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Formulation and evaluation of amoxicillin trihydrate oral lozenges for treating upper respiratory tract infections

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Abstract

In the present investigation an attempt has been made to develop Amoxicillin Trihydrate oral lozenges in treatment of upper respiratory tract infection. The lozenges were formulated by soft lozenges method employing Amoxicillin Trihydrate alone and in combination with natural antiseptic ingredients. The lozenges were prepared employing PEG 4000 as matrix base, Stevia (natural sweetener), Acacia (polymer), MCC (disintegrate) other excipients. The prepared medicated lozenges were characterized for Weight uniformity, hardness, Drug content, and dissolution by standard pharmacopeia methods. The results of the evaluation tests obtained were within the limits. Formulations were tested for drug Excipient interactions by FTIR spectral analysis. The results revealed that there were no major interactions between the drug and polymers used for the preparation of lozenges. Antimicrobial activity studies were performed for different lozenges formulations. AMF2 formulation showed greater Zone of inhibition. This may be due to synergistic antimicrobial effect of Amoxicillin Trihydrate, Tulsi and Ginger. Accelerated stability studies were conducted as per ICH guidelines and found that there wasn't any substantial change in the prepared formulations

Key words: Amoxicillin Trihydrate, Ginger, Tulsi, PEG 4000, MCC, Stevia.

Introduction

Oral dosage forms have advantages over other dosage forms. They are economical and safe to the patient. They are appropriate for any patient, whatever the age is. Oral dosage forms have disadvantages as well. They are not the first choice of drugs if the patient suffers chronic vomiting. They are not good choice in case of uncooperative patients as children and infants. They are not suitable in emergency and for unconscious patients (1). In the 19th century, physicians discovered morphine and heroin, which suppress coughing at its source—the brain. Popular formulations of that era included Smith Brothers Cough Drops, first advertised in 1852, and Luden's, created in 1879. Concern over the risk of opioid dependence led to the development of alternative medications (2). Lozenges historically have been used for the relief of minor sore throat pain and irritation and have been used extensively to deliver topical anesthetics and antibacterials. Today they are used for delivering the drugs for analgesics, anesthetics, antimicrobials, antiseptics, antitussives, aromatics, astringents, corticosteroids, decongestants, and demulcents and other classes and combinations of drugs. Both chewing gum and lozenges may be considered as alternatives to current dosage forms. They are easy to handle, the dose has been apportioned, and the excipients have a demulcent effect on a sore throat since the ingredients are released slowly and spread uniformly over the affected mucosal membrane (3). Lozenges are placed in oral cavity. Since the sublingual lozenges may be impractical due to their size, buccal lozenges are formulated and have been extensively used and are intended to

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be placed between the cheek and the gums. Though the lozenge dissolution time is about 30 minutes, it also depends on the patient, as patient controls the rate of dissolution and absorption by sucking on lozenge until it dissolves. Depending on the type of lozenge, they may be prepared by molding or by compression (4). Amoxicillin is a broad-spectrum, pharmacologically active beta-lactam antibiotic effective against Gram-positive and Gram-negative bacteria. It is a widely used antibiotic in human and veterinary medicine for the treatment and prevention of respiratory, gastrointestinal, urinary and skin infections due to its pharmacological and pharmacokinetic properties. Based on the above physicochemical and biopharmaceutical properties, amoxicillin trihydrate was selected as a drug candidate (5). An herb is a plant or part of a plant valued for its medicinal, aromatic or savourly qualities. Nature produces several food items for every season. Their use in that particular season proves to be highly beneficial for the mankind which is packed with enormous medicinal advantages. Herbal drugs play a major role in systems of health in India; almost 70% of modern medicines in India is derived from natural products. In last few years there is an increment occur in the use of herbal medicines. The herbs used in herbal candy are selected on the basis of their role in the treatment of altitude health problems with lesser side effects, also the selection based on their availability and their preferences. The herbal products are much better than the allopathic medicines. Herbal products have lesser side effects and more therapeutic effects (6). Of all the herbs used within Ayurveda, tulsi (*Ocimum sanctum* Linn) is preeminent, and scientific research is now confirming its beneficial effects. There is mounting evidence that tulsi can address physical, chemical, metabolic and psychological stress through a unique combination of pharmacological actions. Tulsi has been found to protect organs and tissues against chemical stress from industrial pollutants and heavy metals, and physical stress from prolonged physical exertion, ischemia, physical restraint and exposure to cold and excessive noise. Tulsi's broad-spectrum antimicrobial activity, which includes activity against a range of human and animal pathogens, suggests it can be used as a hand sanitizer, mouthwash and water purifier as well as in animal rearing, wound healing, the preservation of food stuffs and herbal raw materials and traveler's health (7). On the other hand, ginger (Zingiber officinale) which is a member of the Zingiberaceae (ginger) family, occurs in horizontal, laterally flattened irregularly branching piece; 3-16cm long, 2-4cm wide, up to 3cm thick, sometimes split longitudinally, pale yellowish buff or light brown externally striated, somewhat fibrous, branches known as fingers arise obliquely form the rhizome, are flattish, obviate, short, about 1-3cm long, fracture, short and starchy with projecting fibers (8) .

Materials and Methods

Amoxicillin Trihydrate (AMT)was obtained as gift sample from Apotex pharma Ltd, Bangalore; Tulsi and Ginger Powder were obtained from Patanjali Super Market, Guntur, Andhra Pradesh; Poly ethylene Glycol 4000, Hydrochloric acid, Acacia and MCC were obtained from S.D Fine Chem, Ltd, Mumbai; Silica gel and Citric acid monohydrate were obtained from High-Pure fine Chem, Chennai; Stevia Natural Sweetener was obtained from Procarvit Food Products (India) Private Ltd, Coimbatore.

Estimation of amoxicillin trihydrate: Standard solution of pure drug containing 100mg of amoxicillin trihydrate /100ml was prepared using 0.1N HCl. The working standards were obtained by dilution of the stock solution in corresponding 0.1N HCl. The standard curve for amoxicillin trihydrate was prepared in concentration range of 5-25ìg/ml at the selected wave length of 227nm. Their absorptivity values were to determine the linearity. Solutions were scanned and beer lamberts law limit was obeyed in concentration range of 0, 5, 10, 15, 20, 25ìg/ml using 0.1N HCl as blank.

Solubility Studies (9): Saturated solubility studies of AMT were performed in different

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dissolution media. 100mg of AMT was weighed and transferred into different conical flasks containing 10ml of different dissolution media i.e., Water, 0.1N HCl, 6.8pH, 7.2pH Phosphate buffer and were closed appropriately. All the conical flasks were placed in a REMI incubator shaker at 50 rpm, 37º C ± 1º C for 24 hrs. The conical flasks were removed from the incubator shaker and samples were filtered using whattman filter paper. The clear solution obtained by filtration and was suitably diluted with appropriate dissolution media and the absorbance values were noted at 227 nm by using corresponding dissolution media as blank solutions. The absorbance values were noted. The solubility of amoxicillin in different media like 0.1N HCl (1.2 pH), pH 6.8 phosphate buffer, pH 4.6 acetate buffer and in distilled water (Table 1).

Preparation of soft lozenges: The quantity of each ingredient needed for compounding the preparation was calculated for 20 lozenges and the required material for two extra lozenges were calculated and weighed. Soft lozenges were prepared by melting and mold technique (2). The PEG (Grade of 4000) was placed into a small beaker (50 ml) and heated without stirring. The remaining powders were mixed in the geometric dilution technique by using mortar and pestle. The powder mixture was passed through a 40 mesh sieve onto a glassine sheet. Once the PEG was melted, the heat was reduced and a stir bar was added with lowest spin rate. The powders were sprinkled onto the melted PEG ensuring each addition is wetted before adding additional powder. Once the powders were added to the PEG, the beaker was removed from the hotplate and colour, flavor were added and allowed to cool until it is "just cool to the back of the hand." The lozenge mold(s) were placed on an electronic balance and the weight of the mold(s) was tarred out. The lozenge material was poured into each mold cavity to the calculated desired weight per lozenge using the digital balance (Table 2 and Fig. 1).

Evaluation of Lozenges: The prepared formulations were evaluated for drug content uniformity, hardness, thickness and diameter, weight variation, friability and *in- vitro dissolution* by pharmaceutical standard methods (Table 3).

In vitro Dissolution Studies for Formulated Lozenges: Dissolution studies were performed on lozenge formulations in a calibrated 8 station dissolution test apparatus (LABINDIA DS8000) equipped with paddles (USP apparatus II method) employing 900 ml of 0.1 N HCl as dissolution medium. The paddles were operated at 50 rpm and temperature was maintained at 37º C ± 1º C throughout the experiment. The samples were withdrawn at 5, 10, 15, 20, 30 and 45 minutes and replaced with equal volume of same dissolution medium to maintain the constant volume throughout the experiment. Samples were withdrawn at various time intervals and suitably diluted with same dissolution medium and the amount of the drug dissolved was estimated by ELICO double beam U.V spectrophotometer at 227 nm. The dissolution studies on each

Figure 1: Formulated Lozenges

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formulation were conducted in triplicate. From the dissolution profiles various parameters like $T_{50,}$ and $DE_{30\%}$ were calculated (Table 4 and Fig 2).

Antimicrobial activity: Antimicrobial activity was tested by Cup Plate Method depends on the diffusion of an antibiotic from a vertical cavity, through the solidified agar layer in a Petri plate. The microbial growth inhibited by the compound will despite as circular zone around the cavity. The nutrient agar is melted, cooled suitably, poured into Petri dish. Spread 0.2 ml of known concentration of inoculums on the surface of the solidified agar. Cavities are made by using a sterile borer. The lozenges formulation was dissolved in 6.8 pH phosphate buffer solution and poured into the cups of agar plate and then incubated for 18 hrs and the zone of inhibition (Fig. 3).

Fourier Transform Infrared Spectroscopy: Infrared spectra of drug and optimized lozenge formulations were recorded by KBr pellet method using Fourier Transform Infrared Spectrophotometer. Pure drug and optimized lozenge formulations were subjected for FTIR analysis using FTIR spectrophotometer to study any interactions between drug and excipients and spectra's (Fig. 4).

Accelerated stability studies: The formulations which showed good *in-vitro* performance were subjected to accelerated stability studies. These studies were carried out by investigating the effect of temperature on the physical properties of lozenges and chemical stability of lozenges containing drugs. The lozenge formulations such as AMF_3 and AMF_4 were subjected to accelerated stability studies. The above said formulations were kept in petridishes after preparation and stored in thermostatic oven at a temperature and relative humidity of 25 ± 2^0C, 60 ± 5% RH for 6 months

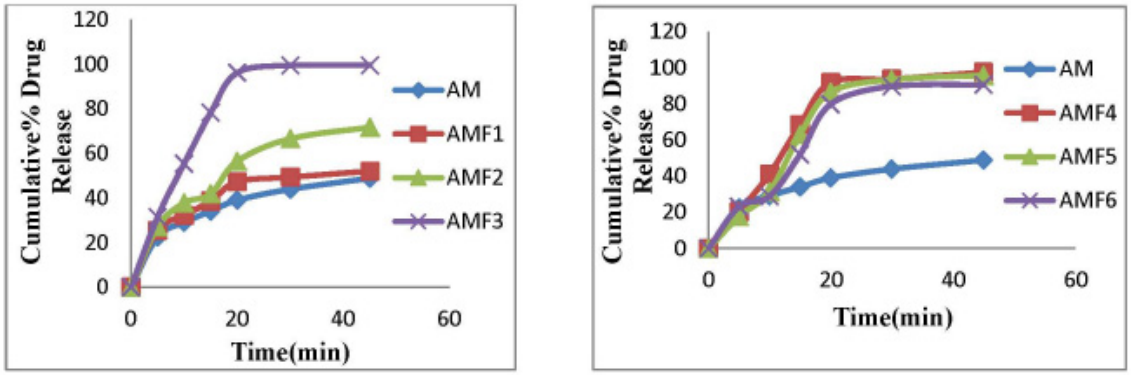

Figure 2: Drug Release Profiles of AMT Lozenges

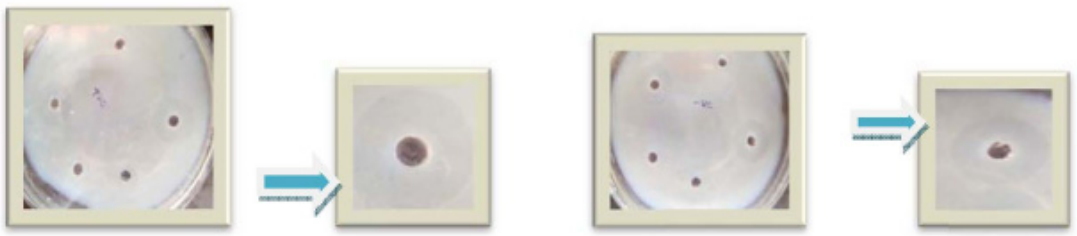

Figure 3: Zone of Inhibitions observed for Anti Microbial Activity.

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and 40 ± 2^{0}C, 75 ± 5% RH for 3 months. Then the samples of each type of formulations were evaluated for the earlier mentioned physical parameters. Further these lozenges were subjected to drug release studies as stated earlier (Fig. 5). The formulations subjected to Accelerated Stability studies were also characterized by FTIR the results revealed that there was no major interaction between the drug and excipients.

Results and discussion

The calibration curve for the estimation of Amoxicillin Trihydrate in 0.1 N HCl was found to be linear and obeyed Beer's law in the concentration range of 5 - 25 µg/ml. Saturated solubility studies were conducted for Amoxicillin Trihydrate using different dissolution media. Amoxicillin Trihydrate showed maximum solubility in 0.1 N HCl medium. Preformultion studies were

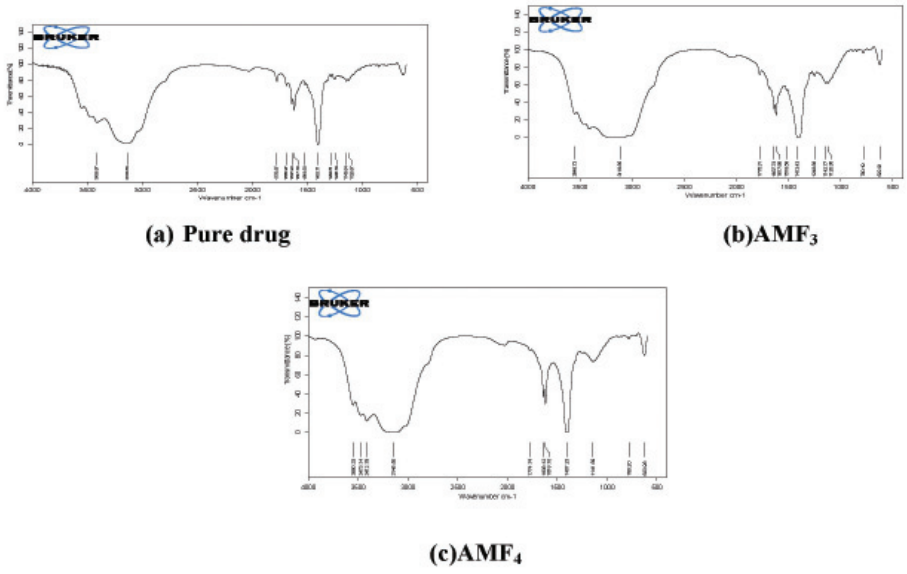

Figure 4: FTIR Spectroscopy (a) pure drug (b) AMF_3 (c) AMF_4

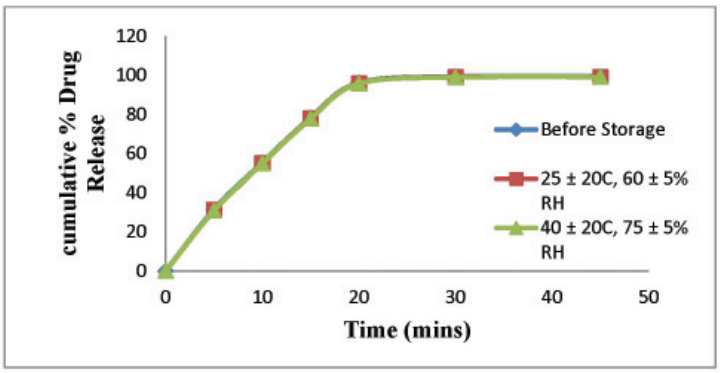

Figure 5: Drug Release Profiles of AMT Lozenge Formulation (AMF_3) Before and After Storage at Different Conditions

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performed on the drug and excipients used in the formulations and were found to be compatible. No drug and excipient reactions were observed.

The drug content of prepared Lozenges was found to be in the range of 246.8 – 249.8±0.3/mg. The melt and mold technique was found to be suitable for molding the soft lozenge formulations. All the batches of lozenges were compressed under identical conditions to minimize processing variables. The soft lozenges were prepared by using PEG 4000 in different concentrations i.e., 80 and 85%. All the prepared lozenge formulations were further evaluated for physical parameters. All the lozenge formulations were found to be stable and meeting I.P specified limits for weight uniformity and drug content. The hardness of all the lozenge formulations were in the range of 1-2 kg/cm^2.Weight uniformity of all the lozenge formulations were in the range of 125.14-142.25 ± 3mg. Drug content estimated for all the lozenge formulations were highly uniform with less than 2.5% variation. The *in-vitro* dissolution studies for all the lozenge formulations were found to release the drug at a faster rate than compared to pure drug. It was found that the lozenge formulations AMF_3 and $AMF_4$with 2.5 % and 2.6 % of silica gel and acacia as suspending agent showed the slow drug release when compared to other formulations. The rate of drug release of lozenge

Table 1: Saturated Solubility Studies of Amoxicillin Trihydrate in Different Dissolution Media

S.No	Solvent	Amount solublein mg/ml
1	Distilled Water	2.46
2	0.1N HCl (1.2 pH)	7.89
3	6.8 pH Phosphate Buffer	4.46
4	7.2 pH Phosphate Buffer	2.68

Table 2: Composition of lozenges formulations

S.No	Ingredients	Lozenges formulations(in mg)						
		AM	AMF_1	AMF_2	AMF_3	AMF_4	AMF_5	AMF_6
1.	Amoxicillin	250	250	250	250	250	250	250
2.	Ginger	—	—	30	30	30	—	60
3.	Tulsi	—	—	30	30	30	60	—
4.	PEG 4000	—	1000	1100	1000	900	900	900
5.	MCC	—	—	—	100	200	200	200
6.	Stevia	—	15	15	15	15	15	15
7.	Silica gel	—	5	5	5	5	5	5
8.	Citric acid	—	5	5	5	5	5	5
9.	Acacia	—	10	10	10	10	10	10
10.	Total weight(in mg)	250	1285	1445	1445	1445	1445	1445

*[one lozege containing 250mg of amoxicillin trihydrate]

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Table 3: Physical Parameters of Amoxicillin Trihydrate Lozenges

S.No	Lozenge formulation	Weight uniformity (g/loz)	Weight of Individual lozenge	Hardness (kg/cm^2)	Drug content (mg)
1	AMF_1	1.25 ± 0.3	1.28	1.1±0.3	247 ± 0.3
2	AMF_2	1.42 ± 0.1	1.44	1.2±0.3	247 ± 0.3
3	AMF_3	1.42 ± 0.3	1.44	1.1±0.1	248± 0.2
4	AMF_4	1.42 ± 0.1	1.44	1.1±0.2	247± 0.1
5	AMF_5	1.42 ± 0.2	1.44	1.3±0.1	248± 0.2
6	AMF_6	1.42 ± 0.2	1.44	1.1±0.2	248± 0.3

Table 4: Dissolution Parameters of AMT Lozenges

S.No	Lozenge Formulations	T_{50}	DE 30 %	First order		Hixoncrowell	
				K (min^{-1})	R^2	K($mg^{1/3}$/min)	R^2
1.	AMF_1	30	35	0.0257	0.937	0.0124	0.856
2.	AMF_2	17.5	40	0.0278	0.879	0.0395	0.919
3.	AMF_3	9	70	0.0310	0.957	0.1185	0.820
4.	AMF_4	12	63.3	0.0898	0.921	0.0992	0.910
5.	AMF_5	19	56.6	0.0794	0.929	0.0992	0.859
6.	AMF_6	14	56.6	0.0776	0.939	0.0976	0.875

formulations was found to be linear with first order rate constant. The R^2 values of all lozenge formulations were in the range of 0.87 to 0.93. The rate of drug release of lozenge formulations was found to be linear with Hixon Crowell rate constant. The R^2 values of all lozenge formulations were in the range of 0.812 to 0.951. FTIR studies were performed for pure drug, polymers and optimized lozenge formulations. In FTIR studies, the groups in pure amoxicillin trihydrate and optimized formulations were having similar fundamental peaks and pattern. This indicates that there were no drug-excipient interactions in the formulations. Taste was masked effectively for the formulation AMF_3&AM F_4 prepared by melt and mold technique. Antimicrobial activity studies were performed for different lozenges formulations. AMF2 formulation showed greater Zone of inhibition. This may be due to synergistic antimicrobial effect of Amoxicillin Trihydrate, Tulsi and Ginger. Accelerated stability studies were carried out for some selected lozenge formulations. There was no significant change observed in physical parameters such as weight uniformity, friability, hardness, and drug content. Drug release from the lozenges after storage at

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different conditions remained unaltered and found to be quite stable. The formulations subjected to Accelerated Stability studies were also characterized by FTIR, the results revealed that there was no major interaction between the drug and polymers.

Future scope of work

Formulation AMF_3 and AMF_4 was found to release the drug at slower rate and is suitable for preparing as lozenges. Further studies can be focused on the Amoxicillin Trihydrate by using newer natural ingredients, polymers and matrices and even in the combinations of these. Investigation can be extended by employing newer techniques for the preparation of lozenges. *In-vivo* pharmacokinetic and dynamic studies can be performed on a suitable animal model.

Conclusion

The present study showed that it is possible to formulate the amoxicillin trihydrate as lozenges. Of all the soft lozenge formulations, the formulations AMF3and AMF4 containing 1000 and 900 mg of PEG 4000 and 100 and 200mg of MCC showed the slow release of the drug i.e. up to 45 minutes. The formulations containing Stevia as sweetener are also stable after storage. Hence the natural sweeteners such as Stevia can be considered as an alternative replacement for the artificial sweeteners in the preparation of lozenges. AMF2 formulation showed greater Zone of inhibition. This may be due to synergistic antimicrobial effect of Amoxicillin Trihydrate, Tulsi and Ginger. Accelerated stability studies were carried out for some selected lozenge formulations. There was no significant change observed in physical parameters such as weight uniformity, friability, hardness, and drug content. Drug release from the lozenges after storage at different conditions remained unaltered and found to be quite stable.

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