristics of drug loaded polymeric strips for cross-linked and uncross-linked strips*

Strip Code	Tensile strength (g/sq.mm)	Hardness (g)	Weight (mg)	Thickness (mm)	% Drug Content (µg)/strip
	Before C. L.	Before C. L.	Before C. L.	Before C. L.	
CP	61.5159 ± 3.0941	245 ± 6.645	1.20 ± 0.031	0.5666 ± 0.0516	-
EP_1	703.98 ± 2.96	85.5 ± 2.88	1.27 ± 0.02	0.127 ± 0.005	-
HP_2	443.08 ± 2.70	152.3 ± 4.50	1.46 ± 0.02	0.193 ± 0.006	-
PP_3	735.31 ± 5.95	61.3 ± 3.88	1.56 ± 0.014	0.142 ± 0.006	-
ELP_4	549.98 ± 4.22	132.3 ± 5.42	1.32 ± 0.02	0.149 ± 0.007	-
ESP_5	514.30 ± 3.51	118.5 ± 3.78	1.34 ± 0.01	0.164 ± 0.006	-
HCP_6	459.80 ± 3.41	190.1 ± 3.76	1.41 ± 0.02	0.179 ± 0.007	-
GC 10	31.0817 ± 4.1464	340 ± 5.298	1.25 ± 0.019	0.8666 ± 0.05163	87.10
GC 20	23.7890 ± 4.5878540	441 ± 2.286	1.30 ± 0.017	0.9166 ± 0.0916	86.42
GC 30	21.4908 ± 5.1492	540 ± 5.491	1.40 ± 0.027	1.05 ± 0.1751	73.77

	After C.L.		After C.L.		After C.L.		After C.L.		After C.L.	
GC 30	**2 hr**	**4 hr**	**2 hr**	**4 hr**	**2 hr**	**4 hr**	**2 hr**	**4 hr**	**2 hr**	**4 hr**
	9.0456 ± 1.9129	9.7289 ± 0.7782	668.33 ± 6.673	800.00 ± 6.285	1.42 ± 0.026	1.43 ± 0.025	1 ± 0.1264	1.116 ± 0.183	69.59	59.03
GE_1	477.9 ± 4.2214		95.5 ± 3.5355		1.42 ± 0.03535		0.139 ± 0.0042		-	
GH_2	383.45 ± 2.9698		165.5 ± 4.9497		1.66 ± 0.01414		0.190 ± 0.0049		88.80	

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GC 30	After C.L.		After C.L.		After C.L.		After C.L.		After C.L.	
	2 hr	4 hr	2 hr	4 hr	2 hr	4 hr	2 hr	4 hr	2 hr	4 hr
GP_3	682.04 ± 4.1507		65 ± 2.8284		1.77 ± 0.02828		0.144 ± 0.0042		90.07	
GEL_4	394.73 ± 4.1648		142.3 ± 3.0550		1.48 ± 0.04242		0.152 ± 0.0035		89.05	
GES_5	417.31 ± 3.6840		124.6 ± 3.2145		1.54 ± 0.05656		0.165 ± 0.0028		89.95	
GHP_6	365.07 ± 4.9992		228.3 ± 3.5118		1.57 ± 0.02828		0.180 ± 0.0035		86.34	

*Each value is a mean and standard deviation of 6 determinations

Table 4: Mass balance studies data for polymeric strips containing gatifloxacin cross-linked and uncross-linked strips*

Strip code	% Drug release			% Residual drug content			% Total drug content		
	Before C.L.	After C.L.		Before C.L.	After C.L.		Before C.L.	After C.L.	
		2 hrs	4 hrs		2 hrs	4 hrs		2 hrs	4 hrs
GC 10	92.16	-	-	6.02	-	-	98.18	-	-
GC 20	84.80	-	-	13.47	-	-	98.27	-	-
GC 30	76.20	67.87	65.00	22.21	29.57	32.62	98.41	97.38	97.62
GE_1	91.24			6.11			97.35		
GH_2	95.86			2.25			98.10		
GP_3	93.84			4.15			97.99		
GEL_4	98.20			0.41			98.61		
GES_5	79.78			17.98			97.76		
GHP_6	96.14			2.11			98.25		

*****Each value is an average of six determinations

Table 5: Stability studies for drug content at various temperatures for polymeric strips containing gatifloxacin of cross -linked and uncross-linked strip

Temp. (°C)	Strip code	Initial drug conc. (μg)	2 Weeks		4 Weeks		6 Weeks		8 Weeks		10 Weeks	
			A.F.	B.P.	A.F.	B.P.	A.F.	B.P.	A.F.	B.P.	A.F.	B.P.
0	GC 10	94.28	94.27	94.25	94.22	94.20	94.17	94.12	93.90	93.65	92.90	92.62
	GC 20	163.34	163.32	163.28	163.27	163.23	163.18	163.11	163.92	162.80	162.12	161.85
	GC 30	209.15	209.01	209.05	208.92	208.83	208.57	208.13	207.71	207.09	206.89	206.52
	GC 30-2 hrs	197.28	197.28	197.27	197.26	197.22	197.24	197.21	197.23	197.12	197.15	197.08
	GC 30-4 hrs	167.33	167.32	167.30	167.29	167.28	167.25	167.22	167.20	167.15	167.07	167.02
	GE_1	49.72	49.69	49.63	49.53	49.47	49.21	49.13	48.89	48.76	48.66	48.53
	GH_2	50.44	50.41	50.37	50.22	50.17	50.02	49.76	49.64	49.53	49.44	49.38
	GP_3	44.87	44.83	44.80	44.71	44.65	44.37	44.28	44.16	44.09	43.78	43.62
	GEL_4	50.37	50.32	50.31	50.19	50.11	50.08	49.86	49.72	49.65	49.32	49.16
	GES_5	48.35	48.29	48.26	48.22	48.17	48.10	47.98	47.75	47.67	47.55	47.38
	GHP_6	51.36	51.34	51.31	51.28	51.22	50.99	50.71	50.62	50.54	50.27	50.19
27	GC 10	94.28	94.26	94.21	94.19	94.09	94.16	94.01	94.02	93.82	93.98	93.53
	GC 20	163.34	163.33	163.28	163.27	163.25	163.25	163.18	163.15	163.06	162.89	162.75
	GC 30	209.15	209.12	209.15	209.11	209.13	209.09	209.07	208.99	208.73	208.61	208.32
	GC 30-2 hrs	197.28	197.26	197.24	197.20	197.22	197.15	197.14	197.10	197.12	197.04	196.98
	GC 30-4 hrs	167.33	167.30	167.29	167.27	167.23	167.20	167.17	167.04	167.01	166.88	166.60
	GE_1	49.72	49.64	49.58	49.48	49.35	49.16	49.04	48.83	48.61	48.57	48.38

Cont…

Temp. (°C)	Strip code	Initial drug con c. (μg)	2 Weeks		4 Weeks		6 Weeks		8 Weeks		10 Weeks	
			A.F.	B.P.	A.F.	B.P.	A.F.	B.P.	A.F.	B.P.	A.F.	B.P.
27	GH_2	50.44	50.37	50.34	50.18	50.08	49.82	49.63	49.58	49.32	49.31	49.22
	GP_3	44.87	44.81	44.77	44.67	44.52	44.36	44.24	44.11	43.91	43.67	43.61
	GEL_4	50.37	50.30	50.27	50.14	50.07	49.86	49.79	49.68	49.51	49.24	49.12
	GES_5	48.35	48.27	48.24	48.16	48.10	47.95	47.81	47.52	47.41	47.33	47.18
	GHP_6	51.36	51.32	51.29	51.21	51.16	50.88	50.69	50.56	50.42	50.19	50.11
40	GC 10	94.28	94.27	94.23	94.19	94.18	94.12	94.09	94.01	93.98	93.77	93.78
	GC 20	163.34	163.30	163.27	163.21	163.22	163.17	163.16	163.08	163.03	162.93	162.83
	GC 30	209.15	209.19	209.15	209.11	209.09	209.02	209.00	208.87	208.82	208.76	208.75
	GC 30-2 hrs	197.28	197.20	197.17	197.13	197.11	197.02	197.01	196.90	196.88	196.81	196.72
	GC 30-4 hrs	167.33	167.30	167.29	167.27	167.23	167.20	167.17	167.04	167.01	166.88	166.60
	GE_1	49.72	49.61	49.55	49.42	49.31	49.15	48.93	48.77	48.52	48.51	48.31
	GH_2	50.44	50.34	50.30	50.14	49.96	49.78	49.61	49.52	49.29	49.25	49.19
	GP_3	44.87	44.79	44.76	44.55	44.43	44.27	44.21	44.04	43.84	43.59	43.46
	GEL_4	50.37	50.28	50.23	50.09	49.96	49.82	49.73	49.50	49.43	49.23	49.10
	GES_5	48.35	48.26	48.20	48.13	48.03	47.86	47.73	47.50	47.39	47.29	47.13
	GHP_6	51.36	51.30	51.23	51.17	51.02	50.75	50.62	50.51	50.37	50.13	50.00

*Each value is an average of six determinations

A.F. = Packed in aluminum foil, B.P. = Packed in butter paper.

A static dissolution was carried out by sets of 6 strips of known weight and dimension (7 × 2 mm) placed separately into small test tubes containing 1.0 mL phosphate buffer, pH 6.6. The tubes were sealed and kept at 37°C for 24 hours. The buffer was then drained off and replaced with a fresh 1.0 mL phosphate buffer, pH 6.6. The concentrations of drugs in the buffer were measured at 290 nm.

Following the *in vitro* drug release, mass balance studies were carried out on the drug content left in the strip. Each strip was dissolved in acetic acid 1% (v/v), diluted suitably and the absorbance was measured. Amount of drug released into the dissolution medium and residual drug content in the films were accounted and compared for the actual drug content.

RESULTS AND DISCUSSION

The strips of ethyl cellulose with different co-polymers or (strips code-E), (GE_1, GH_2 GP_3, GEL_4, GES_5 and GHP_6) and chitosan polymer (biodegradable) strips were prepared by solution casting method. The optimum loading for good, flexible strips was found to be 30% or less than that. For the present study, the prepared strips were chitosan containing gatifloxacin with three different concentrations i.e., 10%, 20% and 30% to the weight of the polymer. Strips containing 30% gatifloxacin were cross-linked. If the cross-linking time was increased more than 4 hours, the strips were very hard, brittle and hence, not fit for further studies. Therefore, 2 and 4 hours cross-linking period was selected.

The physicochemical evaluation data are included in Table 3, which indicates that the thickness of each strip of size 4 × 4 cm was reported in for strip code-E drug loaded with 3.07% w/w. The thickness also varies with different co-polymers used. The thickness values of strips code-E vary from 1.39 mm to 1.80 mm for all six formulations. Strip code-E showed high thickness as compared to chitosan strips loaded with gatifloxacin. GC 30% (1.05 mm) showed high thickness compared to GC 10% and GC 20% (0.8666 to 0.9166) and hence, as the drug concentration increases, thickness also increases. The cross-linked drug loaded chitosan strips did not alter in the thickness significantly and thus, thickness range is satisfactory from the point of insertion into periodontal pockets. Individual weight of 20 strips of size 7 × 2 mm for E code, strips is between 1.42 mg to 1.77 mg. The weight of the strips varies according to co-polymer used. For chitosan strips, 1.25 mg to 1.40 mg and cross-linking has no effect on the weight. The weights are quite uniform. The results were encouraging and particularly important.

The tensile strength of plain strips (E- 457.39 to 731.09, CP- 61.5159 g/sq mm) was much higher than the drug loaded strips followed by strip code E (365.07 to 682.04 g/sq mm)

and GC 10%, GC 20% and GC 30%. (61.5159 to 21.4908 g/sq mm). The drug decreases the tensile strength because of less elongation, indicating the brittleness. It might be due to linear structural feature of the polymer chains. Cross-linking (9.0456 to 9.7289 g/sq mm) has reduced the tensile strength of the strips. There was no considerable elongation of the strips, when force was applied compared to uncross-linked films. The data obtained revealed that incorporation of drug reduces the physical strength of the strip; however; this may not cause any problem during their clinical application. In contrast to tensile strength, the hardness of the strips increases as the drug loading increases, In case of cross-linked films, the hardness was more compared to the films without cross-linking and the results are summarized in the Table 3.

The UV scan studies confirmed the absence of any chemical interaction between the drug and the polymer. The stability studies carried out by drug content estimation and UV scanning for the films i.e., strip code-E and GC 10, GC 20, GC 30 under various conditions of temperature for a period of 10 weeks showed that there was no degradation and drug content did not differ from initial drug content by more than 5%. It should be noted that the drugs stable similar behaviour was observed in case of cross-linked chitosan drug loaded strips (data given in Tables 4 and 5).

In vitro release of gatifloxacin from chitosan strips and ethyl cellulose strips

In vitro dissolution rate studies were carried out by static dissolution method for uncross-linked and cross-linked films for a period of 9 days and 19 days for chitosan stips, respectively. For E strips, the release study were conducted for 11 days. Results showed that a burst release initially, followed by a progressive fall in the release of the drug. The uncross-linked strips GC 10, GC 20 and GC 30 showed 92.16%, 84.80%, and 76.20%, respectively at the end of 9 days. The cross-linked strips (GC 30-2 hours and GC 30-4hours) showed 67.81% and 65.00%, respectively at the end of 19 days, For E strips GE_1, GH_2, GP_3, GEL_4, GES_5 and GHP_6, 91.24, 95.86 93.84, 98.20, 79.78 and 96.14, respectively at the end of 11 days static dissolution period. Initial burst effect was reduced by more than 40%, once the strips were cross-linked and also release of drug was extended, controlled up to 19 days and 11days. The comparative release time profile for drug from chitosan strips and strips E is given in (Figs. 1, 2 and 3), From day three onwards, the release of gatifloxacin was more uniform and constant (GC 10% 2.634, GC 20% 3.067 and GC 30% 2.760, about GC 30%-2 hours 2.622 and GC 30%-4 hours 2.580 and E strips GE_1 2.412, GH_2 2.764, GP_3 2.902, GEL_4 3.012, GES_5 3.231, GHP_6 2.430, respectively per day,). After nine days for uncross-linked, 19 days for coss-linked strips and E strips for 11 days lost integrity and hence, were not fit for the release study. The residual drug content in the strips after 9, 19 and 11 days was determined (after the static dissolution study). The data are reported in the Table 4.

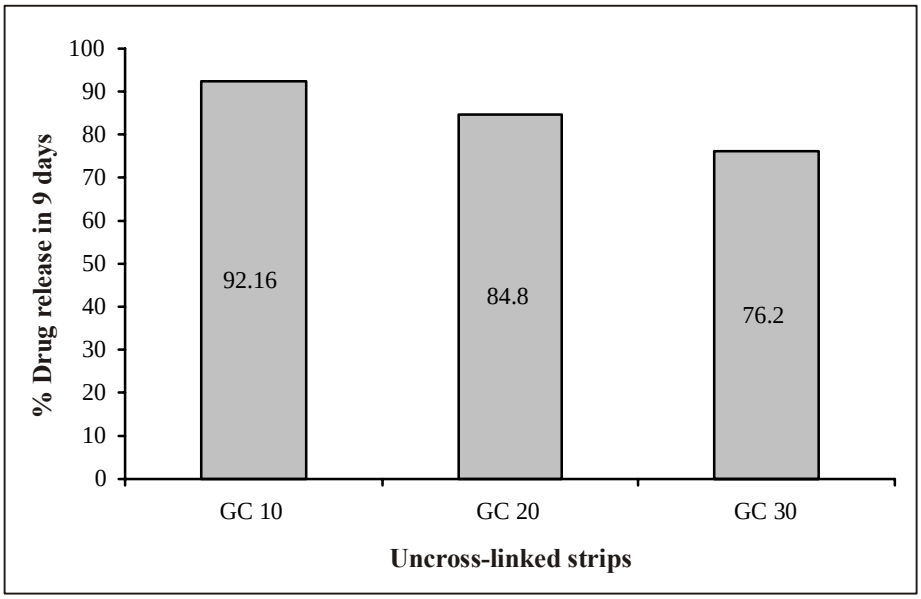

Fig. 1: Comparative release profile of polymeric strips containing gatifloxacin for uncross-linked strips*

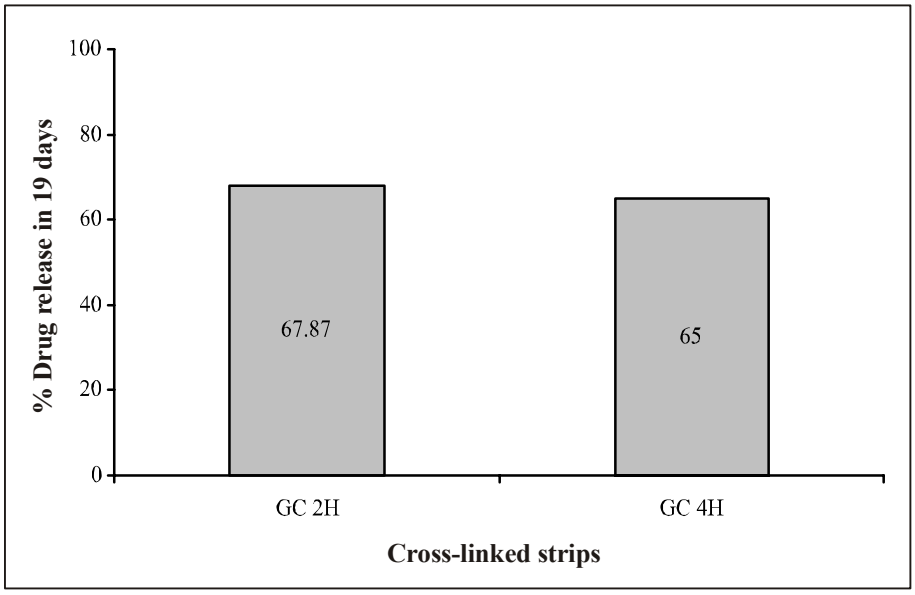

Fig. 2: Comparative release profile of polymeric strips containing gatifloxacin for cross-linked strips*

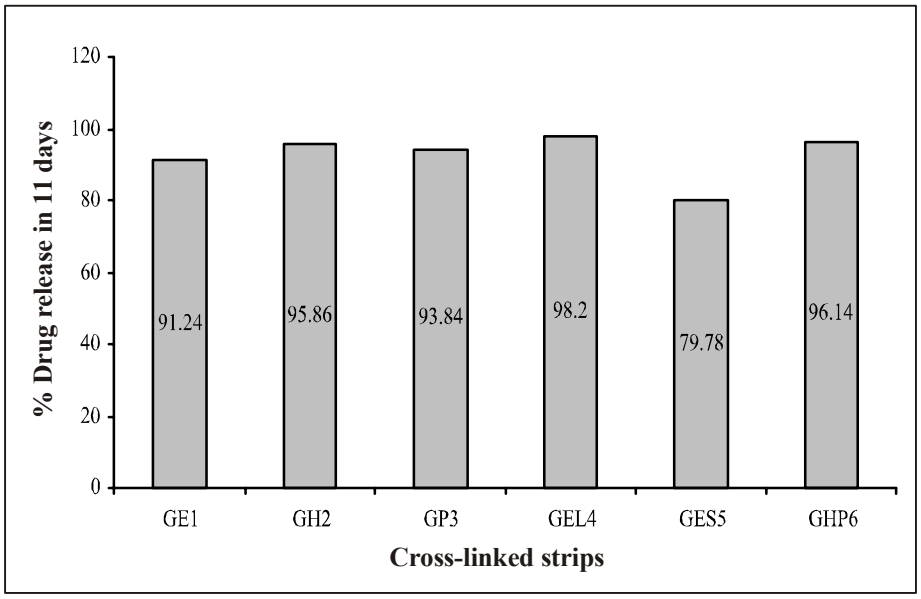

Fig. 3: Comparative release profile of polymeric strips containing gatifloxacin for cross-linked E strips*

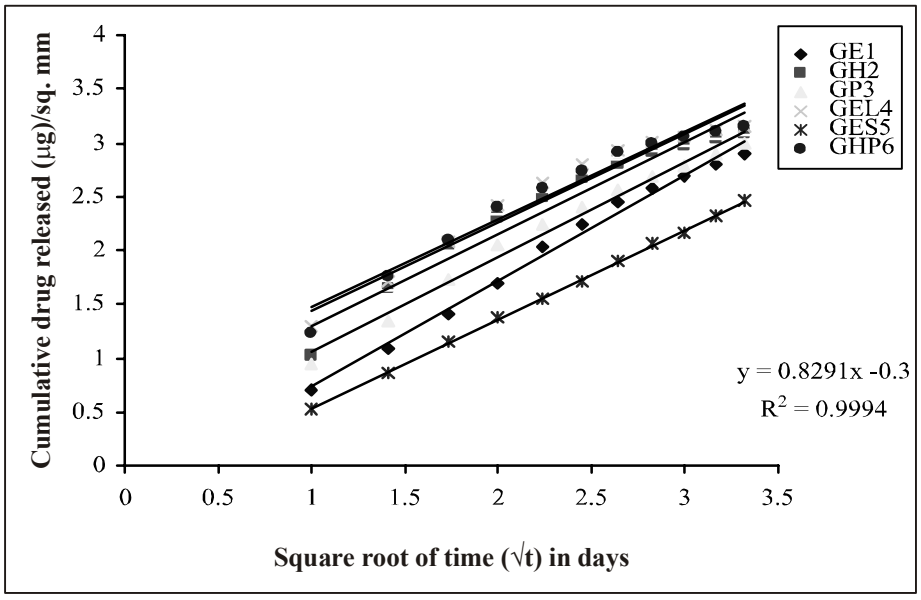

Fig. 4: Best fit lines for Higuchi's equation for cross-linked different polymeric strips containing gatifloxacin for cross-linked E strips

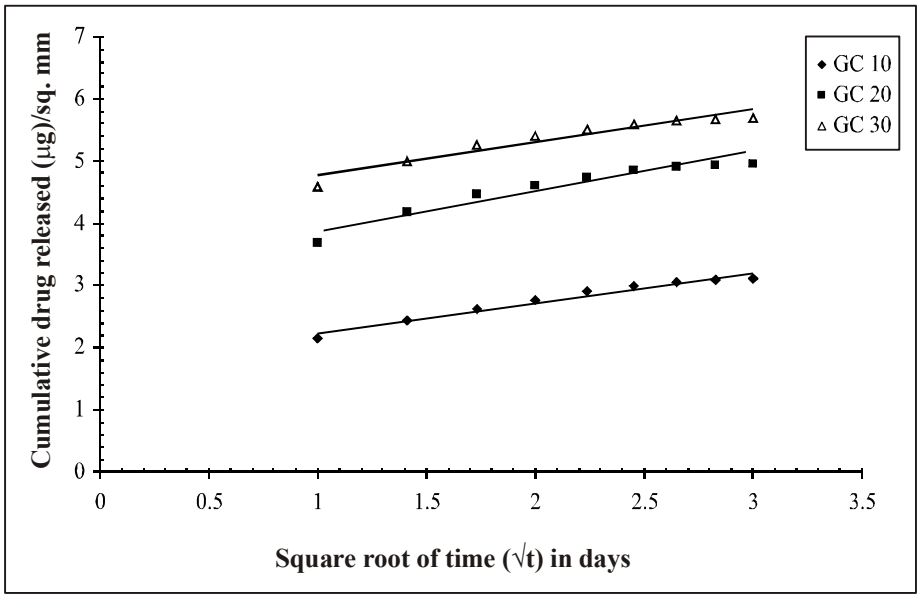

Fig. 5: Best fit lines for Higuchi's equation for uncross-linked chitosan strips containing gatifloxacin

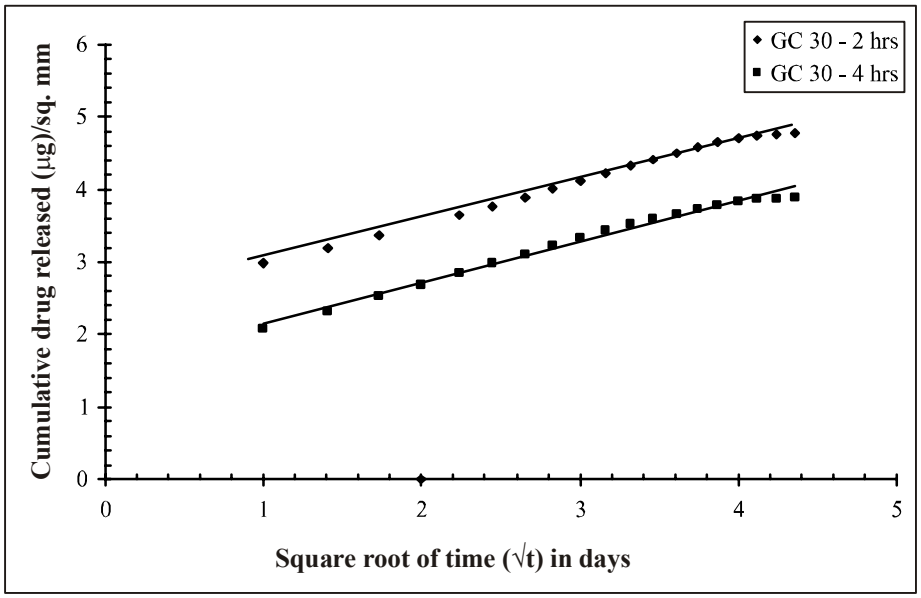

Fig. 6: Best fit lines for Higuchi's equation for cross-linked chitosan strips containing gatifloxacin

Release kinetics

The data were processed for regression analysis using MS-EXCEL statistical functions. A perusal of Figs. 4, 5 and 6 indicated that the release kinetics of gatifloxacin from chitosan strips followed zero order. (R^2 = 0.8956, 0.8269 and 0.8243 for GC 10%, GC 20% and GC 30%; R^2 = 0.9600 and 0.9294 for GC 30%-2 hours and GC 30%-4 hours, R^2 = 0.9918, 0.9692, 0.991, 0.9617, 0.9927 and 0.9647 for GE_1, GH_2, GP_3, GEL_4, GES_5 and GHP_6, respectively).

The mass balance studies done after the *in vitro* dissolution studies (static dissolution) showed that the percentage of drug released plus the percentage of residual drug content did not deviate by more than 3% from the experimental drug content. The data are presented in Table 4. This confirms that the drug is in free form rather than chemically or physically bound to the polymer. Throughout the release study, the strips remain intact without any disintegration, the average release (from day 3 onwards) is 3 µg per day for both; cross-linked and uncross-linked strips, which was above the minimum inhibitory concentration of gatifloxacin.

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