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Design and Evaluation of Mucoadhesive Buccal Patches of Diclofenac Sodium

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The goal of the present investigation was to design and evaluate mucoadhesive buccal patches of diclofenac sodium, which is used as analgesic and antiinflammatory agent. Patches were fabricated by casting technique with different polymer combinations and were evaluated for *in vitro* release, bio-adhesion strength, duration of bioadhesion, folding endurance, surface pH and percentage of elongation. The release profile and test for adhesion were found to be the function of the type of polymer used. The formulation containing hydroxy propyl cellulose and carbapol 934 P was found to give the better results.

The usage of most of the NSAIDS by oral route associated with potential disadvantages such as peptic ulceration and gastric bleeding[1]. Diclofenac sodium is a new generation NSAID, which is widely used in the long-term treatment of rheumatoid arthritis. Short biological half-life of 1-2 h necessitates multiple dosing for maintaining therapeutic effect throughout the day. Diclofenac sodium suffers from several drawbacks like irritation, peptic ulceration and gastric bleeding, this may eventually cause wall perforation. Such effects usually are associated with chronic high dose treatment[2,3]. There is also a substantial first pass effect, only about 50 % of drug is available systemically[4]. These severe drawbacks create a potential need for development of mucoadhesive patches, which are capable of avoiding the first pass effect and gastrointestinal side effects with delayed release system. The development of technology for release of drug at a controlled rate in to systemic circulation using buccal cavity as port of entry has become popular. In the present study an attempt was made to design the mucoadhesive buccal patches of diclofenac sodium with various polymers. The patches were characterized by keeping uniformity, flexibility, clarity and homogenecity as the tools.

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Diclofenac sodium IP and polymers such as carbapol-934P, hydroxypropylmethyl cellulose (6 cps), hydroxypropyl cellulose (HPC), sodium carboxymethyl cellulose and ethyl

cellulose were obtained as gift sample from Zydus Cadila Health Care Ltd, Ahmedabad.

Method of casting on the PVC and aluminum foil surface was adopted for the preparation of patches. The patches were prepared using various polymers in different composition (Table 1). Carbapol 934 P and diclofenac sodium were dispersed in 30 ml of dichloromethane by continuous stirring for 2 h. The other polymers were separately dispersed in 15 ml of isopropyl alcohol and then two polymeric solutions were mixed by stirring for about 2 h. Propylene glycol (1 ml) was mixed with polymeric solution as a plasticizer and was stirred for 4 h. The above mixture was taken into a pipette and poured into plastic rings placed on two different substrates namely a thin PVC sheet and aluminum foil and it was allowed to dry at room temperature for 24 h, the dried patches were removed slowly from the substrate surfaces. The patches of 15 mm diameter were punched out so that each patch contains 75 mg of diclofenac sodium[5].

A simple apparatus was used for *in vitro* release study. A 250 ml glass beaker was filled with isotonic phosphate buffer, (IPB, pH 6.6) simulating the salivary pH. A strip of mucosal membrane was washed with buffer solution and was stabilized to wash out soluble components. After stabilization the patch was fixed on the mucosal membrane and it was tied with a thread to simple funnel in an inverted position. The patch attached to the membrane was dipped into the buffer solution into a flask in a position just below the dissolution fluid. The flask was kept on magnetic stirrer, the whole assembly was maintained at 37°. Samples were collected at fixed time intervals by replacing same volume of dissolution fluid, samples with suitable dilutions were analyzed by using UV-spectrophotometer (Systronics-108) at 450 nm[6]. Samples collected from placebo patches were used as blank.

The duration of bioadhesion was studied by measuring the time required for the formulation to erode completely or the time for which the formulation was maintained at its position without dislodging so bioadhesive strength is an index of adhesive strength of a film to the buccal mucosa till the complete drug releases. Bioadhesive strength of the patch was measured using a modified double beam balance described by Gupta *et al*[7]. Percentage elongation at break was determined using universal testing machine as described by Khanna *et al*[8]. The parameters longitudinal strain (LS, increase in length/initial length) and percentage elongation at break (LSx100) were calculated. A small strip of patch was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gives the values of folding endurance. The surface pH was measured by the method similar to that used by Bottenberg *et al*[9]. A combined glass electrode was used. The patches were kept in contact with 0.5 ml of distilled water for 1 h. pH was noted by bringing the electrode near the surface of the patch and allowing it to equilibrate for 1 min.

All the polymers used for the fabrication of patches gave good quality films. Method of casting on the aluminum foil was found to be satisfactory. Buccal films with different polymers were transparent, smooth and flexible. Initially dummy films were prepared in order to determine the best combination of polymers, plasticizers and solvents required to get good formulations. Then, the formulations, which showed complete homogenous, smooth, flexible and non-sticky were selected for further studies and evaluated for *in vitro* drug release, bioadhesion strength, surface pH, percentage elongation and folding endurance. The results revealed that the

TABLE 1: COMPOSITION OF DIFFERENT MUCOADHESIVE FORMULATIONS.

Ingredients	F_1	F_2	F_3	F_4
Diclofenac sodium	2250 mg	2250 mg	2250 mg	2250 mg
Carbapol 934 P	200 mg	200 mg	200 mg	200 mg
Hydroxypropylmethylcellulose	150 mg	—	—	—
Hydroxypropylcellulose	—	—	—	150 mg
Ethyl cellulose	—	—	150 mg	—
Sodium carboxymethylcellulose	—	150 mg	—	—
Propylene glycol	1 ml	1 ml	1 ml	1 ml
Isopropyl alcohol+dicloromethane (1: 2)	30 ml	30 ml	30 ml	30 ml

TABLE 2: DATA OBTAINED FROM EVALUATION OF MUCOADHESIVE FORMULATIONS.

Formulation Code	Adhesion Time (min)*	Bioadhesive Strength (g)*	Surface pH**	Folding Endurance**	Percent Elongation*	Tensile Strength (kg)*
F1	360 (0.26)	26.1 (0.05)	6.12	180	37.2 (0.21)	19.1 (0.35)
F2	321 (0.18)	20.2 (0.08)	6.20	168	40.0 (0.24)	19.0 (0.23)
F3	293 (0.11)	15.3 (0.15)	6.72	172	27.1 (0.16)	18.9 (0.55)
F4	308 (0.21)	11.1 (0.11)	6.42	163	36.4 (0.41)	18.5 (0.16)

Table 2 depicts the data obtained from evaluation of Mucoadhesive films. *Indicates the values are average of three observations, **Indicates the values are average of five observations, the numbers in parenthesis are standard deviation values.

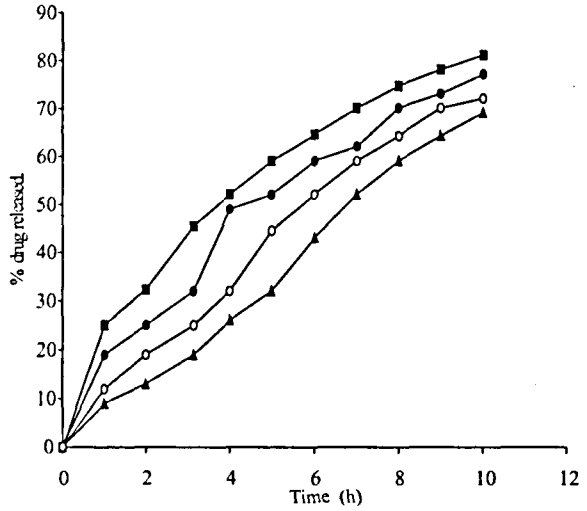

Fig 1: *In vitro* release profiles from different mucoadhesive films.

***In vitro* release of diclofenac sodium from different polymeric mucoadhesive formulations, formulation F_2 (-■-), formulation F_4(-●-), formulation F_1 (-○-) and formulation F_3 (-▲-).**

release of drug is depended on the polymer type as well as on their concentration. Films containing HPC and carbapol 934 P (Code-F_2) released the drug in a sustained manner. The rank order of the drug release from different formulations is $F_4>F_2>F_1>F_3$. Formulation F_4 showed 81% drug release at the end of 12 h, followed by F_2 (77.2%), F_1 (72.7%) and F_3 (69.8%), respectively. The values of amount of drug released at 12 h, with standard deviation are F_1 77.2 % (0.27), F_2 81.0 (0.42), F_3 69.8 (0.35) and F_4 72.7 (0.38).

The bioadhesive strength of the formulations was found to be dependent on the type of polymers used. Results show that, the formulation F_4 (Carbopol934P and hydroxypropyl cellulose) exhibited maximum bioadhesion strength. The order of bioadhesion strength for all formulations is $F_4>F_2>F_1>F_3$. The adhesion time for all the formulations was in the range of 293 to 360 min, minimum adhesion time was observed for the formulation F_3. All the formulations showed that the folding endurance is in the range of 163 to 180. The surface pH was determined in order to investigate the possibility of any side effects, in the oral cavity. As acidic or alkaline pH is bound to cause irritation to the buccal mucosa. Attempt was made to keep the surface pH close to the neutral pH. The surface pH of all formulations was found to be within ±1.5 units of neutral pH. The surface pH of all formulations is very close to the neutral pH, hence it is assumed that, these formulations cause no irritation in the oral cavity. Flexibility of the film affects muco-adhesion[10]. Films with good percentage elongation and tensile strength are essential. Formulation F_2 showed maximum percentage elongation and formulation F_3 showed minimum percentage elongation. The rank order of the percentage of elongation is $F_2>F_1>F_4>F_3$. The results obtained in the present investigation indicate that the film containing Carbopol-934 and hydroxypropyl cellulose could perhaps be considered as the best among all other films prepared.

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Antibacterial Activity of Some 4-*N*-Substituted Thiosemicarbazides and Thiosemicarbazones

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A series of synthesized 4-*N*-substituted thiosemicarbazides and thiosemicarbazones were screened for antibacterial activity. The compounds Ie and IIa were most active compounds of the series against *Staphylococcus aureus* and *Escherichia coli*.

Some new 1H indolo [3,2-c]isoquinolin-5-ylthiosemicarbazide and its derivatives were found to show antibacterial, anthelmintic, antiinflammatory and analgesic activity[1]. Copper and zinc chelates of 4-(4*n*) substituted thiosemicarbazones of 6-methyl-5-nitropyridine-2-carboxaldehyde showed good activity against both gram positive and gram negative bacteria[2]. Schiff and Mannich bases derived from isatin derivatives and *N*-[4-(4′-chlorophenyl)thiol-2-yl thiosemicarbazides were investigated for antimicrobial activity. Among the compound tested 1-[*N*,*N*-dimethylaminomethyl]-5-bromoisatin-3-{1′-4″-*p*-chlorophenyl)thiaxol-2″-yl} thiosemicarbazone showed significant antimicrobial activity[3]. New 4-acetyl-antipyrine-4-alkyl/aryl-3-thiosemicarbazones were tested for *invitro* antimicrobial activity[4]. A series of 3-benzylthiazolidine-2,4-dione-4-thiosemicarbazones was synthesized as potential antimicrobial agents[5]. In the view of these above compounds possessing antibacterial properties, a series of thiosemicarbazides (Ia-e) and thiosemicarbazones (IIa-e) were synthesized by using different isothiocyanates and screened for their antibacterial activity.

Equimolar quantity (0.1 mol) of cyclohexylisothiocyanate and hydrazine hydrate (2) was refluxed in ethanol for 1 h. The mixture was cooled, washed with pet. ether, dried and crystalized from ethanol to yield 4-*N*-cyclohexylthiosemicarbazide Ib. Equimolar quantity (0.05 mol) of 2-hydroxy acetophenone (1) and hydrazine hydrate (2) was stirred for 5-10 min. The yellow solid obtained as 2-hydroxyacetophenone hydrazone (3) was dissolved in 25 ml of ethanol and refluxed with cyclohexyl isothiocyanate in equimolar quantities (0.01 mol). The solid obtained was washed with pet. ether and recrystalized from a mixture of ethanol and acetone to give 2-[hydroxy] acetophenone-4-*N*-(cyclohexyl) thiosemicarbazone IIb (Scheme 1).

Two test strains of bacteria, *S. aureus* (NCTC 10418) and *E. coli* (NCTC 6571) were used. The standard drug used for comparison of test compounds were a disc of vancomycin (for *S. aureus*) and ofloxacin (for *E. coli*). A series of 4-*N* substituted thiosemicarbazides (Ia-e) and thiosemicarbazones (IIa-e) were synthesized and characterized by their melting point, TLC and IR data (Table 1). All the compounds were screened for their antibacterial activity against *S. aureus* and *E. coli* by filter paper disc technique[6]. The results are presented as in Table 2. The antibacterial activity of 4-*N*- substituted thiosemicarbazides and thiosemicarbazones was tested against *S. aureus* and *E.*

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