In Vitro Study of Release of Metronidazole Tablets Prepared from Okra Gum, Gelatin Gum and their Admixture

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Abstract

The aim of this study is to evaluate the ability of okra gum to release it's medicament in bioadhesive polymer-based drug delivery system. Bioadhesive studies using the tensiometer were done to evaluate its bioadhesivenes. Conventional tablets were made with okra gum as binder and in-vitro release studies carried out using gelatin as a standard reference. Okra gum was seen to have a comparable result with gelatin in term of the bioadhesive property the amount of drug released.

Keywords: Bioadhesive, Okra gum, Gelatin gum, Metronidazole, Release studies

Introduction

The use of bioadhesive polymers and co-polymers as means of delivering therapeutically active drugs, to or via mucous membrane has been the focus of attention in recent years (Park and Robinson, 1984). Bioadhesion may be defined as a state in which two materials, at least one of which is of biological origin, are held together for extended periods of time by interfacial force (Longer and Robinson, 1986). For drug delivery system, the term bioadhesion means attachment of a drug carrier system to a specific biological location, which can be the epithelia tissue or the mucous coat on a tissue surface (Khanna et al., 1998), thus increasing drug absorption and overall bioavailability.

The materials with this property are generally hydrophilic macromolecules that contain numerous hydrogen bond forming groups, and hydrates that swells when placed in contact with an aqueous solution. These materials need to hydrate to become adhesive, but over-hydration usually results in the formation of a slippery mucilage and loss of adhesive properties (Kinloch, 1980). Intimate contact between the adherents is a prerequisite for a strong adhesive bond. As a result, static adhesion behaviour has been described as a function of the spreading coefficient, S_{12}; this thermodynamic parameter has been suggested as the driving force for the wetting process (Wu, 1978):

$$S_{12} = Y_{23} – Y_{13} – Y_{12} \quad \text{Eq 1}$$

Where: Y_{23} is the interfacial tension between substances 2 and 3 and $Y_{12\ is}$ the interfacial tension between substances 1 and 2 and Y_{13} is the interfacial tension between substances 1 and 3.
The specific work of adhesion, W_{12}, can be described by the Dupre's equation:

$$W_{12} = Y_{13} + Y_{23} – Y_{12} \quad \text{Eq 2}$$

Here, W_{12} represent the energy required to reversibly separate two substrates to infinite separation. When the two substrates are identical, this expression reduces to

$$W_{12} = W_{11} = 2Y_{13} \quad \text{Eq 3}$$

where W_{11} is the energy of uniformly mixed substances.

The interfacial tension between phases 1 and 2 can be re-expressed by separating the dispersed, and the polar contributions to the surface tensions of the two phases. A modification which is most appropriate for polymer systems has also been discussed (Wu, 1978). Adhesion is thermodynamically favoured when the polarities of the two phases match. This occurs when the spreading coefficient is maximized; i.e. when

$$Y^p_1/Y_1 = Y^p_2/ Y_2 \quad \text{Eq 4}$$

where P is the index of distribution or partitioning during spreading. Wetting of a substrate is best when the measured contact angle approaches zero (Mikos and Peppas, 1990).

Delivering mucosa-adhesive dosage forms offers the advantage of good drug absorption as a result of increase in resident time, enhanced patient acceptance and compliance. Bioadhesion has been employed in treating toothache in dentistry and in orthopaedics purpose which eventually healed fracture bones. In the field of skin grafting, bioadhesion has been utilized for artificial replacement of soft tissue. Bioadhesion has been extensively used in ophthalmology cases for the adhesion of the conjunctiva of the eye or upon surgery to put together intra-ocular lenses with the eye. Genetically, bioadhesion is highly favoured in target macromolecules (Derjaguin et al., 1977)
In this study, attempts were made to establish the mucoadhesive and bioadhesiveness of okra gum prepared from *Albelmoschus esculentus (*Family: Hibiscus), alone and in combination with an auxiliary animal gum (gelatin) co-precipitated.

*Albelmoschus esculentus (*Okra) is a member of the mallow family related to cotton, hibiscus. Okra is an annual tropical herb cultivated for it edible green pod it is grown in warm climates. It has a heart shaped laves and the pods are 3-10 inches long tapering. The pod grew rapidly being ready to harvest in about 60 days (Adikwu, 1993).

Gelatin is a protein obtained from collagenous material such as animal skins, tendons, ligaments, and bones. There are two types of gelatin: Type A is gelatin obtained from an acid treated precursor (with isoelectric point between 7 and 9), and Type B is gelatin derived from an alkali treated or hydrolysis of precursor from animal bone (with isoelectric point between 4.7 and 5.2). Gelatin is soluble in hot/warm water, but insoluble in cold water. However, when placed in cold water, gelatin softens and gradually absorbs about 5 – 10 times its own weight of water. It has been proved to be insoluble in alcohol, chloroform, ether, fixed or volatile oil, but readily soluble in biological fluid at body temperature. It is a good film forming material and is non-toxic. It is widely employed in foodstuffs, as tablet film coats and binders (Adikwu, 1995).

The research work is aimed at evaluating the release of metronidazole from drug loaded granules prepared from a gelatin-okra gum. It also looks into usefulness of okra gum in bioadhesive drug delivery system and its role in in-vitro release of drug from the preparation.

Materials and Methods

Gelatin (Merck, England), acetone (BDH, England), concentrated Hydrochloric Acid, Lactose, Maize Starch (Merck, England), monobasic potassium phosphate and Sodium chloride (BDH, England), and metronidazole sample (BDH, England), were used as such. All laboratory reagents were freshly prepared.

Methods: Fresh *okra* pods were cut in to small sizes and soak in water for 24 h. the gel was squeezed out of the fruit and passed through a muslin bag. The mucilaginous material was subsequently precipitated with 5000 ml of acetone. This volume of acetone was five times the volume of the mucilage. The precipitate was washed severally with fresh acetone and decanted until no slight change in colour was noted. The gum was air-dried and comminuted into fines with an end-runner mill (Model 331245G, Erweka, England). The fines of the gum were passed through 250 µm sieve and stored in an air-tight container until used.

Preparation of metronidazole tablets: Batches of metronidazole tablets were produced using the different ratios of the gums as show in Table 1. Each tablets contain, maize starch 40 mg, lubricant 3 mg and lactose to 300 mg. Wet granulation method of tablet production was employed and the granules compressed in a tablet press (F-3 Manesty) with a force of 48 kgf. The ratio of gums used for the different batches are stated in Table 1, together with the amount of drug used.

Tensiometric bioadhesive test: A tensiometer (Lecomte Du Nuoy Tensiometer, Model Nr 3124, A. Kruss Germany) was used for this study. A clean polythene support was placed on a platform with an adhesive. A freshly excised pig jejunum was washed of its waste material. A small portion of the jejunum with diameter 2 cm and length 5 cm was used. The mucus surface of the intestine was

Table 1: Ratios and quantities of the polymers used in metronidazole tablets

Batch	Ratios Gelatin: okra	Amount Of Polymer Okra (mg)	Gelatin (mg)	Drug (mg)
A	gelatin	0	3	40
B	okra	3	0	40
C	1:1	1.5	1.5	40
D	1:2	1	2	40
E	1:4	2.4	0.6	40
F	1:5	2.5	0.5	40

exposed and used immediately for the test. The tissue was fixed unto the polythene support of the bioadhesive instrument placed on a metal support. The instrument was zeroed and the bioadhesion of the clean glass plate determined in degrees by gradually raising the platform such that the plate on the lever arm of the tensiometer was in contact with the tissue and 5 min contact time allowed for interaction to take place. The glass plate was raised by screw gently until it just detached from the surface of the tissue. The force required to remove the glass plate from the surface of the tissue was read off from the microform balance.

The glass plate was washed, dried and subsequently coated to a thickness of 1.5 mm with the different aqueous dispersion of the weighed bovine mucin and allowed to dry for 10 min. This was repeated three times for each sample, and the average reading was taken. The force to detach the glass plate and conversion of these to tension were done using the equation below:

$$T = mg/2L \times F \quad \text{.....Eq. 5.}$$

where T = tension, m = weight in kg, g = acceleration due to gravity (10 m/s^2), L = perimeter of the plastic plate and F = constant = 0.94.

In vitro release study of metronidazole from the preparation: Batches of tablets preparations of metronidazole were placed into the appropriate basket of the dissolution apparatus (Serial No 00721, VEEGO) containing 700 ml of simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) one after the other. The medium was maintained at 37 ± 1^0C for all the test. At zero time, the preparation was put inside the dissolution medium. At predetermined times of 0, 5 10, 20, 25, 30, 45, 60, 75, 90, min, 5 ml of the dissolution medium was withdrawn, adequately diluted and assayed spetrophotometrically at 270 nm. Each amount of dissolution medium withdrawn was immediately replaced with equivalent amount of fresh dissolution medium. This was repeated three times and was done for all the batches. The concentration of metronidazole released during each period was determined with reference to Beer's plot in SIF and SGF. The data generated was further analysed graphically.

Measurement of absolute drug content of the tablets: Five tablets from each batch was taken and weighed individually. The average weight was weighed out from the crushed tablets and poured into a beaker (250 ml) containing 50 ml of SIF. A 1 ml volume of the SIF from each of the tablets was

withdrawn and 9 ml of SIF was added to make a 10 fold dilution. The absorbance of each dilution was read using a spectrophotometer. The procedure was repeated in SGF for the batches.

Measurement of contact angle: The tilted drop measurement was used. In this technique, a droplet is added to the surface and the advancing and retreating contact angle are measured as the surface is tilted up until the droplet reaches a point where it almost moves. This technique is useful to measure both the receding and advancing contact angles at same time.

An inclined plane with smooth surface was used to study for the different dispersion of the okra-gelatin admixtures. A drop of 1 % w/v of the dispersions for each gum was placed on the smooth surface until it formed a plateau. The plane was tilted until the droplet reached a point where it almost moved. The adjacent and hypotenuse length were measured and the angle was calculated using Cosine Formula.

Results and Discussion

Bioadhesive strength: Bioadhesion/mucoadhesion is expressed as the force required for detaching 50% of the tablets (Giovanni et al., 1991). Okra gum alone showed highest bioadhesive strength with the percentage mucoadhesion of 97.7 % as shown in Table 2, the okra : gelatin gum in 1:1 combination showed a convincing high bioadhesive property. Gelatin is known to possess good bioadhesive properties. Bioadhesive force occurs between the mucus surface and the polymer. The stronger the bioadhesive interaction between a polymer and a mucus membrane, the greater the force required to detach the polymer film from the mucus (Dodou et al., 2005). It showed that okra gum alone gave the highest tension result of 97.7 ± 0.2. This can be attributed to the ready hydration properties of the okra gum which facilitated fast spreading over the mucosal surface to produce an interpenetrating layer and entanglements (Huang et al., 2000).

Table 2: Bioadhesive test

Polymer concentration 1 % w/v (gelatin : Okra)	Bioadhesive strength (dynes/cm)
A (gelatin)	78.4 ± 0.2
B (Okra)	97.7 ± 0.2
C (1:1)	80.4 ± 0.4
D (2:1)	78.4 ± 0.3
E (1:4)	78.4 ± 0.2
F (1:5)	86.1 ± 0.5

The ratio combination of 1:5, also exhibited a high-tension result next to okra alone than gelatin, 1:1, 1:4 and 2:1 gelatin: okra combinations. Gelatin showed lower bioadhesive values than the okra gum alone and its combinations.

Gelatin alone showed an appreciable level of bioadhesiveness from the study. However, it takes a longer period to gel than okra gum and the molecular structure weakens on gelling. This may be responsible for the low bioadhesive values recorded for the material. The results of the contact angle of all the batches shown in Table 3, above were below 90 $^{0.}$ Thus the effectiveness of wetting process may be related to the contact angle which the solid makes with the liquid interface. The condition for complete wetting of a solid surface is that the angle should be zero. The total decrease of surface free energy per unit area of surface is due to the spreading of the liquid. The condition for complete immersion of the solid in the liquid is that there should be a decrease in surface free energy as a result of the wetting process.

Table 3: Contact angle studies

Polymer Conc. Gelatin: Okra (0.25)	Contact Angle in degree
1 :0	15.6 ± 0.2
0:1	23.7± 0.2
1:1	17.8± 0.1
2:1	19.4±0.1
1:4	24.4± 0.4
1:5	22.4± 0.4

Figures 1 and 2 showed the results of the in vitro release studies of the various batches of the tablets from the normal release studies. In Figure 1, the maximum percentage release among the batches was recorded with the okra- gelatin ratio of 1:4 (Batch E).

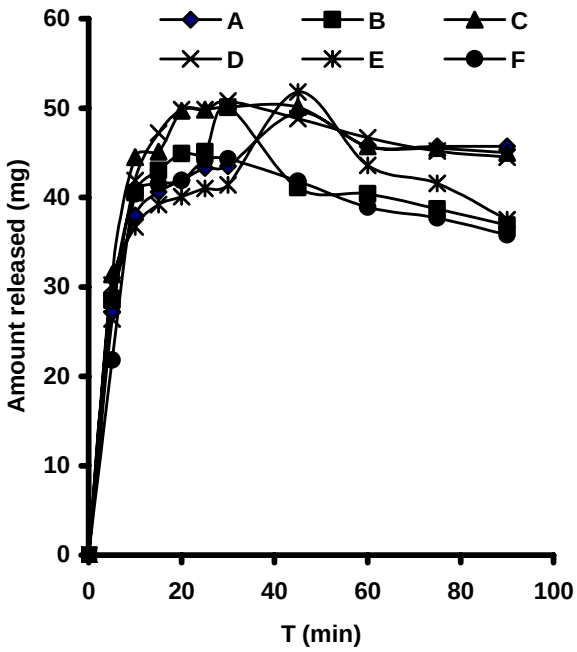

Fig.1: Plot of amount released against time in SIF

All the batches showed a gradual decrease in percentage amount released within 30-60 min. In Figure 2, batch D containing ratio of 2:1 okra-gelatin was seen to have the highest % amount released in SGF. Higher release was show in SGF than SIF; this could be due to the type of gelatin used and also the acidic medium of the SGF. In the use of polymers in drug formulations it is often essential to use combinations to modify the effect of each of the polymers in the formulations. Many of such combinations may be physically made in the dry state.

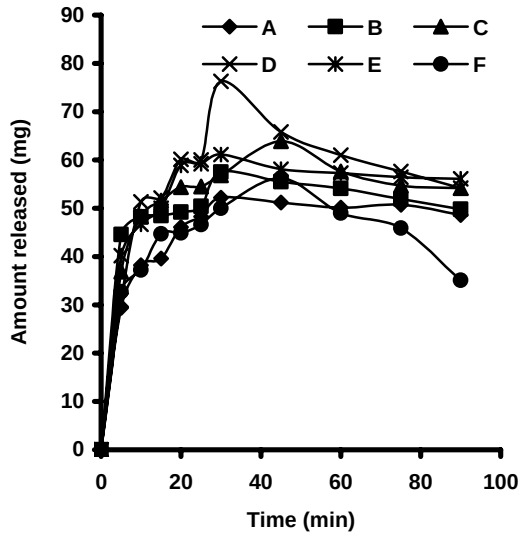

Fig. 2: Plot of amount release against time in SGF

However, this could lead to non uniform mixing or it may need longer mixing or kneading time. The method of mixing the two gum using co-precipitation is novel. This is because the aqueous dispersions when mixed together before drying can lead to molecular mixing and interactions. At this level, many electrostatic forces such as hydrogen bonding may occur resulting in a product that has properties which is intermediate between the two compounds (Mortazayi et al., 1993). The two polymers are of different origin, gelatin is from animal while the okra is plant origin, corpreicipitation of the two will give both plant and animal characteristics properties and this has slight effect on the release properties of the formulated drugs, since release is largely dependent on the hydrophobicity of a material (Huang et al., 2000).

Conclusion: This study showed that okra gum alone has the highest bioadhesive strength from the *in vivo* study. Similarly, the gum co-precipitated with gelatin gave a good result in terms of adhesion at the ratios of 1:1 and 1:4 okra: gelatin.

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