In-vitro Evaluation of Bioadhesive and Release Properties of Thiamine hydrochloride formulation from Gelatin, Gellan as their Admixtures

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Abstract

This study was aimed at determining the effect of gelatin on the bioadhesive strength and release properties of gelatin gum. Bioadhesive strength determination was carried out using tensiometric methods. Thiamine tablets was prepared by wet granulation method and used for the study. Tablets properties evaluated include: weight uniformity, friability, disintegration time test and release studies in simulated intestinal fluid (SIF) and simulated gastric fluid (SGF). The study showed that the gelatin alone had the highest bioadhesive strength, while gellan had the least. Admixture of both gelatin and gellan showed values that were intermediate to those obtained for the two polymers differently. The release study showed a better release with batch A (1:1) highest in SIF, followed by F (0:1), and the reverse was the case in SGF.

Key words: Bioadhesive, Gellan gum, Gelatin gum, Thiamine hydrochloride, Release studies

Introduction

Bioadhesion is referred to as the attachment of natural or synthetic macromolecule to a biological substrate. It is referred to as a state in which a material of a biological nature is held together with another material (biological or otherwise) for extended periods of time by interfacial forces (Peppas and Bury, 1985). With reference to drug delivery, it implies the attachment of a drug carrier system to a specific biological location or tissue; which can be an epithelial tissue or the mucus coat on the tissue surface. When attachment occurs more specifically to a mucosal epithelium, this phenomenon is referred to a "mucoadhesion", because the biological layer responsible for adhesion is the mucus layer (Gu *et al.,* 1988). The mucoadhesive process has been described as beginning with establishment of intimate contact between the mucoadhesive substance and the mucus gel. The second stage involves the physical entanglements of both polymer chains to allow the formation of secondary chemical bonds (Mortazavi, 1995).

In the context of their medical and pharmaceutical use, the term bioadhesion refers to the adhesion of synthetic and biological macromolecules to a biological tissue. The biological substrate may be cells, bone, dentine, or the mucus coating the surface of a tissue. Many examples of bioadhesion exist in nature, including such diverse events as cell-to cell- adhesion with a living tissue, and bacteria binding to tooth enamel. In healthcare, bioadhesives were first used as fixatives. Over the last two decades, bioadhesives have been of interest within the pharmaceutical sciences because of their potential to optimize drug delivery. Such drug delivery may be optimized at the site of action (e.g. on the cornea or within the oral cavity) or at the absorption site (e.g., in the small intestine or nasal cavity). Bioadhesives may also be used as therapeutic agents in their own right, to coat and protect damaged tissues (gastric ulcers or lesions of the oral mucosa) or to act as lubricating agents (in the oral cavity, eye and vagina). Skin adhesives, tissue sealants, and dental and bone adhesives and cements are also defined as bioadhesives (Lee *et al*., 2000).

The performance of a bioadhesive can be evaluated in terms of various parameters, such as adhesion strength, adhesion number, and/or duration of adhesion. Measuring the mechanical properties of a bioadhesive is the most direct way to quantify the bioadhesive properties. The most commonly used types of stress to measure the force of adhesive joints are tensile shear and peel stress (John, 2004).

The objective of this research was to study tablets properties disintegration time test and release in simulated intestinal fluid (SIF) and simulated gastric fluid (SGF).

Materials and Methods

Materials: The materials used were procured from their local suppliers without further purification. All the reagents used were of certified analytical grade. The following materials were used in the course of this work; Thiamine hydrochloride, Gelrite (Keleo, England), Gelatin and Monobasic potassium Phosphate (Merck, India).

Preparation of gum dispersions: Aqueous gum dispersions were prepared at a concentration of 5 % in the different ratios (1:1, 1:2, 2:1, 1:5 1:0, 0:1) using distilled water. The dispersions were stirred very well and left to hydrate for 5 hrs. These were then used to study bioadhesion.

Table 1: Ratios and quantities of gums used for coating beads

Ratio	Gellan (g)	Gelatin (g)	volume of water (ml)
1:1	0.25	0.25	10
1:2	0.17	0.33	10
2:1	0.33	0.17	10
1:5	0.08	0.42	10
1:0	0.5	0	10
0:1	0	0.5	10

Preparation of coating beads: Glass beads of average diameter 3 mm and mass 60 mg respectively, were thoroughly cleaned with distilled water and then with acetone to maximize the roughness factor (Bamba *et al.,* 1979). The beads were then immersed in the aqueous dispersion of 5 % of the polymers to ensure uniform coating and air-dried.

Bioadhesive determination using coated beads: This was done using isolated intestinal mucus surface. The apparatus designed and used in this study was made of separatory funnel clamped on a retort stand with a rubber tube attached to the end of the separatory funnel and a metal support used to position a plastic support at an angle of 30 ^{0}C. Freshly excised hog ileum of about 10 cm was pinned on the plastic support. A container was placed, directly below the set-up to collect the detached beads. Ten coated glass beads were placed on the exposed mucus surface of the tissue, 10 min. was allowed for mucus-polymer interaction. Simulated intestine fluid (SIF) without pancreatin (250 ml) was then allowed to flow over the beads at a rate of 30 ml / min. the number of detached beads were noted and used as a measure of bioadhesion. This was repeated and the average taken as the number of beads detached.

Preparation of thiamin hydrochloride tablets: Batches of thiamine hydrochloride tablets were produced using the different ratios of the gums. 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 2 together with the amount of drug used.

Table 2: Ratios and quantities of the polymers used in thiamine hydrochloride tablets

Batches	Ratios	Amount of polymer		Drug (mg)
		Gellan (mg)	Gelatin (mg)	
A	1:1	7. 5	7.5	50
B	1:2	5	10	50
C	2:1	10	5	50
D	1:5	3	12	50
E	1:0	15	0	50
F	0:1	0	15	50

Evaluation of tablet properties

Weight uniformity: Ten tablets were selected from each batch at random and weighed individually with an electronic weighting balance (Ohaus, England). The different weights were noted. The mean standard deviation and coefficients of variation were calculated for each tablet batch.

Crushing strength/hardness test: Three tablets were used from each batch. The test was carried out using a hardness tester (Manesty) and the load/ pressure required to crush each tablet when placed on its edge was recorded. Average of the values obtained for the three tablets in each batch was calculated.

Friability: This was carried out using a friabilator (Erweka, England). Ten tablets from each batch were dedusted, weighed and subjected to rotation for 4 minutes at 25 rpm. The tablets were removed from the chamber, dedusted and reweighed. The initial and final weights (Wo and W respectively) were noted. The abrasion resistance B was calculated using the equation;

$B = 100 (Wo – W / Wo)$ ……..Eq. 1

Disintegration time test: This test was carried out using Erweka disintegration unit (model TO 88T 175). Four tablets were selected from each batch and placed individually in four tubes whose lower ends were closed by a screen of 2 mm nominal aperture. The tubes were raised and lowered in SIF maintained at 37 ± 1 ^{0}C. The time taken for the tablets to disintegrate was noted, and an average of the four tablets taken as the disintegration time for that batch.

Table 3: Result of bioadhesive determination using coated beads

Ratios	No. of beads used	Number Detached	Number Undetached	Percentage Bioadhesion (%)
1:1	10	5	5	50
1:2	10	4	6	60
2:1	10	6	4	40
1:5	10	4	6	60
1:0	10	7	3	30
0:1	10	2	8	80

Measurement of bioadhesive strength of thiamine hydrochloride tablets: The Leconte du Nouy tensiometer (model 1 Nr 3014, Akruss Hamburg Germany) was used for the study. Hog ileum of about 5 cm long and 2 cm wide was longitudinally slit to expose the mucus surface. The ileum was pinned on a cork placed on the metal support of the tensiometer. A flexible constantan wire on which a plastic plate of width 2 cm was attached was hung at the place meant for it on the lever. The plastic plate was made to gently touch the intestinal mucus surface. The plate was thereafter raised by means of a screw until it just detached from the surface of the mucus. The tension required for this was read off. Some weight was used to return the lever back to zero and the weight determined.

One tablet from each batch was glued to the plastic plate of the tensiometer using a cyanocrylate adhesive. The plate with the tablet was then hung on the lever which was then zeroed. The hog ileum on the metal support was raised to establish contact with the glued tablet.

Table 4: Results of tablet evaluation properties

Formulation Ratio (Gellan:Gelatin)	Crushing strength (Kgf)	Friability %	Disintegration time (min)	Weight uniformity (mg)
A (1:1)	5.7 ±1 (0.085)	0.8± 0.1	13.75± 1	294.8
B (1:2)	7.2± 1(0.085)	0.93± 0.1	27. 25± 1	297.4
C (2:1)	6.8 ±1 (1.085)	1.0± 0.1	11. 25± 1	299.3
D (1:5)	8.7±1 (0.585)	0.9± 0.1	21. 25± 1	292.7
E (1:0)	5.2±1 (1.085)	1.1± 0.1	7.5± 1	283. 7
F (0:1)	7.3±1 (0.085)	0.9± 0.1	12 ± 1	300.8

Table 5: Result of tensiometry test on tablets

Batch	Tension in degree	Tension in nm^{-1}
A (1:1)	41.35	$1.12x\ 10^{-1} \pm 0.1$
B (1:2)	76.00	$2.\ 06x\ 10^{-1} \pm 0.1$
C (2:1)	55.55	$1.51x\ 10^{-1} \pm 0.1$
D (1:5)	80.85	$2.19x10^{-1} \pm 0.1$
E (1:0)	31.55	$8.54x10^{-2} \pm 0.1$
F (0:1)	93.35	$2.53x\ 10^{-1} \pm 0.1$

A time interval of 5 min was allowed for tablet mucus interaction. Thereafter, the plate was raised by means of a screw until the tablet just detached from the surface of the mucus layer. The tension required for the tablets removal was read off from the tensiometer in degrees. An average of two determinations was recorded. The procedure was repeated for all the tablet batches. The respective averages in degrees were thereafter converted to tension equivalent of bioadhesive strength using the formula below:

$T = mg / 2L \times F$ ……Eq. 2.

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

Release study of thiamine hydrochloride from the formulations: In vitro release of the thiamine hydrochloride from the tablet was measured according to the USP XXIII paddle apparatus (Model 1324R Erweka, England) at 37 ^{o} C ±1 ^{o}C and at 50 rpm using 500 ml of SGF or SIF in each batch as the dissolution medium. Samples (10 ml) were withdrawn at predetermined time intervals of 5 min for 20 min, and then 10 min till 80 min. Absorbance of the corresponding samples was read spetrophotometrically at the wavelength of 446 nm and 445 nm in SGF and SIF respectively. An equal volume of fresh dissolution medium, maintained at the same temperature, was added after withdrawing each sample to maintain the initial volume constant. Percentage of drug dissolved at different time intervals was calculated using the equation generated from the standard curve.

Results and Discussion

From the data obtained (Table 3), the 0:1 (gelatin only batch) gave the highest bioadhesive property, gelatin is known to possess good bioadhesive properties. Bioadhesive force occurs between the mucus surface and the polymer (Higuchi, 1961). 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. Gellan alone gave the least value, this can be accounted for by the fact that gellan in a minute quantity and the combination absorbs water to form a tacky film which exhibits a maximum bioadhesion but when it is over-hydrated it forms a slippery mucilage, thus releasing the beads and providing an easy glide of the beads from the intestinal mucus surface (Jimenez, *et al.,* 1983). Although this test could be said to be subjective, it gave an insight into the relative bioadhessiveness of gellan gum and gelatin. Bioadhessiveness determined using this method is dependent on the mucus content of the hog intestinal surface, speed of washing and angle of inclination of the surface on which the hog item is place. The percentage friability of all admixtures of gums, except at D (1:0) was within the acceptable limits of 0.8 - 1 %. However, tablets containing Gellan and gelatin were less friable.

The percentage friability of all admixtures of gums, except at D (1:0) was within the acceptable limits of 0.8 - 1 %. However, tablets containing Gellan and gelatin were less friable. Similarity was also found between crushing strength and friability of tablet. The highest crushing strength was D (1:5) which is an indication that gelatin improve the binding property of gellan (Table 4).

The disintegration time also confirmed that gellan alone is not as good as gelatin as it disintegrate earlier than the acceptable time. Tablets made of gellan : gelatin in the same ratio disintegrated within the official limit of 15 min and not much better than gelatin when use alone as showed in F (0:1). Except, higher ratio as for B (1:2) and D (1:5), the disintegration times were far beyond the official limit.

Gelatin only gave the highest tension of $8.54x10^{-2} \pm 0.1\ Nm^{-1}$ property; this was followed by the 1:2 ratios, and the 1:5 ratios (Table 5). The batch containing gellan alone gave the least bioadhesive effect. These are indications that gelatin has a better bioadhesive property than gellan. The result also conformed to the disintegration time and friability tests that were function of good bioadhesive property.

Drug release: The data in Figures 1 and 2, showed that gelatin alone (0:1) released faster and higher in SGF than gelan gum or the combination, while the release was slower in SIF. Gelan: gelatin in the ratio of 1:1 released highest in SIF which shows the effect of pH in the release pattern of most polymers. All these could be attributed to the type of gelatine. In this study gelatine type B, used showed higher water uptake than gelan gum and its combinations (Higuchi, 1963).

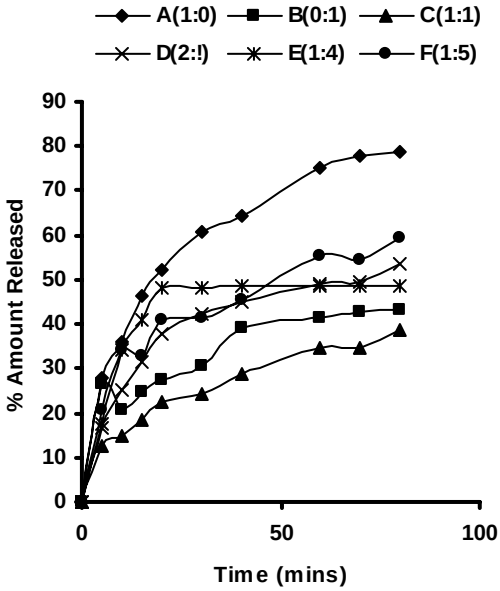

Fig. 1: Graph of percentage amonut of drug released against time in SIF

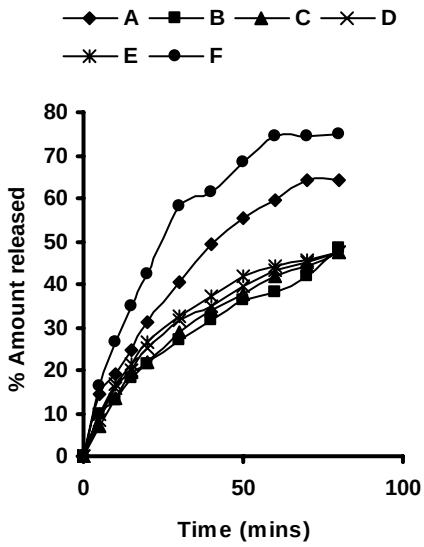

Fig 2: graph of percentage amount of drug released against time in SGF

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. However, this could lead to non uniform mixing or it may need longer mixing or kneading time.

Conclusion: Gelatin gave the highest bioadhesive properties of the two gum and their admixtures studies. Gellan is relatively low; it could be preferably in eye preparation also gelatin could be employed in preparations intended for the gastrointestinal tract, buccal or rectal use. Batch containing gelatin alone gave a higher release of the drug in SGF than in SIF, this further suggesting it use in preparation for gastric mucosa. All the tablet batches gave appreciable release of the drug.

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