

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## Insight into the Release kinetics of Amoxicillin trihydrate from Buccoadhesive tablets with a Natural gum .

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### ABSTRACT

To get successful buccal controlled release dosage form hydrophilic, swellable polymeric material alone or in optimum combination is essential. This dosage form should get stuck to the oral cavity and release the drug at a controlled rate under the conditions prevailing in the mouth. Here a natural gum (Gum karaya) has been selected to prepare buccal tablets of Amoxicillin trihydrate. The tablets were prepared by wet granulation method. After granulation the pre-compression evaluation tests were performed. The granules showed good flow ability and compressibility. Physicochemical tests of the tablets revealed satisfactory results. Buccoadhesive strength of the tablets was found to be in the range of 28-39 gm, which is good enough to hold the tablets in the buccal cavity. Percent Swelling of the tablets was 10-107.87 % in phosphate buffer pH-6.8. The release data were fitted in kinetic models of zero order, first order and Higuchi. The tablets with 10- 15 % of gum karaya showed zero order kinetics whereas the other formulations (2.5-7.5% and 17.5- 20 % of gum karaya) showed mixed and higuchi kinetics of drug release respectively. Optimum concentration of gum karaya (10-15%) reveals the dominance of the highly swollen layer which controls the drug release from the buccoadhesive tablets.

**Keywords:** Buccoadhesion, Amoxicillin trihydrate, Gum karaya, zero order kinetics

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## INTRODUCTION

Among the various routes of drug delivery, the oral route can be considered as preferred route for patients and clinicians. Based on our current understandings of biochemical and physiological aspects of absorption and metabolism, many drugs, cannot be delivered effectively through the conventional oral route, because after administration they are subjected to pre-systemic clearance extensively in liver, which often leads to a lack of significant correlation between membrane permeability, absorption, and bioavailability. The buccal area has rich blood supply that drains directly into the jugular vein and bypasses the liver. During last few years mucoadhesive dosage forms have promoted an area of drug delivery system that renders the treatment more effective and safe, not only for topical disorders but also for systemic problems. Successful buccal drug delivery using buccal adhesive systems should have good bioadhesion to retain the formulation in the oral cavity and maximize the intimacy of contact with mucosa. This formulation needs a vehicle that is responsible for releasing the drug at an appropriate rate under the conditions prevailing in the mouth and successful strategies should be implemented to overcome the low permeability of the oral mucosa [1].

Bioadhesion can be defined as a phenomenon of interfacial molecular attractive forces in the midst of the surfaces of the biological substrate and the natural or synthetic polymers, which allows the polymer to adhere to the biological surface for an extended period of time.

Gum Karaya is a vegetable gum produced as an exudate by trees of the genus *Sterculia*. Gum Karaya is a negative colloid and a high-molecular-weight complex acidic polysaccharide. It is a partially acetylated complex polysaccharide composed of galacturonic acid, beta-D-galactose, glucuronic acid, L-rhamnose, and other residues obtained as the calcium and magnesium salt [2]. It is used as a thickener and emulsifier in foods, as a laxative, and as a denture adhesive. It is also used to adulterate Gum Tragacanth due to their similar physical characteristics [3]. A gumkaraya particle placed in water does not dissolve but absorbs water and swells to many times its original size. The general utility of GK is based on its viscosity. GK showed its suitability in the preparation of hydrophilic matrices mini-matrices [4], microcapsules and transdermal buccal tablets. It has good mucoadhesive property due to its swelling behavior. The swelling behavior of karaya gum is dependent upon the presence of acetyl groups in its structure. Deacetylation through alkali treatment results in a water soluble gum. When used in higher concentrations in water (up to 4%), karaya forms gels or pastes. Unlike other gums, karaya swells in 60% alcohol, but remains insoluble in other organic solvents. Karaya may absorb up to 100 times its weight in water.

Amoxicillin ( $\alpha$ -amino hydroxyl benzyl penicillin) is a semi synthetic antibiotic, belonging to the  $\beta$ Lactam family, which is effective for bacterial infection treatment, especially for *Helicobacter pylori* infection. *Helicobacter pylori* is a major causative agent of diseases such as Tonsillitis, Pneumonia, Bronchitis, Gonorrhoea, ear infections, urinary tract infection and skin infection. It is a  $\beta$ -lactam antibiotic agent which is chemically 7-[2-amino-2-(4-hydroxyphenyl) - acetyl] amino-3, 3-dimethyl-6-oxo -2-thia-5-azabicyclo [3.2.0] heptane -4-carboxylic acid.

Amoxicillin trihydrate acts by inhibiting the cross-linkage between the linear peptidoglycan polymer chains of the cell wall of grampositive bacteria such as *Streptococcus spp.*, *Staphylococcus. spp.* and *Enterococcus spp.* and gram-negative organisms such as Haemophilus, Neisseria, Escherichia, Proteus and *Salmonella spp* [5]. Amoxicillin in trihydrated form is available in capsules, chewable and dispersable tablets, and syrup and paediatric suspension, for oral use and as sodium salt for intravenous administration [6].

In order to extend the residence period, a gastro retentive system of amoxicillin based on non effervescent mechanism has been developed. Sustained release is a kind of controlled release system that provides medication over an extended period. In other words, a sustained release system controls the drug concentration in the target tissue. Due to rapid degradation of amoxicillin, a sustained release dosage form that maintains therapeutic concentration in the blood for a longer period of time is desirable.

A suitable buccal drug delivery system should be flexible with good bio adhesion, so that it can be retained in the oral cavity for the desired duration releasing the drug in a predictable manner to elicit the required therapeutic response. Gum karaya, is good buccoadhesive in nature. It swells in water and has profound effect on the release kinetics of controlled release dosage form [7].

## MATERIALS AND METHODS

### Materials

Amoxicillin trihydrate was a gift from Unimerk Remedies, Birganj, Nepal. Gum karaya powder # 150 was obtained as gift sample from Nutriroma, Hyderabad, India. All other materials used were of analytical reagent grade.

### Methods and Methodology:

Buccal tablets were prepared by wet granulation method. All the powders were passed through 80 mesh sieve. Required quantity of drug and excipients were mixed thoroughly. Mixture was then granulated using gum karaya paste. Granules were dried at 40°C for about 15 min. After drying talc and magnesium stearate were finally added as glidant and lubricant respectively. The mixture was then compressed into tablets using an 8 mm, round-shaped flat punch in a single-stroke using 10 station rotary machines (Karmavati Ahmedabad, India). The tablets were prepared with different concentrations of Gum karaya (Table-1).

### Drug-Excipient Interaction Study Using

#### FTIR Spectroscopy

Drug-excipient interaction, one of the most essential parameters, is studied before development of the formulations. Amoxicillin trihydrate, Gum karaya and its mixture with drug

were mixed with IR grade KBr in the ratio 1:100. Corresponding pellets were prepared by applying 5.5 metric ton of pressure in a hydraulic press. The pellets were scanned over a wave number range of 4000 to 400  $\text{cm}^{-1}$  in FTIR spectroscope (ALPHA T, Bruker, USA).

### Differential Scanning Calorimetry (DSC)

The DSC analysis of pure drug, Gum karaya, and physical mixture of the Gum karaya with the drug was carried out separately using Pyris Diamond TG/DTA Thermo gravimetric /Differential Thermal Analyzer (Perkin Elmer Inc, PerkinElmer SINGAPORE) to study any possible drug-polymer interaction at the molecular level. The ratio of drug to polymer chosen was same as that in the final formulation. Platinum crucible was used with alpha alumina powder as reference.

About 6 to 10mg sample were kept in platinum pans at a rate of 12°C/min from 10°C to 300°C temperature range under a nitrogen flow of 150 ml/min. The changes in the DSC curves were evaluated both with the positions of peak maxima and minima. The peak areas represent the phase-transition enthalpies [7].

### Buccoadhesion Test

To determine the buccoadhesive strength of the experimental tablets, each formulation to be tested was attached to goat-buccal mucosa. A small physical balance having two circular pans (diameter, 2 cm) hanged from a rod which was balanced with a fulcrum on a stand, was used as a modified buccoadhesion test assembly [8].

Lower end of a circular pan was attached to the tablet. Immediately after the attachment weights were placed on the other pan. Placing of weights was continued till the pan got detached.

### Swelling Index

The buccal tablets were weighed ( $W_1$ ) and placed separately in petri dishes containing 25 ml of Phosphate buffer (pH-6.8) and allowed to swell at  $37 \pm 0.5^\circ\text{C}$ . The dishes were stored at room temperature. After 4 h the tablets were removed and the excess water on their surface was carefully removed using filter paper. Percent swelling was calculated in terms of water uptake and presented as percentage of water uptake [9].

The swollen tablets were weighed ( $W_2$ ) and the percentage of swelling was calculated by the following formula.

$$\text{Swelling index} = (W_2 - W_1)/W_1 * 100$$

### **Moisture content capacity**

To determine the moisture content capacity of the tablets they were kept in desiccators for 24 hours with Silica beads. The percentage moisture content was calculated from the weight differences relative to the final weight after exposing prepared matrix tablets to activated silica in vacuum desiccators.

### **Surface pH Study**

The surface pH of the buccal tablets was determined in order to investigate the possibility of any side effects in vivo. As an acidic or alkaline pH may irritate the buccal mucosa, we sought to keep the surface pH as close to neutral as possible. For determination of surface pH the buccal tablets were allowed to swell for 2 h on the surface of an agar plate. Then the surface pH was determined by touching the electrode of a pH meter (Toshniwal Instruments, Ajmer, Rajasthan, India) in the excess phosphate buffer pH-6.8 present at the surface of the tablets. A mean of three readings was recorded [10].

### **Drug Content Analysis**

A buccoadhesive tablet was taken in 100 mL phosphate buffer pH 6.8 in a volumetric flask, and the mixture was stirred for 48 h at room temperature using a magnetic stirrer (Remi Equipments, Mumbai, India). The drug content analysis for Amoxicillin trihydrate was done by UV method. Initially, the time of analysis of the method was standardized by taking formulation with measured amount of drug in phosphate buffer pH-6.8 and determination of amount of drug released with the duration. It was found that 100% release of the drug was found by 24 h. Therefore, the time of drug content analysis was chosen up to 24 h [11].

### **In vitro drug release (Dissolution Study)**

The United States Pharmacopeia (USP) XXIII rotating paddle method used to study the drug release from the buccoadhesive tablet. The dissolution medium consisted 900 ml of phosphate buffer pH 6.8. The release was performed at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ , with a rotation speed of 50 rpm. In this study sinker was used to prevent the float of the tablet and retain at the bottom of the dissolution vessel. Samples (5 mL) were withdrawn at predetermined time intervals and replaced with fresh medium. The samples filtered through Whatman filter paper and analyzed after appropriate dilution (1ml of Sample in 10 ml) by UV spectrophotometer (UV – 1800 Shimadzu) at 273 nm (pH- 6.8) [12].

### **Kinetics of Drug Release**

To investigate the drug release kinetics from buccoadhesive tablets of Amoxicillin trihydrate the release rate obtained from dissolution studies were fitted to various kinetic equations. The kinetics models used were a, Zero order equation ( $Q_t = Q_0 - K_0t$ ), First order equation ( $Q_t = \ln Q_0 - K_0t$ ) and Higuchi's equation ( $Q_t = Kht_{1/2}$ ).

### ***In vitro* drug permeation study [13]**

The *in-vitro* permeation studies of buccoadhesive tablets of drug through excised layer of goat buccal mucosa were carried out using Franz diffusion cell having 3.14 cm<sup>2</sup> effective diffusional area. It consists of two compartments one is donor compartment and another is receptor compartment of 70 ml capacity. The cell contents were stirred with a magnetic stirrer and temperature of 37±2°C was maintained throughout the experiment. The separated buccal epithelium was mounted between two chambers and in receptor chamber phosphate buffer of pH 6.8 (37°C) was filled and epithelium was allowed to stabilize for the period of 1 h. After stabilization the tablet was placed into the donor compartment and was wetted with 1ml of phosphate buffer. The amount of drug permeated through the membrane was determined by drawing samples periodically and replaced with same volume of phosphate buffer pH-6.8. Then the samples were analyzed by using UV-Visible spectrophotometer at  $\lambda_{max}$  of 273 nm.

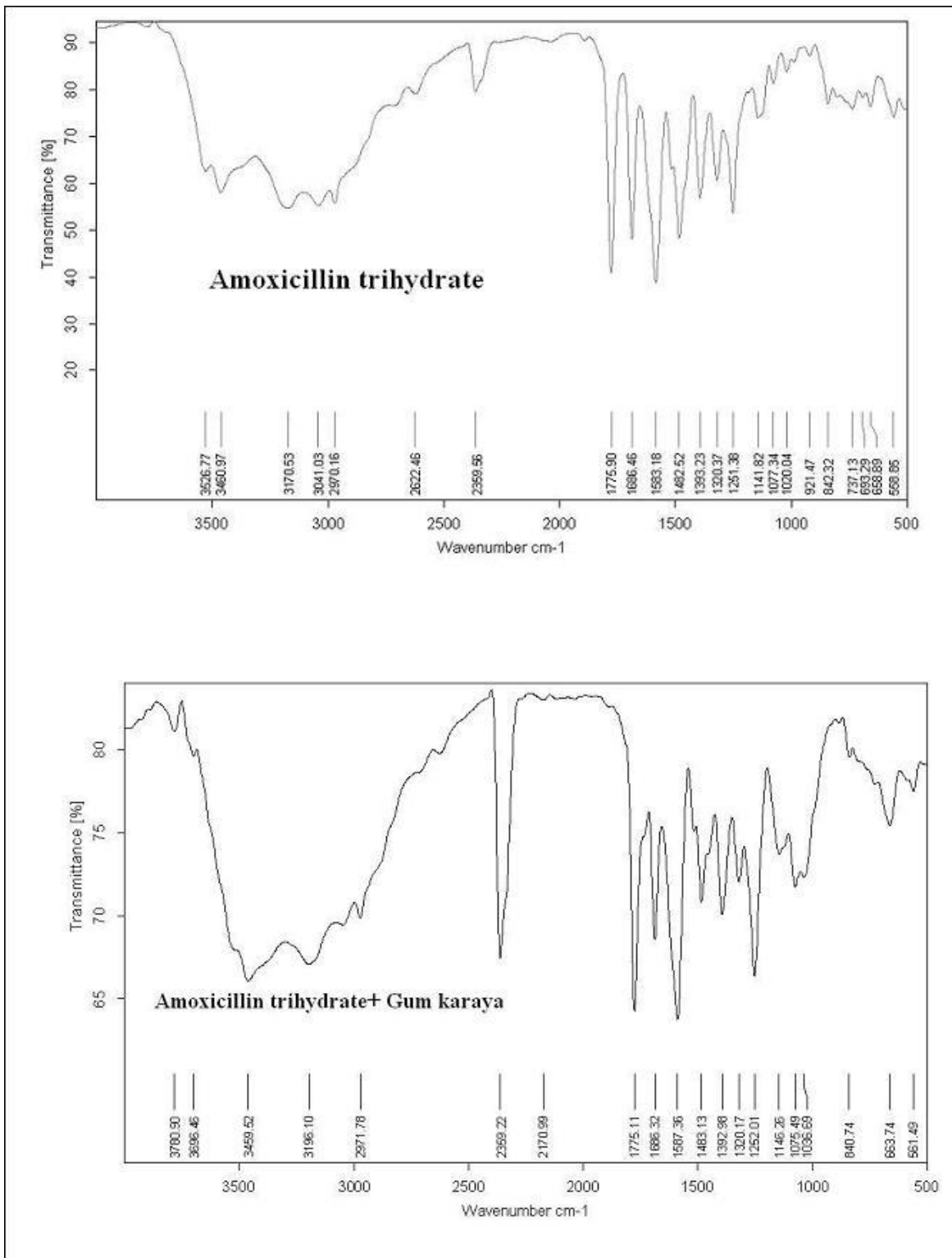
### **Statistics**

Data were assessed by one-way ANOVA followed by Tukey HSD Test using Vassar Stats software (USA). P<0.01 has been considered as statistical significance.

## **RESULTS AND DISCUSSION**

The present study was intended to develop buccoadhesive tablets containing Amoxicillin trihydrate. After a screening with several combinations of various polymers and evaluating the different physico-chemical parameters and *in vitro* drug release, the best polymeric composition achieved has been reported in this study. The drug-polymer ratio by weight was taken 1:10 for each of the formulations, containing Amoxicillin trihydrate, respectively. Drug-excipient interaction is a very important pre-formulation study to develop a new formulation [14]. Among the various methodologies available to understand the drug excipient interaction, common approaches are FTIR spectroscopy, DSC, IR-spectra etc. FTIR-spectroscopy shows the interaction between the molecules at the level of functional groups. Here drug-excipient interaction was studied using FTIR-spectroscopy and DSC. FTIR spectroscopy shows interaction between molecules at the level of functional groups [15]. An FTIR spectrum of pure Amoxicillin trihydrate shows that all the characteristic peaks of Amoxicillin trihydrate are present [16]. Figs. (1A, 1B) show the IR spectra of drug and mixture of drug and polymer respectively. Between 3200 cm<sup>-1</sup> and 2800 cm<sup>-1</sup> and between 1800 cm<sup>-1</sup> and 1000 cm<sup>-1</sup> wave numbers, variations at transmission spectroscopy data were noted. Alkenyl (-C=C-) (3020 cm<sup>-1</sup>- 3100 cm<sup>-1</sup>), amide (-NH) (1000 cm<sup>-1</sup>- 1250 cm<sup>-1</sup>) ketonyl (-C=O) (1710 cm<sup>-1</sup>-1720 cm<sup>-1</sup>), phenolic (-OH) (970 cm<sup>-1</sup>-1250 cm<sup>-1</sup>) stretches are mainly responsible for those regions. This suggests that there may be physical interactions related to the formation of weak to medium intensity bonding since no major shifting of peaks was noted [17]. Polymers may change the rate and pathway of diffusion of drug molecules by varying entanglement in polymeric network [18]. Thus the physical interactions might be helpful in sustaining the release of drug molecules from the experimental formulations.

Figure 1: FTIR spectra of a. Amoxicillin trihydrate b. mixture of Amoxicillin trihydrate and Gum karaya





DSC measurement was carried out to provide better evidences whether predicted physical interaction would lead to drug amorphous formation in the formulations. Figure 2A shows the DSC and TGA of Amoxicillin trihydrate. Figure 2B and 2C Show the DSC and TGA of Gum karaya and Drug-Gum karaya mixture respectively.

Figure 2: DSC and thermograms of a. Amoxicillin trihydrate b. Gum karaya c.mixture of Amoxicillin trihydrate and Gum karaya

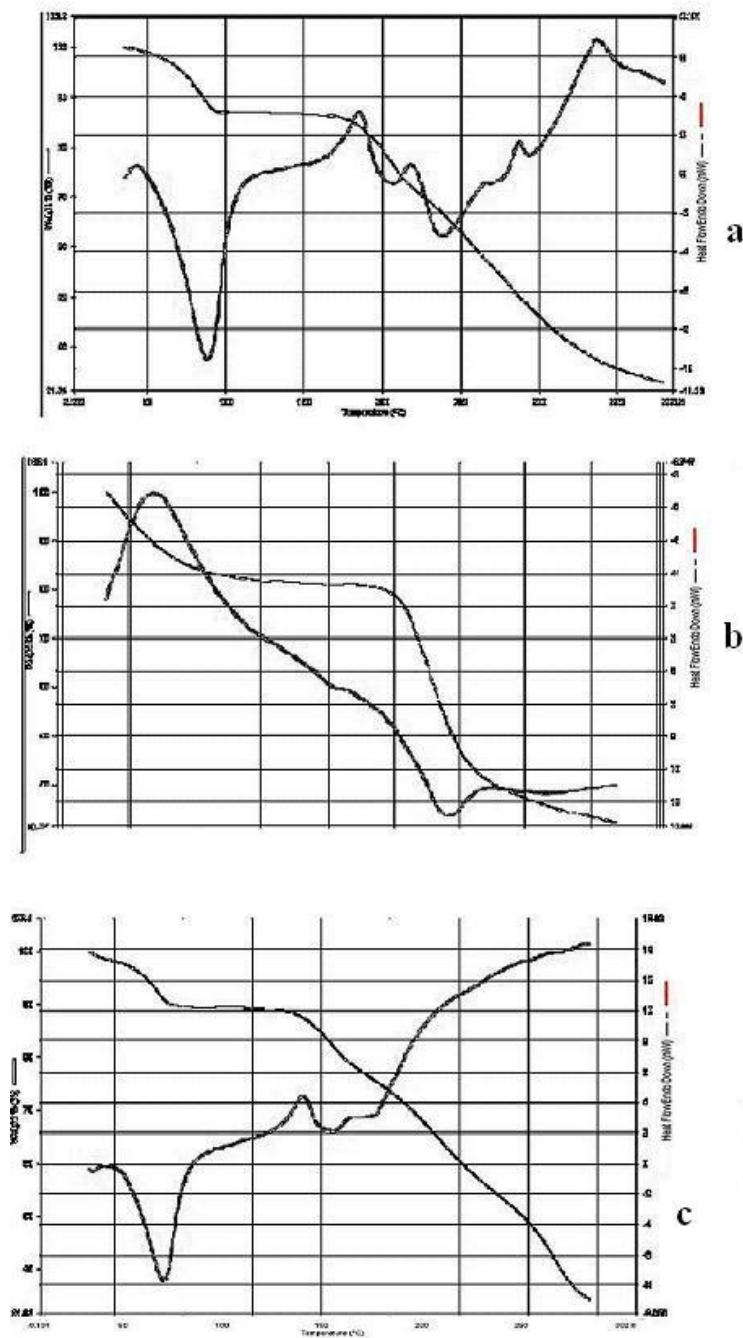


Figure 2C shows dipping of curve at 84.27°C claiming the loss of water molecule from Amoxicillin trihydrate. This was followed by the crystallization of Amoxicillin trihydrate



molecules at 182 °C and then immediately it was followed by the melting of the drug molecules at 194 °C. When the drug molecules reached at 182°C they gained enough energy to move into very ordered arrangement for crystallization. Thus 182°C temperature gave the crystallization temperature of Amoxicillin trihydrate. This was followed by endothermic transition of melting phenomenon started at 194 °C and this was followed by degradation of the molecules. The data are further supported by TGA curve of the drug in the same figure. The changes in all the DSC thermograms correspond to the changes at the respective TGA shown in the Fig. 2.

**Table 1: Composition, Drug content and physical-mechanical characters of the formulation.**

Formulation	Gum karaya (Concentration %)	Drug content	Surface pH	Buccoadhesive strength (g)	Moisture content
F2.5	2.5	99.17 ± 0.6 %	6.8± 0.1	25.3± 1.3	8.17%
F5	5	98.17 ± 0.6 %	6.8± 0.1	28.2± 1.3	11.06%
F7.5	7.5	98.17 ± 0.6 %	6.8± 0.1	30.12 ± 1.5	12.89%
F10	10	98.17 ± 0.6 %	6.8± 0.1	32.05 ± 1.4	14.87%
F12.5	12.5	98.17 ± 0.6 %	6.8± 0.1	34.14 ± 1.4	15.23%
F15	15	98.15 ± 0.4 %	6.8± 0.1	37.16 ± 1.5	16.71%
F17.5	17.5	98.17 ± 0.6 %	6.8± 0.1	38.08± 1.1	16.98%
F20	20	98.23 ± 0.4 %	6.8± 0.1	39 ± 1.2	18.50%

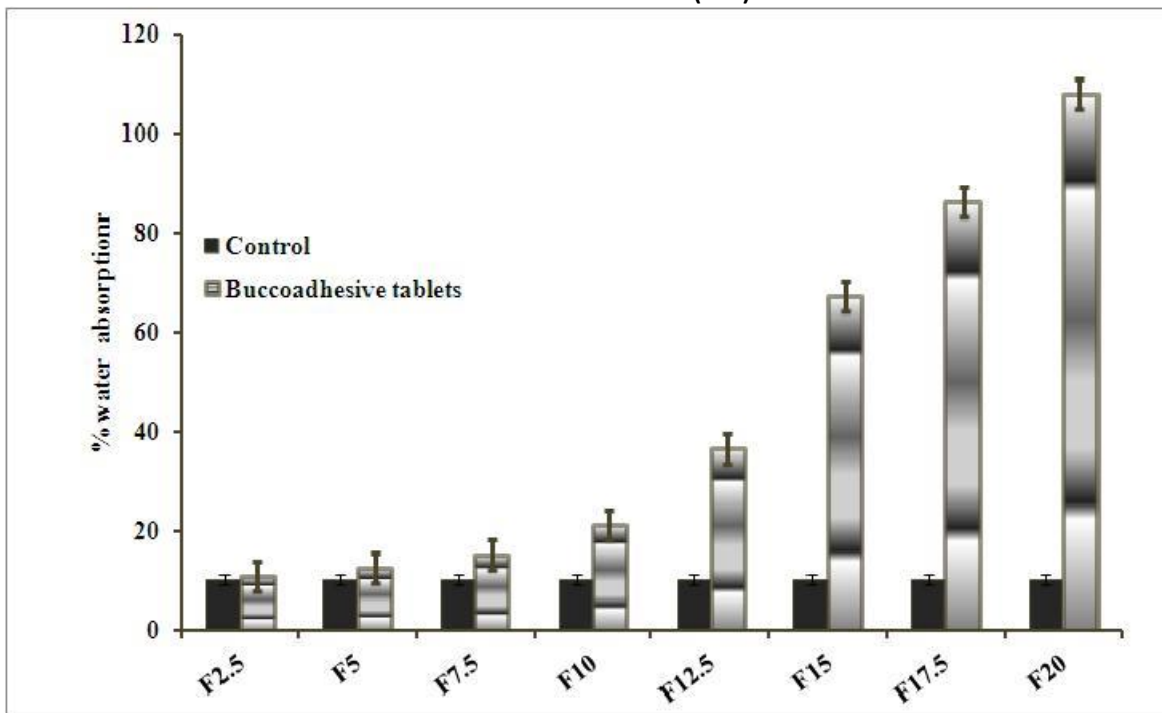
**Table-2: Release rate constants for the drug Amoxicillin trihydrate from the Buccal tablets obtained from different kinetic models**

Batch	R <sup>2</sup>	K <sub>1</sub>	R <sup>2</sup>	K <sub>2</sub>	R <sup>2</sup>	K <sub>3</sub>
<b>Zero Order</b>						
F2.5	0.9771	0.0872				
F5	0.9733	0.0882				
F7.5	0.9721	0.0871				
F10	0.9743	0.0952	-	-	-	-
F12.5	0.993	0.0851				
F15	0.9916	0.0587				
F17.5	0.980	0.0582	-	-	-	-
F20	0.9759	0.06	-	-	-	-
<b>First order</b>						
F2.5	-	-	0.943	1.629		
F5	-	-	0.949	1.231		
F7.5	-	-	0.946	1.611		
F10	-	-	0.9903	1.001	-	-
F12.5	-	-	0.960	1.514		
F15	-	-	0.9798	1.001	-	-
F17.5	-	-	0.925	1.303		
F20	-	-	0.9175	1.14	-	-
<b>Higuchi</b>						
F2.5	-	-	-	-	0.990	2.721
F5	-	-	-	-	0.975	2.718
F7.5	-	-	-	-	0.976	2.703
F10	-	-	-	-	0.9443	2.977
F12.5	-	-	-	-	0.981	2.61
F15	-	-	-	-	0.9744	1.727
F17.5	-	-	-	-	0.986	1.726
F20	-	-	-	-	0.9987	1.824

The buccal tablets show satisfactory physical-mechanical properties (Table 1). In the entire three formulations drug content is above 98% and the low values of standard deviation and coefficient of variation (<1) indicate uniform distribution of the drug within the buccoadhesive tablets. The surface pH obtained in this study was within the limits and showed hardly any variation from time to time which omits the chances of irritation in the buccal mucosa upon application.

All the three types of buccal tablets exhibited good buccoadhesive strength; those were found to be 32.16, 34.25 and 39.32 g respectively. In case of the mucoadhesive polymers desired strength was reported to be about 30 g [19]. Hence the buccoadhesive strengths were found to be satisfactory to hold the buccal tablet inside the oral cavity. That strength of tablet was found to be a function of the nature of polymer used and their concentration in the formulation.

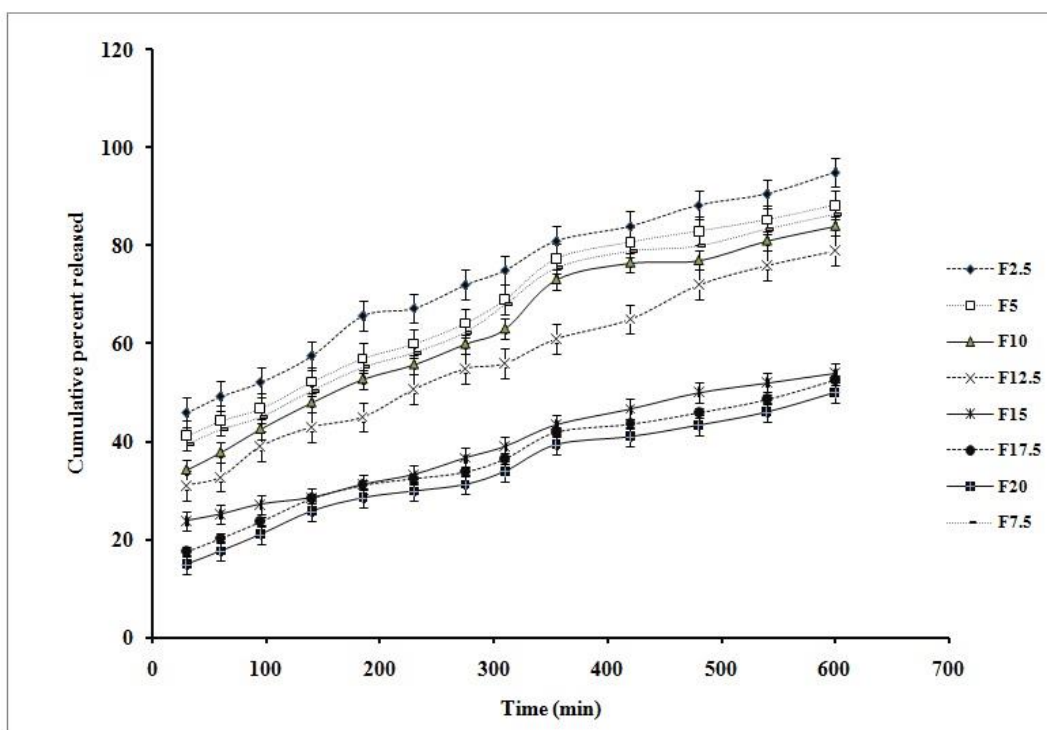
**Figure 3: Swelling index in terms of water absorption capacity of buccoadhesive tablets at phosphate buffer pH-6.8 for 4 h. Data show mean (n=6) ± SD**



Swelling depends on the polymer concentration and the moisture absorption capacity of the polymers [20]. The swelling results were expressed in terms of percentage water uptake at 37°C [21]. For F15 the swelling was 51.43 % and for F2.5 and F20 they were 10.35 % and 107.85 % respectively. The swelling ability increases with increase in percentage of Gum karaya. The highest hydration (swelling) was observed with the formulation F3 (Fig 3). Flexibility of polymer chain from individual polymer is important for interpenetration and entanglement, and in presence of water molecules, polymers become crosslinked and the mobility of individual polymer chain decreases and swelling occurs with the time [22].

In this study, the buccoadhesive strength was determined by measurement of the force of detachment or force of adhesion [23]. These parameters are the most frequently studied adhesive properties. Buccoadhesion occurs shortly after swelling [24] but the bond formed between mucosal layer and polymer is not very strong at the beginning. The adhesion will increase with the degree of hydration to an optimum value. Results indicate that swelling is maximum in where GK is 20%. Buccoadhesive strength is also maximum in those formulations and that supports the above findings.

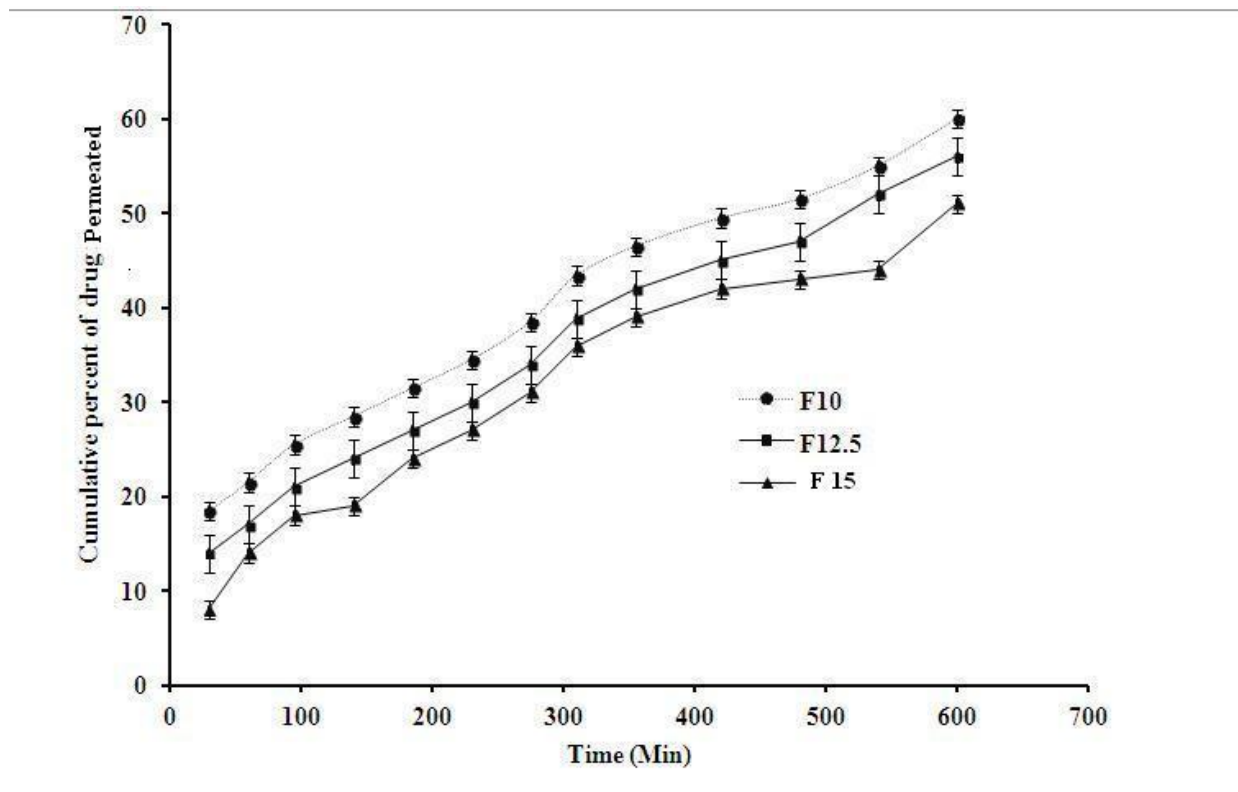
**Figure 4:** *In vitro* release of Amoxicillin trihydrate from buccoadhesive tablets containing Amoxicillin trihydrate in phosphate buffer (pH-6.8). Data show mean (n=6) ± SD, Values were significantly different as accessed by one-way ANOVA followed by Tukey HSD test (p<0.01).



The result of moisture content was 8.17% to 15.50%. Fig 4 represents the graph consisting of cumulative percentage of drug release vs time. Drug release is the slowest one in F20. In formulation F10,  $t_{50\%}$  value is 2 hr 35 minutes whereas in F15 and F20 they are 8 hr and 10 hr 5 minutes respectively.

The release profile and kinetics of drug release are important because they correlate the in vitro and in vivo drug responses by comparing results of pharmacokinetics and dissolution profile patterns. Hence, the cumulative drug release results of the formulations were fixed into various mathematical models.

Figure 5: *In vitro* skin permeation study of buccoadhesive tablets of Amoxicillin trihydrate through goat buccal mucosa at phosphate buffer pH-6.8 . Data show mean (n=6)  $\pm$  SD



The drug release pattern of formulation F10, F12.5 and F15 were found to be highly linear, and close to infinity as indicated by their high regression value as 0.9843 0.9930 and 0.9916 respectively. Therefore it was ascertained that the drug release from these formulations could follow either zero or near zero order kinetics. These forms released the same amount of drug by unit of time irrespective of the drug concentration. F2.5, F5 and F7.5 drug release followed mixed kinetics. It was noted that drug release gradually changed from concentration independent “zero-order” release kinetics to concentration dependent “first-order” kinetic pattern. At the beginning, because of the matrix structure drugs is released by zero-order kinetic pattern later due to erosion of the structure drug released via first order kinetics Where as F17.5 and F20 followed Higuchi kinetics throughout the study ( $r^2=0.9860$  and  $0.9987$  respectively). Here more amount of Gum karaya is responsible for maximum swelling of the matrix. That swollen layer behaves like a complete matrix structure and controlled the drug release showing higuchi kinetics of drug release as with the time, the swelling of the polymers varied the entanglement of polymeric pathways to control the diffusion of the drugs from the formulations [25]. In F10, F12.5 and F15 drug release is concentration independent -zero order. Here concentration of Gum karaya was such that it showed promising drug release pattern. That was optimum enough to hold the matrix structure till the experimental work to release the drug in zero order fashion. On the basis of drug release profile F10, F12.5 and F15 formulations were selected for *in vitro* permeation study.

In vitro permeation study the results are in conformity with the in-vitro drug release. Drug permeation through goat buccal mucosa was steady and that also maintained a steady state throughout the experiment. It can be concluded that sufficient concentration gradient were maintained for drug diffusion across the skin.

### CONCLUSION

The prepared buccoadhesive tablets of Amoxicillin trihydrate can help to bypass extensive hepatic first-pass metabolism and improve bioavailability. These tablets exhibited buccoadhesion time of more than 8 h.

Buccoadhesive tablets with 10, 12.5 and 15 % concentration of Gum karaya showed zero order kinetics of drug release when subjected to dissolution study in phosphate buffer pH-6.8. Similarly, *in-vitro* permeation studies showed almost 50-70% of drug permeation in 8 h. Hence a conclusion can be drawn that formulation F10, F12.5 and F15 could be used to release the drug uni directionally in buccal cavity in controlled manner.

### ACKNOWLEDGEMENT

The authors are thankful to NSHM Knowledge campus, Kolkata for providing necessary facilities to carry out the research work and Metallurgy Department, Jadavpur University to provide DSC facility.

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