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Formulation, Characterization and In - Vitro Drug Release Studies of Gastrointestinal Mucoadhesive Patches Bearing Anticancer Drug - Delivery for Colon Cancer

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Abstract-In an attempt to prolong the release and improve the absorption of the drug, we developed gastrointestinal mucoadhesive patches containing the anticancer drug - 6-mercaptopurine. We observed that all formulations prepared for the fabrication of gastrointestinal mucoadhesive patches were smooth, translucent, and flexible in nature. There was a regular distribution of weights and thicknesses across all formulations of the formulation. Furthermore, on these Gastrointestinal Mucoadhesive Patches, the following parameters were also measured: pH, folding endurance, swelling percentage (%S) and in vitro disintegration time. The release profiles of Gastrointestinal Mucoadhesive Patches have been shown to be enhanced in vitro, and the release patterns are pH- dependent, which suggests they provide faster absorption and increased reach. The method of solvent cast technology has proven to be an efficient method of preparing the patch for administering 6- mercaptopurine to the gastrointestinal tract through the application of gastrointestinal mucoadhesive patches.

Introduction

Worldwide, gastrointestinal cancer is one of the most common types of cancer [1, 2] and the main means of treating it is through invasive surgical resections. [3,4,5] As a result of the invasiveness of surgery and the resulting sequelae of an affected area, surgical resection can be a significant burden on the patient due to the long recovery period that is associated with it. [6,7,8] Numerous studies have been conducted in order to determine the effectiveness of using wireless structures for the treatment of the gastrointestinal tract, as an alternative to gastrointestinal treatment. When compared to conventional resections, wireless structures would be considered noninvasive, although they have advantages such as being patient-friendly and easy to operate

relative to conventional resections. [9,10,11] A capsule that delivers insulin-loaded microneedles to the small intestine, in which the capsule can be moved in the digestive tract by using peristalsis, has been reported as delivering insulin to the small intestine. On the other hand, the drug loaded on the microneedles in the capsule could only be exposed and delivered in a specific pH range when the capsules were placed in a certain position. [12] The capsule delivery method of delivering the drug directly to the small intestine is considerably less invasive compared to a subcutaneous injection, which demonstrates better absorption efficiency when compared to subcutaneous injection. Despite this, drug-loaded microneedles cannot be delivered rapidly to a lesion because the capsules are moved by peristalsis, and they can only be exposed and delivered to the lesion site within a specific pH range, because peristalsis is not the fastest process. Using a mucoadhesive patch delivery method using capsules as a drug-loaded multilayer delivery system, we recently presented a novel concept. [13] Drugs could be delivered quickly and accurately to multiple lesion sites because multilayer mucoadhesive patches are equipped in capsule. The mussel-inspired adhesive patch is a verified tissue adhesion material in a wet environment and has been widely studied and utilized in various fields. [14, 15, 16]

A mucoadhesive patch that has been proposed has the following features.

1. The drug can be actively delivered to a number of target lesion sites in the body.
2. As the product is manufactured with a mussel-inspired hydrogel, it exhibits biocompatibility, biodegradability, and increased adhesion to wet surfaces on the gastrointestinal tract, which are all important characteristics in this application.
3. A Colon Cancer treatment can be performed using this device.

Various experiments were performed to validate the delivery and therapeutic feasibility of the proposed mucoadhesive patch. First, adhesion force measurements were taken to confirm the sequential delivery of the proposed patches to the gastrointestinal tract. In addition, the temperature change and active drug release of the proposed patch were tested under AMF stimulation.

Currently, the exact etiology of colon cancer is unknown. However, both environmental factors and genetic susceptibility may contribute to the development of it. As a result of immune-modulators and monoclonal antibodies being introduced to the market, a great deal of progress has been made in the management of the disease, but a cure has not yet been found. For the induction and maintenance of remission in cancer of the colon with meshalazine or sulfasalazine formulations, these are the top drugs for this type of cancer. A dose of glucocorticoids (CSs) may need to be added depending on the condition. [17] In severe acute UC, cyclosporine may be an option for the patient if the intravenous steroid treatment fails to result in remission.[18] There is some evidence that Azathioprine (AZA) and 6-mercaptopurine (6-MP) can both help maintain remission although the amount of evidence that they are effective is rather limited.Among the various methods available to pharmaceutical

companies to administer drugs for systemic effects, the oral route of administration is the most convenient and important method because of the patient's acceptance and the ease of administering the drug. An oral Gastrointestinal Mucoadhesive patch is intended to retard the drug release in the stomach and small intestine for the treatment of disease like colorectal cancer. It is designed to retard the release of the drug in the stomach and small intestine. [18, 19, 20]

It has been found that 6-mercaptopurine (6-MP) is an effective immunosuppressant and anticancer agent at the same time. It has become increasingly common to prescribe the drug in human and veterinary medicine to treat inflammatory diseases such as Crohn's disease, ulcerative colitis, rheumatological disorders, etc., as well as leukemias such as acute lymphoblastic leukemia, and acute myelosuppressive leukemias.[21, 22] On the other hand, under short- and long-term therapeutic conditions, the metabolites [6-thioguanine (6-TGN) and 6-methylmercaptopurine (6-MM)] of 6-MP accumulate in the cells and cause myeloid, renal, and hepatic toxicities without much benefit to survival. [23, 24] There is still a lack of understanding of the specification mode of 6-MP, but it has been suggested that its cytotoxicity and immunosuppressive properties are due to the incorporation of its metabolite, 6-TGN/6-MM, into the DNA, resulting in poor DNA synthesis and the death of cells. [25] A mucoadhesive drug delivery system may preferred dosage forms for the treatment of CC since the drug is placed in a specific region and is maintained there for a long period of time. There are many benefits associated with mucoadhesive patches, including the fact that they are able to effectively localize the drug and its effects.[26, 27] During the bowl movement, these formulations should be strong enough to withstand the movement of the bowl while preserving the integrity of drug delivery, yet flexible enough to prevent interference with the normal movement of the bowl.[28, 29] Upon interaction of the mucoadhesive patch with porcine intestine, the mucoadhesive patch showed excellent mucoadhesion and mucoadhesion strength. Until now, research on colon cancer the drug release using patches delivered in the gastrointestinal tract has not been reported. The proposed mucoadhesive patch consisted of pectin and Eudragit S100 layers.

Materials And Methods

Materials

6-mercaptopurine monohydrate (Assay purity =98.0%), Pectin (Mw= 194.14 g/mole), Eudragit S 100 (Mw=172.18 g/mol), and Monobasic sodium phosphate (NaH_2PO) and Dibasic sodium phosphate (Na_2HPO_4)4 and Sodium Hydroxide (NaOH) were purchased from Emplura®, Merck Specialities Private Limited.

Preparation of 6-mercaptopurine Gastrointestinal Mucoadhesive Patches (GIMAPS)

In a volume of 100ml of distilled water, an appropriate quantity of pectin was

dissolved. The pectin solution was mixed with a sufficient amount of glycerin, and the mixture was subjected to sonication for a duration of 1 hour. Following the sonication process, the resulting polymeric solution was carefully poured into pre-lubricated petri plates. These plates were then set aside at room temperature until complete drying occurred. Once dried, small circular sections with a diameter of 0.5cm were meticulously excised from the material.

For the drug layer, the second stratum was formulated by dissolving 20 mg of 6 mercaptopurine in 1ml of methanol. This solution was vigorously mixed using a vortex for a duration of 5 minutes. Subsequently, 10µl of the drug solution was delicately applied onto the miniature circular patches (0.5cm in diameter) and left to undergo the drying process.

Moving on to the pH-sensitive layer, the third layer within the GIMAPS construct was fashioned by dissolving an appropriate quantity of Eudragit S 100 in methanol, thus creating a coating solution. The circular patches containing the drug were immersed in the Eudragit solution approximately 4 to 5 times and were then dried using a hair dryer. The resultant patches were earmarked for subsequent physico-chemical analyses.

Surface pH

Using a combination of pH electrodes, the surface pH of the Gastrointestinal Mucoadhesive Patches containing 6-mercaptopurine was evaluated. A segment of the film patch was dampened with milli-Q water, and the pH measurement was taken at the interface where the film and water made contact.[30]

Thickness

The measurement of the transdermal patch thickness was conducted using a micrometer screw gauge. The rectangular patch (2x2 cm) was measured at three distinct points, and the average thickness was subsequently computed. The absence of notable variations in thickness is crucial for the efficacy of the patches. [31]This identical procedure was also implemented for the evaluation of other patches.

Folding Endurance

To ascertain the folding endurance, the film was repetitively folded at a single point until it reached a point of fracture. The folding endurance value represents the count of successful folds the film can endure at that specific point before breaking. This process was repeated in four separate tests, and the mean value was subsequently derived from these four tests. [32]

Swelling Percentage (S %)

The patch's swelling index was computed under simulated conditions mirroring the pH of mucous membranes. This investigation involved weighing a patch (with a surface area of 4 cm^2) and placing it onto a designated petri-plate containing buffer media. At specific time intervals (15 seconds), the films were taken out, rapidly blotted using absorbent paper, and then re-weighed.[33] The percentage of water uptake was

determined using the following formula

$$\%S = \frac{w_f - w_i}{w_i}$$

The calculation of water uptake involves the utilization of two parameters: Wf, representing the weight of the wet grafted patch, and W*i*, signifying the weight of the dry grafted patch. These parameters are essential for determining the percentage of water uptake in the patch.

Drug Content

A 3 cm^2 sample was dissolved in 10 ml of a 0.1N sodium hydroxide solution using vortex mixing for a duration of 5 minutes, in order to extract the drug from the film. The resulting solution was then filtered through a Whatman filter paper. Subsequently, the solution was subjected to spectrophotometric analysis at a wavelength of 325 nm, with methanol being used as the reference blank.[34]

Tensile Strength

Tensile strength measurements served as practical means to assess the mechanical characteristics of the patches.[35,36] The process involved using a specially designed apparatus for measuring tensile strength. This assembly was constructed by suspending a pan using a robust thread, with the patch affixed to the opposite end of the thread. Weights were then placed on the pan, and the entire assembly was handled similar to a beam balance.

The calculation of tensile strength was derived from the following formula: Tensile Strength= Break Force/ a. b (1+ ΔL/L)

Where: a = width of the patch,

b = thickness of the patch, L = length of the patch,

ΔL = elongation of patch at break point,

Break Force= weight required to break the patch (Kg).

This method allowed for the determination of the patches' tensile strength, a crucial aspect of their mechanical behavior.

Moisture Content

Following the individual weighing of the patches, they were introduced into a desiccator containing calcium chloride and left at room temperature for a duration of 24 hours. At predetermined time intervals, the patches were re-weighed until their weight remained consistent. Utilizing the subsequent formula, the percentage of moisture content was computed. [37]

$$\text{Percent Moisture Content} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100\%$$

This approach facilitated the determination of the patches' moisture content, a vital parameter in their overall quality assessment.

***In Vitro* Drug Release**

A kinetic study was undertaken employing USP Apparatus-I, operating at 50 rpm, utilizing 600 ml of PBS (Phosphate Buffered Saline) sustained at 37°C and maintained at pH levels of 3.4, 6.4, and 7.4. In this study, separate dialysis tubes were employed, each containing 10 mg (with a concentration of 2 mg/ml) of both pure drug and Pectin-Eudragit 100 mucoadhesive patches. These tubes were immersed in PBS at a pH of 7.2.

At predetermined intervals (0, 15, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 8 hours), a 4 ml aliquot of the release medium was withdrawn. The concentration of the withdrawn sample was subsequently determined using UV spectroscopy at a wavelength of 325 nm. Importantly, to maintain a constant volume, fresh buffer (4 ml) was added without replacing the entire dissolution medium.

This methodology allowed for the examination of the release kinetics of the substances under various pH conditions, shedding light on their dissolution behavior over specific time intervals. [38]

Results

In this study, we developed gastrointestinal mucoadhesive patches containing 6-mercaptopurine, designed to target the colon, using the solvent casting technique. Pectin and Eudragit S 100 were selected as colon-targeting polymers for patch preparation. Various formulations with different ratios of drug to polymer were created and subjected to a comprehensive assessment of physical characteristics, including Surface pH, Thickness, Folding Endurance, Swelling Percentage (S%), Drug Content, Tensile Strength, Moisture Content, and in vitro drug release profiles.

Observing the amounts of polymer coating, it was evident that the weight of the film increased in correlation with a slight rise in film thickness. This phenomenon could be attributed to the higher concentration of polymer used. Meanwhile, the surface pH values of all formulations closely resembled the pH of the colon. This observation suggests that all formulations were devoid of any potential mucosal irritation.

In order to assess the flexibility and tensile strength of the patches, two tests were conducted: the folding endurance test and the tensile strength test. The findings of these studies indicated that as the polymer concentration was elevated, both the flexibility and tensile strength of the patches exhibited an increase. This effect could potentially be attributed to the presence of robust covalent bonds between the polymer and the drug.

Furthermore, the drug content across various formulations demonstrated a consistent and uniform range. This outcome suggests that the drug was effectively dispersed in a

uniform manner throughout the Gastrointestinal Mucoadhesive Patch (GIMAP) matrix. (Table 1).

Achieving appropriate swelling behavior in films is a crucial characteristic for ensuring uniform and sustained drug release, coupled with effective mucoadhesion. Upon conducting swelling studies, it was discerned that within the initial hour, approximately 35% swelling transpired across all four formulations. Subsequently, there was minimal alteration in both swelling and moisture sorption observed in the films from M1 to M9. This consistent behavior in swelling and moisture absorption across the different formulations suggests a stable and reliable property in these films.

The Figure 1 illustrates the drug release profiles of 6-mercaptopurine from formulations M1 to M9. The findings from the drug release studies unequivocally demonstrate that the release of the drug was influenced by the concentration of the polymer. Notably, there was no observable lag time when the patch was directly exposed to the dissolution medium. In the initial hour, approximately 5-12% of thedrug was released. This rapid initial release could be attributed to the presence of an erodible and

hydrophilic polymer layer. The hydrophilic nature of Eudragit S 100 polymer contributes to its dissolution, resulting in the formation of pores and channels that facilitate the diffusion of the drug from the patches.

It's worth noting that the highest drug release was observed with formulation M8, leading to its selection for further studies. This selection was likely based on its desirable drug release characteristics that align with the intended purpose of the study.

The assessment of both bioadhesive force and mucoadhesive residence time revealed that the films of formulation M8 displayed commendable mucoadhesive properties. This characteristic is highly favorable for drug administration through this route. In vitro permeation studies unveiled a gradual rate of drug permeation, with approximately 89.38% of drug permeating after a duration of 8 hours. (Table 2).

Table 1: Evaluation of drug loaded patch Evaluation of drug loaded patch [Thickness, Folding Endurance, Water-vapour transmission rate, Tensile strength] of 6 – Mercaptopurine

FC	ırface pH	Thicknes s	Folding Endurance	Weight variation (mg)	Water vapour transmission rate (gms/cm^2)	Tensile strength (dynes/cm2)	% Drugrelease

M1	6.3	0.08mm	435	56.3	0.132	3.1	70.41±1.99%
M2	6.4	0.10mm	469	43.6	0.139	4.2	83.45±1.44%
M3	6.3	0.10mm	378	44.8	0.110	2.8	74.48±0.56%
M4	6.4	0.10mm	476	46.3	0.189	2.9	93.25±2.10%
M5	6.6	0.14mm	385	48.2	0.199	3.5	89.05±0.38%
M6	6.5	0.14mm	472	46.2	0.129	3.3	82.35±0.46%
M7	6.8	0.08mm	374	71.5	1.12	3.8	67.28±0.84%
M8	6.5	0.11mm	385	64.4	0.112	4.4	91.02±0.55%
M9	6.4	0.10mm	386	63.3	0.121	4.3	90.01±0.54%

Table 2: Cumulative drug release

Time (Min)	**Cumulative drug release**								
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**
0	0	0	0	0	0	0	0	0	0
15	9.05± 0.36	8.72 ± 0.34	7.68 ± 0.37	7.75 ± 0.38	6.38 ± 0.17	6.65 ± 0.05	5.38±0.07	8.21± 0.15	6.75±0.36
30	19.7± 0.76	16.37±0.10	17.20 ±0.28	14.06 ±0.12	13.26±0.11	11.49±0.23	11.41± 0.34	17.6± 0.14	11.08±0.30
60	26.23± 0.07	21.62± 0.31	26.40±0.12	21.31±0.15	24.41±0.16	15.59±0.28	19.83±0.15	35.31±0.38	21.45±0.55
120	35.23± 0.08	29.86±0.46	35.6±0.72	30.70±0.40	32.61±0.08	31.63±0.24	31.76±0.31	47.61±0.47	39.74±0.33
180	48.87±0.32	39.82±0.18	45.11±0.07	44.15±0.10	40.61±0.28	42.53±0.31	50.03±0.10	57.42±0.20	53.02±0.12
240	53.71±0.42	47.37±0.16	56.13±0.24	55.34±0.14	48.87±0.51	53.20±0.14	57.18±0.40	76.74±0.46	64.20±0.25
360	69.82±0.55	68.29±0.13	68.68±0.32	62.14±010	68.12±0.08	71.45±0.20	66.45±0.27	86.81±0.13	78.30±0.25
480	72.6±0.38	68.29±0.37	69.89±0.38	63.12±0.14	77.25±0.13	80.65±0.35	86.85±0.15	89.38±0.37	87.89±0.53

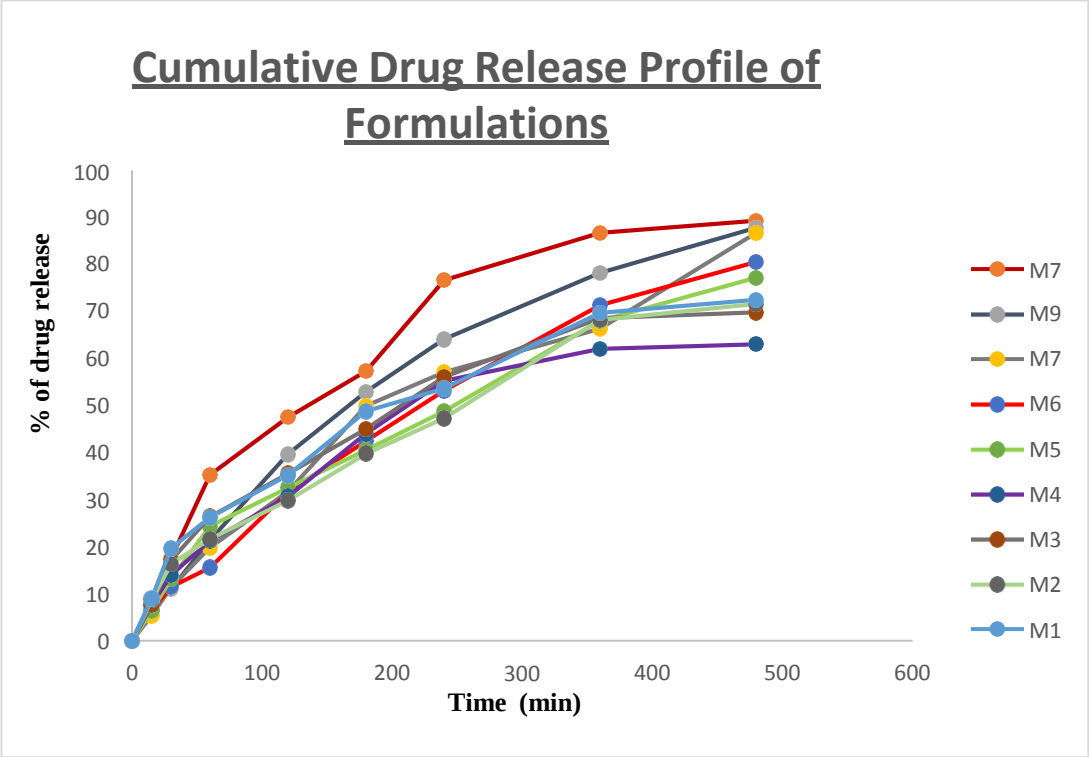

Figure 1: Cumulative Drug Release Profile of Formulations of 6 – Mercaptopurine

Conclusion

Gastrointestinal mucoadhesive patches of 6 – Mercaptopurine using polymers like pectin and Eudragit S-100 in various proportions and combinations showed satisfactory physicomechanical and mucoadhesive characteristics. The proportional amounts of various hydrophilic polymers in various formulations have influence on drug release from these formulated 6 – Mercaptopurine gastrointestinal mucoadhesive patches. From the present investigation, it can be concluded that such gastrointestinal mucoadhesive patches of 6 – Mercaptopurine may provide sustained gastrointestinal mucoadhesion for prolonged periods in the management of colon cancer, which can be a good way to bypass the extensive hepatic first-pass metabolism.

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