FORMULATION AND EVALUATION OF MUCOADHESIVE BUCCAL FILMS OF AMILORIDE HYDROCHLORIDE

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ABSTRACT

A mucoadhesive drug delivery system for systemic delivery of Amiloride, a potassium sparing diuretic and antihypertensive agent, through buccal route was formulated. Mucoadhesive polymers like hydroxypropyl methyl cellulose K-100, Chitosan, Carbopol, polyvinyl alcohol, and polyvinyl pyrrolidone were used for film fabrication. The films were evaluated for their weight, thickness, percentage moisture absorbed and lost, surface pH, folding endurance, drug content uniformity, *In vitro* residence time, *In vitro* release and *ex vivo* permeation. Based on the evaluation of these results, it was concluded that buccal films made of hydroxylpropylcellulose and Chitosan (A-1), which showed better controlled drug release (95% w/w at the end of 11 h) and satisfactory film characteristics could be selected as the best among the formulations studied.

Key words: Amiloride, Mucoadhesive, Buccal route

INTRODUCTION

Development of new drug delivery systems has been one of the major thrust areas of pharmaceutical research these days. The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired concentration. Film type dosage forms create a new dimension in the era of controlled drug delivery system [1]. Film type dosage forms can be used for transdermal therapy, ophthalmic therapy and for buccal or sublingual therapy [2,3]. Remarkable efforts have recently been focused on placing a drug or drug delivery system in a particular region of the body for extended periods of time [4]. This is required not only for the local targeting of the drugs but also for a better control of systemic drug delivery [5,6]. Buccal cavity has a wide variety of functions and it acts as an excellent

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site for the absorption of drugs [7]. The Buccal mucosa provides direct entry of drug molecules into systemic circulation, thus avoiding hepatic first pass effect. The ease of administration and ability to terminate drug delivery when required makes it a potentially attractive route of drug delivery. The effectiveness of a Mucoadhesive formulation is greatly determined by the different nature of the polymer composites used.[8] Mucoadhesive buccal devices, including tablets, films, patches, disks gels and ointments. Buccal films are highly flexible and thus much more readily tolerated by the patient than tablets. Films also ensure more accurate dosing of the drug compared to gel and ointment [9, 10].

Amiloride hydrochloride is a potassium sparing diuretic and antihypertensive agent that acts as Na+ channel blocker present at the luminal site. It is a BCS class III drug, i.e. high solubility and low permeability (log P value- 0.76). The drug is incompletely (15 to 20%) absorbed from gastrointestinal tract, hence there is need to develop a suitable formulation of AMHCL to improve its bioavailability [11].

The goal of present study is to formulate the buccal films of Amiloride by using polymers like Hydroxy Propyl methyl cellulose, Chitosan, Carbopol and Poly vinyl pyrrolidone to enhance the permeability and consequently bioavailability and do deliver the drug in a controlled manner.

MATERIALS AND METHODS

Amiloride was purchased from posh Pvt. Ltd. Hyderabad, Chitosan, HPMCK4M, CP 934, and PVP procured from Drugs India (Hyderabad, India); all materials received and used were of analytical grade.

Drug–excipient interaction study

The pure drug, Amiloride and a mixture of it with the polymers, HPMC, Chitosan, PVP and CP were mixed separately with IR grade KBr in the ratio of 100:1 and corresponding pellets were prepared by applying pressure in a hydraulic press[12] using Thermo Nicolet USA, FTIR instrument.

Casting of Films

The buccal mucoadhesive films were prepared by the method of solvent casting technique employing 'O' shape ring placed on a glass surface as substrate by using different polymers like Hydroxy Propyl Methyl Cellulose (HPMC), Chitosan, Carbopol (CP) and Poly vinyl pyrrolidone (PVP). The calculated quantities of polymers were dispersed in ethanol (70%). The polymeric solutions are levigation with 30% w/w propylene glycol which served the purpose of plasticizer as well as penetration enhancer. The solution was mixed occasionally to get semisolid consistency. Then the solution was subjected to sonication in a bath sonicator to remove the air bubbles [13]. Then these were casted on glass surfaces and to prevent the evaporation of alcohol, medicated gels were filled into the vials and closed tightly by the rubber closures. The dried films were separated and the backing membrane used was aluminium foil. Then the formulations were stored in desiccators until further use [14].

Thickness and weight variation

The thickness of the film at three different points was determined using thickness gauge and the films were then weighed individually using digital balance to determine the weight of each film taken out from the casted film. The films were subjected to weight variation by individually weighing ten randomly selected films. Such determinations were carried out for each formulation [15].

Folding Endurance

The folding endurance was determined manually for the prepared films by repeatedly folding the film at the same place until it broke. The number of times the film could be folded at the same place without breaking or cracking gave the value of folding endurance [16].

Surface pH

To determine surface pH, three films of each formulation were allowed to swell for two hours on the surface of an agar plate. Surface pH was measured by using pH paper placed on the surface of the swollen film as per reported method. A mean of three readings was recorded [17].

Swelling index

The swelling index was measured using the diameter method. The agar solution was prepared by dissolving 0.2 g of agar in 10 mL of warmed simulated saliva fluid, pH 6.8 (50-70 ºC). This solution was then poured into a Petri dish and allowed to cool. After determining the initial film diameter, each film was allowed to swell on its respective surface of gel. The diameters of the film were determined after 2, 5 and 7 hours, and results were recorded as the mean value of three readings. Swelling studies were performed for 7 hours because a residence time of 7 hours was recommended [17]. The observations were recorded for three optimized formulations:

Swelling index = $\{(D_f - D_i) / D_i\}$ x 100,

Where D_i = initial film diameter and D_f = final film diameter.

Measurement of Buccoadhesive Strength

A modified balance method was used for determining the ex-vivo buccoadhesive strength. Fresh sheep buccal mucosa was obtained from a local slaughter house and used within 2 h of slaughter. The mucosal membrane was separated by removing the underlying fat and loose tissues. The membrane was washed with distilled water and then with isotonic phosphate buffer (IPB) pH 6.8 as moistening fluid. Sheep Buccal mucosa was fixed on the plane surface of glass slide attached (with adhesive tape) to bottom of smaller beaker, kept inverted in 500 ml beaker attached to the bigger beaker. Isotonic phosphate buffer pH 6.8 was added to the beaker up to the upper surface

inverted beaker with buccal mucosa. The buccal film was stuck to the lower side of the upper clamp with cyanoacrylate adhesive. The exposed film surface was moistened with IPB and left for 30 s for initial hydration and swelling. Then the platform was slowly raised until the film surface came in contact with mucosa. Two sides of the balance were made equal before study by keeping a weight on the right hand pan. A weight of 5 g was removed from the right hand pan, which lowered the pan along with the film over the mucosa. The balance was kept in this position for 5 minutes contact time. Then weights were slowly added to the right hand pan until the film detached from the mucosal surface. This detachment force gave the buccoadhesive strength of the buccal film in grams [17].

The following parameters were calculated from the bioadhesive strength.

Force of adhesion (N) =
(Bioadhesive strength (g) ×9.8)/1000

Bond strength (N m–2) = Force of adhesion / surface area.

Drug content

The drug content of films was measured without the backing membrane. Hydrochloric acid (0.008 mL) was taken in a beaker and volume was brought to 10 mL with distilled water. The film was dispersed in 5 mL of the solution above and volume was brought to 10 mL with pH 6.8 phosphate buffer. An analysis was conducted for 1 mL of this solution, in order to assess drug content by UV spectroscopy (UV1700, Shimadzu, Japan) at 362nm [17].

Percentage moisture absorption

The percent moisture absorption (PMA) test was carried out to check the physical stability of the buccal films at high humid conditions. In the present study, the moisture absorption capacity of the films was determined as follows. Three 1cm diameter films were cut out and weighed accurately then the films were placed in a desiccator containing saturated solution of aluminum chloride, keeping the humidity inside the desiccator at 79.5%. After 3 days, the films were removed, weighed and percentage moisture absorption was calculated [18].

Percentage moisture absorption =
$$\frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Stability study in human saliva

The stability study of buccal films was performed in natural human saliva. Samples of human saliva were collected from 10 humans (age 18–40 years) and filtered. The films were placed in separate Petri dishes containing 5 ml of human saliva and kept in a temperature controlled oven at 37±0.2°C for 6 hours. At regular time intervals, the films were examined for changes in color, shape, collapse and physical stability [18].

In vitro release study

The USP XXIV six station dissolution apparatus type I, was used throughout the study. One film of each formulation was fixed to the central shaft at just above the basket, using a cyanoacrylate adhesive. The dissolution medium consisted of 900 ml pH 6.6 phosphate buffer (PB). The release study was performed at 37 ± 0.5°C with a rotation speed of 50 rpm. The release study was carried out for 8 h. After every hour, 1 ml sample was withdrawn from each station and the same volume was replaced (with the dissolution medium) back to the stations. Each withdrawn sample was filtered, diluted suitably and then analyzed spectrophotometrically at 362 nm. The data presented were the mean of three determinations [19].

In vitro permeation study

The in vitro buccal permeation study of Amiloride buccal patches through the pig buccal mucosa was performed using Franz diffusion cell at 37 ± 0.2^{0}C. Sheep buccal mucosa was obtained from a local slaughterhouse and used within 2 hours of slaughter. Freshly obtained sheep buccal mucosa was mounted between the donor and receptor compartments. The patch was placed on the mucosa so the smooth surface of the mucosa placed towards receptor compartment and the compartments were clamped together. The donor compartment was wetted phosphate buffer (pH 6.8). The receptor compartment was filled with isotonic phosphate buffer (pH 6.8) stirred with a magnetic bead at 50 rpm.

1 mL sample was withdrawn at predetermined intervals and replaced with fresh buffer solution and assayed by UV spectrophotometer (Shimadzu 1800, Japan) at 362 nm [19].

In vivo drug release study

Six male New Zealand white rabbits (2–2.6 kg) were selected for the in vivo study. The dose of Amiloride was adjusted based on the rabbit weight and the optimized formulations were cut and placed in the buccal membrane with the help of a clip. Dextrose solution was transfused continuously throughout the period of study. Periodically, 1 ml of blood sample was taken by syringe containing 1 ml of heparin solution to prevent blood clotting. These blood samples were centrifuged at 2500 rpm for about 30 minutes. One milliliter of the supernatant was taken, and after suitable dilution, analyzed at 362 nm spectrophotometrically by the method described under in vitro analysis [20].

RESULTS AND DISCUSSION

Buccal films were prepared using a drug Amiloride by using polymers like HPMC, Chitosan, Carbopol and Poly vinyl pyrrolidone. The thickness and weight, surface pH, drug content, folding endurance, percent moisture absorption, stability studies, Bioadhesion strength, in vitro drug release, Invitro drug permeation and in vivo drug release for different formulations were evaluated.

The compatibility studies between the drug and excipients were studied by FTIR spectroscopy. The FTIR spectra of Amiloride, HPMC, Carbopol, Chitosan and PVP and the combination of drug and polymers showed no significant interaction between drug and polymer. The results indicate that there was no chemical incompatibility between drug and excipients used in the formulation.

Thickness was found to vary from 0.29 to 0.48 mm. The weight of patches was found to vary from 165.18±0.91 to 176.37±0.80 g. The folding endurance was found to be greater than 300 times in case of all the formulations. This makes the system acceptable for movement of mouth, indicating good strength and elasticity. Folding endurance test results indicated that the films would maintain the integrity with buccal mucosa when applied. Surface pH was found to be in the range of 6.6 to 6.79 for all the formulations. Surface pH for all formulations was well within range of salivary pH and would not cause irritation in the mouth (Table 2).

The swelling indices (Tables 2) polymer was reported to be crucial for its bioadhesive character. The adhesion occurs shortly after swelling but the bond formed is not very strong. The adhesion increases with the degree of hydration till the point of distanglement at the polymer tissue surface, which leads to abrupt drop in adhesive strength due to over hydration. The formulation A1 (0.5 Chitosan, 1.5 HPMC, 0.5 PVP) shows higher value of Swelling percentage 140.9±0.9 which is due to presence of higher concentration of carbopol.

Checking the physical stability of the film at high humid conditions and integrity of the film at dry conditions, the films were evaluated for PMA (Table 2). The percentage Moisture uptake in the formulation A1 has shown the highest value of moisture absorption 13.02±0.23. This may be due to the presence of higher concentrations of HPMC along with Chitosan. The observed results of content uniformity (Table 2) indicated that the drug was uniformly dispersed and with minimum intra batch variability. Recovery was possible to the tune of 18.1 ± 0.26 to 19.76 ± 0.15.

The stability study of the optimized patches (A1) was done in natural human saliva. The films did not exhibit any significant changes in their color, shape and satisfactory physical stability. The buccoadhesive properties (Table 3) of the fabricated films containing Carbopol gives the highest bioadhesive force. The bioadhesive strength exhibited by Amiloride buccal films was satisfactory for maintaining them in oral cavity. The combination of HPMC and Chitosan shows good adhesion. Upon addition of PVP the bioadhesive strength increases which may be due to hydrogen bond formation and vanderwaal forces.

In-vitro Drug Release studies were carried out in phosphate buffer (pH 6.8) for 8 hours. In order to find out the order of release and the mechanism, which were predominately influences, the drug release from the membrane. The formulation A1 (1.5% HPMC, 0.5% Chitosan, 0.5% PVP) has shown the drug release of 98.6% at 7th hour. The in-vitro drug release plot has shown that the drug release

followed zero order kinetics, which was evinced from the regression value of the above mentioned plot. The *in-vitro* release plots of all other formulations were suggestive of zero order release and are diffusion mediated which was evinced form the regression value Higuchi's plot. All the formulations undergo non-fickian type of release which is confirmed form the slope values obtained from the Peppa's plot.

The oral mucosa represents a barrier to drug permeation and it is intermediate between skin epidermis and the gut in its permeability characteristics. The effectiveness of the buccal barrier and whether buccal absorption could provide means for Amiloride administration can be determined by ex vivo permeation studies. Permeation studies were carried out on optimized formulation.

In vivo buccal diffusion studies that were conducted for the optimized formulation in rabbits showed zero order release pattern. The in vivo studies of buccal films of Amiloride in rabbits did not show any inflammation or any other sensitization reactions at the administration site. In vitro and in vivo correlations were carried out for the therapeutic efficacy of a pharmaceutical formulation and are governed by the factors related to both in vitro and in vivo characteristics of the drug. A graph was plotted by taking cumulative % in vitro release and cumulative % in vivo drug release for the same period of time and the release rate followed zero order, showing the correlation co efficient value to be 0.995, as shown in Figure 4.

CONCLUSION

The Amiloride buccal films were prepared by solvent casting technique using ethanol (70 % v/v) as a solvent, employing 'O' shape ring placed on a glass surface as substrate and by using different polymers like Hydroxy Propyl Methyl Cellulose - 15 cps (HPMC), Chitosan, Carbopol(CP) and Poly vinyl pyrrolidone (PVP). The polymeric solutions are levigated with 30 % w/w propylene glycol which served the purpose of plasticizer as well as penetration enhancer. The prepared Amiloride buccal films were characterized based upon their physico-chemical characteristics like surface pH, PMA, swelling percentage, thickness, weight, folding endurance and drug content. The ex-vivo buccoadhesive strength, in vitro permeation studies, in-vitro release studies and in-vivo release studies in rabbits were performed. The satisfactory results were obtained in all prepared formulation and based on the results A1 (1.5% HPMC, 0.5% Chitosan, 0.5% PVP) was the best one when compared to other. Good correlation was observed between in-vitro and in- vivo profile, revealed the ability of the formulation to reproduce the in-vitro release pattern through the biological membrane. This formulation was found to be suitable candidates for the development of controlled drug delivery for therapeutic use of Amiloride.

Table 1: Composition of Buccal films of Amiloride

Formulation code	Polymers (%)				Solvents (ml)		
	HPMC	Chitosan	CP	PVP	Ethanol (70% v/v)	Distilled water	PG (30% w/w)
A1	1.5	0.5	-	0.5	5.5	4.0	0.6
A2	1.5	-	0.5	0.5	5.5	4.0	0.6
A3	1.5	0.3	-	0.5	5.5	4.0	0.6
A4	-	0.2	0.5	0.5	5.5	4.0	0.6
A5	-	1.0	1.5	0.5	5.5	4.0	0.6
A6	1.0	1.0	1.0	0.5	5.5	4.0	0.6

Table 2: Physico-chemical evaluation of buccal films

Formulation Code	Thickness (mm)	Weight (mg)	Folding endurance	Surface pH	% SW	Drug content (mg)	PMA
A1	0.48 ± 0.02	172.23±0.91	320 ± 5.0	6.73±0.05	140.9±0.9	19.9±0.05	13.02±0.23
A2	0.31 ± 0.01	169.18±0.91	300 ±3.0	6.79±0.005	99.6±0.69	17.9±0.20	9.24±0.09
A3	0.47 ± 0.01	170.53±0.80	315 ± 1.0	6.71±0.015	118.4±0.72	18.1±0.26	7.32±0.11
A4	0.39 ± 0.01	176.31±0.58	298 ± 6.0	6.64±0.050	124.2±0.99	19.76±0.15	10.13±0.09
A5	0.29 ± 0.02	171.37±0.80	281 ± 4.0	6.6±0.015	132.4±0.6	17.76±0.15	11.21±0.06
A6	0.41 ± 0.01	165.12±1.00	318 ± 5.0	6.69±0.03	138±0.85	19.43±0.20	7.86±0.27

*Each value in the table is the mean ± SD of three estimations

Table 3: Buccoadhesive strength of Amiloride buccal films

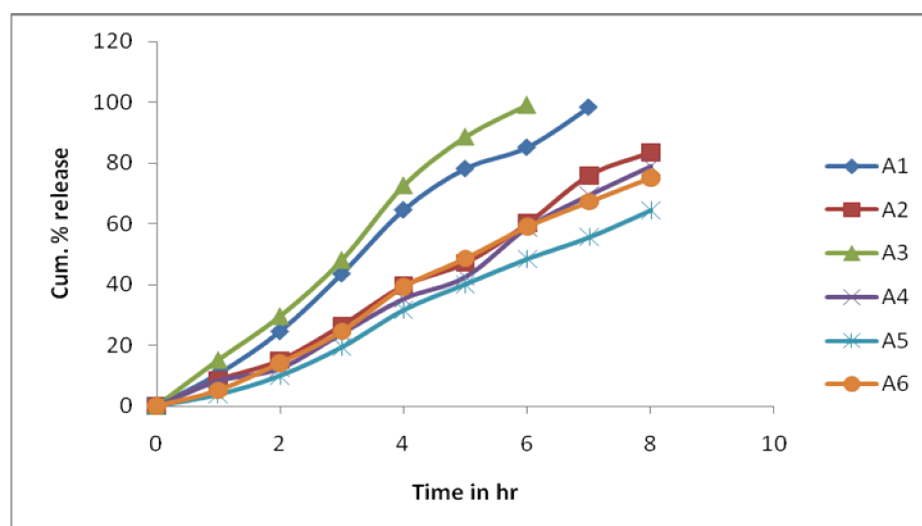| Formulation code	Buccoadhesive strength in g	Mechanical strength in kg/mm^2
A1	36.4	14.23±0.045
A2	33.5	08.89±0.140
A3	35.6	09.78±0.040
A4	30.5	10.64±0.020
A5	24.8	07.76±0.030
A6	32.5	11.25±0.050

Fig.1: Cumulative % release profile for Amiloride hydrochloride buccal Formulations
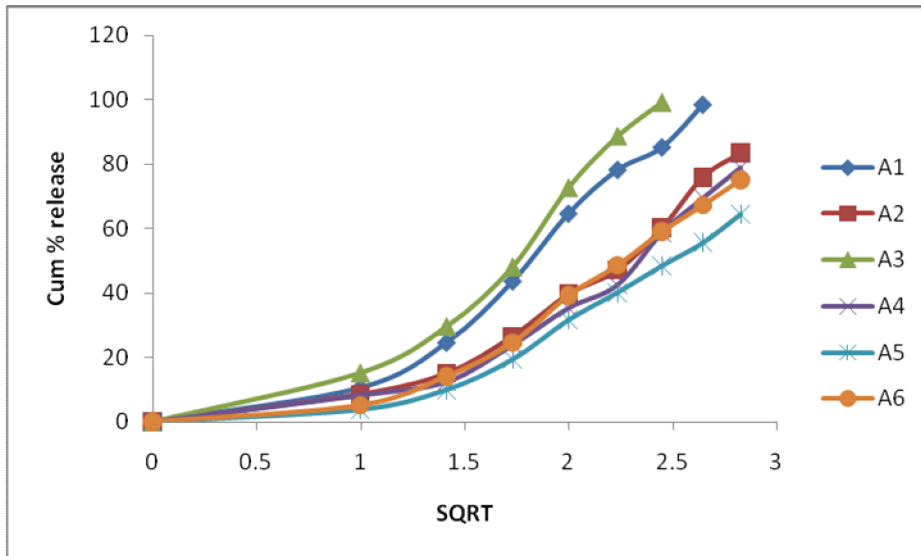
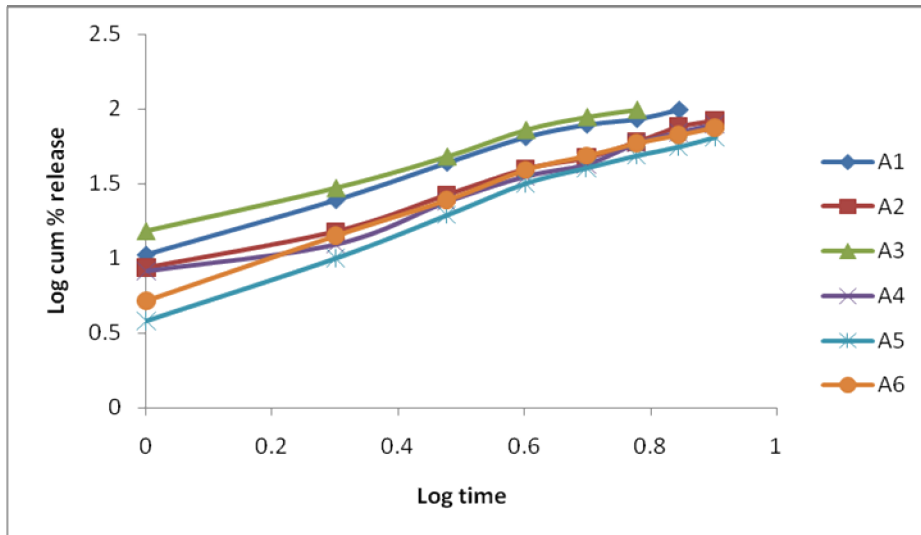**Fig. 2:** Higuchi's plot for Amiloride hydrochloride buccal Formulations

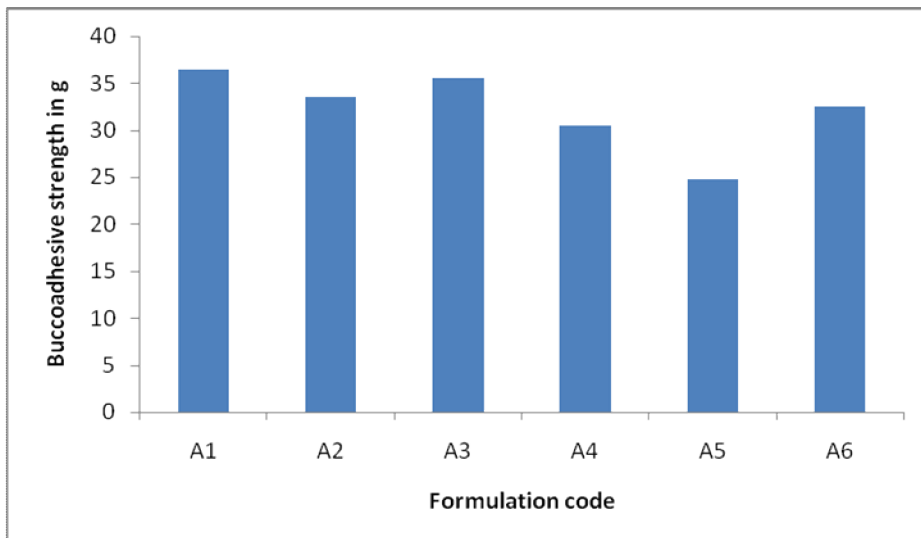**Fig. 3:** Peppa's plot for Amiloride hydrochloride buccal Formulations

Fig. 4: Buccoadhesive strength of Formulations

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