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Research Article

In Vitro and *In Vivo* Evaluation of Taste Masked Chlorhexidine-Releasing Oral Films

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ABSTRACT
The present investigation was undertaken with the objective of formulating chlorhexidine diacetate containing fast dissolving oral films to serve as superior alternative to mouthwash, aiming at consumer compliance. Various film forming agents, plasticizers and taste masking agents were evaluated for optimizing the composition of fast dissolving oral films. The potential of arginine hydrochloride as taste masking agent for fast dissolving oral films containing hydroxypropylmethylcellulose E5 (HPMC E5), propylene glycol and sucralose were formulated. Fast dissolving oral films of chlorhexidine diacetate were evaluated for the *in vitro* dissolution profile and *in vitro* microbiological assay. Oral films exhibited satisfactory *in vitro* dissolution profile and *in vitro* antimicrobial activity. Effect of incorporation of eugenol on the *in vivo* performance of oral films was evaluated in human volunteers. Arginine hydrochloride and eugenol containing oral films improved effectiveness and acceptability of films with respect to taste masking, mouth feel and mouth freshening without compromising the *in vivo* dissolution time.

Keywords: Fast dissolving oral films; Chlorhexidine diacetate; Arginine hydrochloride; Eugenol.

INTRODUCTION

Chlorhexidine diacetate is an antimicrobial and antiseptic agent used in oral hygiene products and dental preparations. It is effective over gram positive and gram negative bacteria and fungi. It has got properties like substantivity that helps to retain on oral mucosal surfaces. Consequently it results in long lasting effect and prevention of plaque formation. Also chlorhexidine diacetate exhibits low mammalian toxicity. This makes it a potential drug to formulate into various dosage forms for treatment of periodontal diseases, malodor, plaque and gingivitis[1].

Chlorhexidine diacetate is available as mouthwash and widely used. There are some limitations regarding usage of mouthwashes. A mouthwash has to be taken in undiluted (15-20 ml) form and twice a day. It has to be expelled and not to be swallowed. So there are many directions which have to be followed.

The use of mouthwashes is often limited by problems related to handling, inconvenience of use during traveling, stability aspects of liquid formulations in certain cases and patient noncompliance. The need of the hour is to have an oral hygiene product superior to mouthwash that would be easy to handle, acceptable; consumer friendly would allow rapid release of chlorhexidine diacetate for instant local action.

Fast dissolving oral films fulfill all the aforementioned requirements of potential oral dosage form for local delivery of chlorhexidine diacetate. Fast dissolving oral film when placed in the oral cavity quickly gets hydrated and then disintegrates and dissolves to release the drug[2]. Thus, a fast dissolving oral film is a unique solid oral dosage form with significant advantages[3-7].

Initial investigations were focused on development of placebo fast dissolving oral films with good peelability, mouth feel, mouth freshening and *in vivo* dissolution time in human volunteers. After choosing the components for the placebo oral film, chlorhexidine diacetate loaded oral films were formulated. Although, fast dissolving oral film is an attractive dosage form for the delivery of chlorhexidine diacetate, the bitter taste of chlorhexidine diacetate was the actual challenge in the formulation of fast dissolving oral films[1]. In order to improve taste of oral films of chlorhexidine diacetate, potential of glycine hydrochloride and arginine hydrochloride was evaluated in the present investigation. The fast dissolving oral films with the taste masking agent were formulated and evaluated for *in vitro* and *in vivo* performance.

MATERIALS AND METHODS

Materials

Chlorhexidine diacetate purchased from Yash Chemicals, Aurangabad. Methocel E15, E5, E3 (Colorcon Asia Pvt Ltd., Goa, India) and Sucralose (Gangwal Ltd.) were received as gift samples. *Streptococcus mutans* MTCC No. 497culture was purchased from Institute of Microbial Technology, Chandigarh. Eugenol (S. H. Kelkar Co., Mumbai, India), glycine hydrochloride, arginine hydrochloride, propylene glycol, polyethylene glycol and glycerin (Qualigens Fine Chemicals, Mumbai, India) were purchased for carrying out various experiments. All the chemicals used were of analytical grade.

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Table 1: Composition of Various Placebo Fast Dissolving Oral Films.

Ingredients	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5(%)	F6 (%)	F7 (%)
HPMC E5	3	3	4	4	5	4	4
Propylene	1.84	1.84	2.45	2.5	3.5	2.5	2.5
Glycol	1	1.25	1	1.25	1.25	1.25	1.25
Arginine hydrochloride	0.5	0.75	0.5	0.75	0.75	0.75	0.75
Sucralose							
Eugenol	-	-	-	-	-	0.005	0.002

Table 2: Compositions of Chlorhxidine diacetate Fast Dissolving Oral Films.

Ingredients	F8 (%)	F9 (%)
Chlorhexidine diacetate	0.005	0.005
HPMC E5	4	4
Propylene Glycol	2.5	2.5
Arginine hydrochloride	1.25	1.25
Sucralose	0.75	0.75
Eugenol	0.005	0.002

Table 3: Scale for Evaluation of bitterness threshold of Chlorhexidine diacetate.

Scale	Taste
0	Tasteless
0.5	Very slightly bitter
1	Slightly bitter
1.5	Slight to moderate bitter
2	Moderately bitter
2.5	Moderate to strong bitter
3	Strongly bitter
4	Very strongly bitter

Table 4: Weight, Thickness, Folding Endurance, Assay of Chlorhxidine diacetate containing Fast Dissolving Oral Films of Formulae F8 and F9.

Film Formula	Weight (g)	Thickness (mm)	Folding Endurance (number of folds)	Assay (%)
F8 (%)	0.0813 ± 0.021	0.210 ± 0.015	301 ± 3	98.22 ± 1.56
F9 (%)	0.0770 ± 0.004	0.1818 ± 0.016	325 ± 3	99.73± 0.8

Table 5: Taste Recognition Threshold Determination.

Concentration µg/ ml	Volunteer 1	2	3	4	5	6	7
5	N	N	N	N	N	N	N
10	Y	Y	Y	Y	Y	Y	Y
20	Y	Y	Y	Y	Y	Y	Y
30	Y	Y	Y	Y	Y	Y	Y
40	Y	Y	Y	Y	Y	Y	Y
50	Y	Y	Y	Y	Y	Y	Y

Y: recognition of bitter taste N: no perception of bitter taste

Screening of the Components for Formulation of Placebo Fast Dissolving Oral Films

Hydroxypropylmethylcellulose (HPMC) is known for its good film forming properties and has excellent acceptability. Hence grades of HPMC namely Methocel E3 and Methocel E5 were evaluated as film forming agents. For formation of oral films, propylene glycol was used as a plasticizer and sucralose was used as a sweetener. Initially, films containing various concentrations of plasticizers and taste masking agents were formulated. The composition of various films is shown in Table I. Oral films were prepared by solvent casting method. Briefly, dispersion of HPMC E5 in distilled water was sonicated for 15 minutes and propylene glycol, arginine hydrochloride and sucralose was added to this dispersion. Eugenol was dissolved in isopropyl alcohol and this alcoholic solution was added to the polymeric dispersion with stirring to obtain homogenous dispersion. 20 ml of the dispersion was casted onto each polypropylene petri plate. The dispersion was air dried. The films were carefully removed from petri plates and cut into strips of dimensions 2×2 sq.cm and stored in an air tight glass bottle. The films were evaluated for imperfections and cuts, peelability without rupturing, folding and cracking endurance and surface roughness.

Preparation of Chlorhexidine diacetate containing Fast Dissolving Oral Films

Chlorhexidine diacetate containing fast dissolving oral films were prepared as per the method described for the development of placebo fast dissolving oral films. Briefly, chlorhexidine diacetate was dissolved in isopropyl alcohol and the rest of the procedure was same as that for formation of placebo fast dissolving oral films. The composition of chlorhexidine diacetate containing oral films is shown in Table II. Chlorhexidine diacetate concentration in the final polymeric dispersion was 0.005% *w*/*v*.

Evaluation of Chlorhexidine diacetate containing Fast Dissolving Oral Films

Determination of Weight and Thickness

Oral films of formula F8 and F9 ($n = 30$) were weighed on electronic balance (AT Series, Shimadzu, Tokyo, Japan). Thickness of films of formula F8 and F9 ($n = 12$) was measured using a micrometer screw gauge (Mitutoyo, Kawasaki, Japan).

Folding endurance

Oral films of formula F8 and F9 ($n = 3$) were evaluated for folding endurance. One film was repeatedly folded at same place until it broke and the number of times the film could be folded at the same place without breaking yields the value of folding endurance.

Table 6: *In vivo* performance of Fast Dissolving Oral films.

Human Volunteer	Mouth feel		Mouth Freshening		*In Vivo* Dissolution Time (s)	
	F8	F9	F8	F9	F8	F9
1	+	+++	++	++	100.17	86.25
2	+	++	++	++	112.85	80.32
3	+	++	++	++	120.02	90.15
4	+	+++	++	++	121.35	83.28
5	+	+++	++	++	105.24	80.21
6	++	+++	++	++	95.25	90
7	++	+++	++	++	96.31	95.23
8	+	++	+	+	100.38	78.20
9	++	+++	++	++	121.06	86.12

Mouth feel: +++ Excellent, ++ Very Good, + Good, Mouth Freshness: +++ Significantly present, ++ Present, + Slightly present

Table 7: Data on Stability Studies.

Parameters	Temperature And Humidity Conditions	Three Month
Folding endurance	25°C / 60% RH	302 ± 3
(number of folds)	40°C / 75% RH	300 ± 3
Assay	25°C / 60% RH	96.42 ± 2.15
(%)	40°C / 75% RH	95.35± 1.95

Surface pH[8]

For determination of surface pH, the oral films ($n = 3$) ere allowed to swell for 2 h on the surface of an agar plate. The surface pH was measured using pH paper placed on the surface of the swollen film. The average of three determinations for each F8 and F9 was taken.

Determination of Chlorhexidine diacetate Content

For determination of chlorhexidine diacetate content, oral films ($n = 10$) were crushed to obtain a fine powder and chlorhexidine diacetate was extracted from the film by using 100 ml distilled water. Further the solution was sonicated for 15 minutes. From above solution 1 ml was pipetted and volume was made up to 100 ml with distilled water and analyzed using UV-Vis spectrophotometer (Incarp SCIAN 2301) at 255 nm.

In Vitro *Chlorhexidine diacetate Release from Oral Films*

For *in vitro* dissolution studies, each film was placed with the help of forceps in a 500 ml glass beaker containing 300 ml of distilled water. The beaker was suspended in a water bath of USP-XXIII dissolution apparatus (Electrolab TDT-08L) and agitation was provided by the shaft of the USP-XXIII Type I Apparatus (Electrolab TDT-08L) at 50 rpm without the basket attached to it. The temperature of the dissolution medium was maintained at 37 ± 0.5 °C. During the study, 1 ml of aliquot was withdrawn at 1, 3, 5, 7, 10 and 15 min and was replaced by fresh distilled water. The aliquots were diluted to 25 ml with distilled water and amount of chlorhexidine diacetate released in the medium was determined using UV-Vis spectrophotometer (Incarp SCIAN 2301) at 255 nm. The *in vitro* chlorhexidine diacetate release data were evaluated by a non-paired, two tailed Student's '*t*' test. Differences were considered statistically significant at $P < 0.05$.

Evaluation of bitterness threshold concentration of Chlorhexidine diacetate[9]

The bitter taste threshold value of chlorhexidine diacetate was determined based on the bitter taste recognized by seven volunteers (three females and four males) in the age group of 21–28 years. Aqueous solutions of chlorhexidine diacetate with different concentrations (5, 10, 20, 30, 40 and 50 µg/ ml) were prepared. One milliliter of solution was placed on the center of the tongue of volunteer for 30 s. The solution was spat out after 30 s, and the mouth was thoroughly rinsed with distilled water. The same procedure was repeated for all solutions and volunteers. A gap of 30 min was maintained in between tasting two different solutions. The threshold value was selected on the basis of the lowest concentration that had a bitter taste. Scale for evaluation of bitterness threshold concentration of chlorhexidine diacetate was prepared and is shown in Table III.

Evaluation of Effectiveness of Taste Masking[10]

5 mg of chlorhexidine diacetate equivalent to dose of chlorhexidine diacetate in oral film was weighed and placed in two 25 ml beakers. 5 ml distilled water was then added and the beakers were allowed to stand for 60 and 120 seconds respectively. After specified time, the solutions were filtered and the resulting filtrates were analyzed for chlorhexidine diacetate content using UV-Vis spectrophotometer (Incarp SCIAN 2301) at 255 nm.

In vitro Microbiological Studies

In vitro microbiological studies were carried out to confirm antimicrobial activity of chlorhexidine diacetate from the oral films. For this purpose, each oral film of formula F8 and F9 was evaluated for its antimicrobial activity. Antimicrobial activity was evaluated against *Streptococcus mutans* MTCC No. 497, a common causative organism of dental plaque and caries[11,12]. The protocol for the antimicrobial studies was in accordance to the study reported by Jagtap and Karkera[13]. The effective zone of inhibition at the end of 24 hours was calculated as a difference between diameters of zone of inhibition of chlorhexidne diacetate loaded oral film and that of placebo film. The values of zone of inhibition observed with the oral films were evaluated by a non-

paired, two tailed Student's '*t*' test (GraphPad InStat Demo Version). Differences were considered statistically significant at $P < 0.05$.

Evaluation of Oral Films of Formulae F8 and F9 in Human Volunteers

The oral films of formula F8 and F9 were evaluated in healthy human volunteers with their consent ($n = 9$; 7males and 2 females) for mouth feel, mouth freshening and *in vivo* dissolution time in oral cavity. The protocol for the human studies was approved by Local Level Ethical Committee for Scientific Research of Saraswathi Vidya Bhavan's College of Pharmacy. Each healthy human volunteer was randomly given oral film of formula F8 or F9 (single blind design) with a potable water rinse at start. The volunteers were asked to place the oral film on the tongue. Volunteers were not restricted later on with respect to tongue movement. The oral films used in the study had dimensions of 2 × 2 sq.cm. The *in vivo* dissolution time observed for the oral films was evaluated by a non-paired, two tailed Student's '*t*' test (GraphPad InStat Demo Version). Differences were considered statistically significant at $P < 0.05$.

Stability Studies

Oral films of optimized formulation F9 were stored at two different storage conditions namely 25°C/60 % RH and 40°C/75% RH. Each oral film was wrapped in an Alu Alu pouch, which was heat-sealed at the end**.** The oral films were evaluated for appearance, weight, chlorhexidine diacetate content and *in vitro* drug release after storage for 90 days. The f_1 (difference factor) and f_2 (similarity factor) values for *in vitro* chlorhexidine diacetate release from the oral films were calculated[14].

RESULTS AND DISCUSSION

Screening of Components for Formulation of Placebo Fast Dissolving Oral Film

HPMC, a polymer with excellent film forming ability, was used as the film forming agent in oral films[15]. Initial studies indicated that amongst the grades of HPMC, HPMC E5 gave oral films with most desired properties at the concentration of 4% *w*/*v*. Amongst various plasticizers, propylene glycol showed best ability to improve film forming properties of HPMC E5 as compared to the other plasticizer like glycerine and polyethylene glycol. Desired and satisfactory taste masking was obtained by arginine hydrochloride in chlorhexidine diacetate oral films. Eugenol is one of the main components of clove oil and finds application as mouth freshener and to improve mouth feel. Therefore eugenol was used as a flavoring agent. Incorporation of eugenol in the oral films did not affect the properties such as film transparency and peelability.

Evaluation of chlorhexidine diacetate containing Fast Dissolving Oral Films

Determination of Weight and Thickness

The average weight and thickness values for oral films of formula F8 and F9 are shown in Table IV.

Folding endurance

Oral Films of formulae F8 and F9 did not show any cracks even after folding for more than 300 times, the values are shown in Table IV. The values were found to be optimum to reveal good film properties.

Surface pH

The films of formulae F8 and F9 were of neutral pH and thus can be concluded to be safe and non-irritating to the oral mucosa.

Determination of Chlorhexidine diacetate Content

Chlorhexidine diacetate content in F8 and F9 was found to be 98.22 ± 1.56 and 99.73 ± 0.8% respectively ($n = 7$). The oral films dissolved almost completely in about 20 to 25 min. The chlorhexidine diacetate release pattern of both the oral films (F8 and F9) was similar. The oral films due to hydration began to disintegrate and release the drug. After about 3 min, the oral film became thinner and around 45-50% of chlorhexidine diacetate was released. Although at the end of 20 min, matrix of F8 and F9 was completely disintegrated.

Evaluation of bitterness threshold concentration of Chlorhexidine diacetate

Threshold bitterness concentration of aqueous solution of chlorhexidine diacetate is 10 µg/ml and shown in Table V. All the volunteers could not recognize the bitter taste of chlorhexidine diacetate at the concentration of 5 µg/ ml and perceived the bitter taste at 10 µg/ ml. Thus, the threshold bitterness of chlorhexidine diacetate is 10 µg/ ml.

Evaluation of Effectiveness of Taste Masking

The bitterness threshold of chlorhexidine diacetate is 10 µg/ml while the concentration of chlorhexidine diacetate released in the study was 0.244 µg/ml and 0.248µg/ml after 60 and 120 seconds respectively which is insufficient to impart bitterness and did not give bitter taste of the drug.

In Vitro *Microbiological Studies*

The ability of chlorhexidine diacetate available from oral films to inhibit growth of *Streptococcus mutans* was evaluated by measuring zone of inhibition observed for formulae F8 and F9. The effective zone of inhibition due to oral film of F8 was 29 ± 0.5 mm and that of F9 was 36 ± 0.2 mm ($n = 5$). F9 demonstrated significantly larger zones of inhibition than that of F8 ($P < 0.05$). Considering this and earlier observations, F9 was selected for stability studies.

Evaluation of Oral Films of Formula F8 and F9 in Human Volunteers

Oral films of formulae F8 and F9 were evaluated in human volunteers. The results of *in vivo* studies are shown in Table VI. Oral films of formula F9 were better than that of F8 with respect to taste masking of chlorhexidine diacetate, mouth feel and mouth freshening properties. However, oral films of formulae F8 and F9 did not differ significantly with respect to *in vivo* dissolution time ($P > 0.05$). Human volunteers preferred oral films of formula F9 over F8.

Stability Studies

The oral films did not show any significant change in appearance, folding endurance and weight loss on storage and chlorhexidine diacetate content. The f_1 values for the oral films ranged from 2.8 to 11.35 and f_2 values ranged from 68.36 to 88.42, which indicated that the *in vitro*

chlorhexidine diacetate release profiles of oral films of formulation F9 was not affected after storage.

CONCLUSION

The taste masked fast dissolving oral films of chlorhexidine diacetate could be easily formulated with the available components such as HPMC E5 and propylene glycol. Chlorhexidine diacetate, a bitter antimicrobial and antiseptic drug could be successfully prepared as taste masked fast dissolving oral films by use of arginine hydrochloride with acceptable mouth feel due to use of eugenol in the oral films. *In vitro* and *in vivo* evaluation of the oral films confirmed the great potential as an innovative dosage form and better alternative to the conventional mouthwashes to improve delivery of chlorhexidine diacetate.

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