Section A-Research paper

Preparation, Characterization and *In Vitro* Drug Release Studies of 6-mercaptopurine Gastrointestinal Mucoadhesive Patches

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Abstract

We developed gastrointestinal mucoadhesive patches containing 6-mercaptopurine and pectin to prolong the release and improve the absorption of the drug. It was observed that all formulations prepared for the fabrication of gastrointestinal mucoadhesive patches were smooth, translucent, and flexible. Throughout the formulations, uniform weights and thicknesses were observed. The following parameters were also evaluated on these Gastrointestinal Mucoadhesive Patches: pH, folding endurance, swelling percentage (%S), and in vitro disintegration time. Gastrointestinal Mucoadhesive Patches have shown enhanced release profiles in vitro compared to pure drugs, and the release patterns are pH-dependent. To conclude, solvent cast technology is an efficient method for delivering 6-mercaptopurine to the gastrointestinal tract via gastrointestinal mucoadhesive patches.

INTRODUCTION

As effective immune-suppressants and anticancer agents, thiopurines, azathioprine, 6-mercaptopurine and 6-thioguanine are prescription medications increasingly used to treat inflammation[1]. Since 1953, 6-mercaptopurine (6-MP) has been approved by the Food and Drug Administration (FDA) as an antitumor drug[2] for treating acute lymphoblastic leukemia and acute myelocytic leukemia[3,4], as well as other diseases such as rheumatologic disorders, prevention of rejection after organ transplantation, systemic lupus erythematosus, non-Hodgkin lymphoma, inflammation (Crohn's Syndrome and Ulcerative Colitis) and other diseases[5,6].

As part of its transcriptional activation, 6-MP is metabolized into methylated thioinosinic acid (MeTIMP) by hypoxanthine phosphor ribosyl transferase (HPRT) in the cells, inhibiting the synthesis of de novo purines[7], which is then converted into thioguanine for DNA intercalation. In contrast, 6-MP undergoes extensive first pass catabolism by XO (Xanthine Oxidase) and TPMT (thiopurine S methyltransferase), which limits its bioavailability. In this instance, the drug may have a low bioavailability (about 16%) [8], a short plasma half-life (0.5–1.5 h) [9], moderate plasma protein binding (19% to 30%)[10], and a decreasing chemotherapeutic effect[10]. This investigation aimed at developing Gastrointestinal Mucoadhesive Patches containing pectin and Eudragit 100 to deliver sustained releases of 6-MP and evaluate their effectiveness.

MATERIALS AND METHODS

Materials

Preparation of 6-mercaptopurine Gastrointestinal Mucoadhesive Patches (GIMAPS)

Suitable amount of pectin was dissolve in 100ml of distilled water. Adequate quantity of glycerin dissolved in pectin solution was added and sonicated for 1hr. After sonication polymeric solution was poured in pre-lubricated petri-plate. Kept a side at room temperature for complete dry and after drying tiny patches of (0.5cm) diameter were cut down. ***Drug Layer***: Second layer was prepared by 20 mg of 6 mercaptopurine was dissolve in 1ml of methanol and vortex for 5 min. 10µl of drug solution was then poured on tiny patches of (0.5cm) diameter and allow to dry. ***pH Sensitive Layer***: The third layer in GIMAPS was prepared by taking suitable amount of Eudragit L 100 dissolved in methanol to prepare coating solution. The drug containing patches were 4-5 times dipped in Eudragit solution and dried by hair drier. The resulting patches were used for further physico-chemical analysis.

Surface pH

Utilizing combined pH electrodes, the surface pH of 6-mercaptopurine Gastrointestinal Mucoadhesive Patches was assessed. A patch of film was moistened with milli-Q-water, and pH was measured at the interphase between the film and the water at the points of contact[11].

Thickness

The thickness of the transdermal patch was measured using a micrometre screw gauge. It was measured at three different points the thickness of a rectangular patch (2x2cms) and its average thickness calculated. In order for

patches to be effective, there should be no significant variance in thickness[12]. A similar process was carried out for other patches as well.

Folding Endurance

In order to determine folding endurance, the film has been folded many times at the same point until a breaking point is reached. The folding endurance value is the number of times the film can be folded at the same point without breaking. There were four tests performed, and the mean was calculated from the four tests[13].

Swelling Percentage (% S)

A patch swelling index was calculated in simulated mucous membrane pH conditions. This study was performed by weighing a patch (surface area 4 cm^2) and transferring it to an individual petri-plate that contained buffer media[14]. At definite time interval (15s), films were removed, blotted quickly with adsorbent paper, and then weighed. The percentage of amount water uptake was calculated as follows

$$\% S = \frac{(W_f - W_i)}{W_i} \times 100$$

Where W_f is the weight of wet grafted patch and W is the weight of dry grafted patch.

Drug Content

A sample of 3 cm2 was dissolved in 10 ml methanol by vortex for 5 minute to extract drug from film and filtered through whatman filter paper and analyzed spectrophotometrically at 325 nm using methanol as a blank[15].

Tensile Strength: Measurements of tensile strength were convenient tools for determining the mechanical properties of the patches [16, 17]. The tensile strength of the patches was measured using an assembly designed for measuring tensile strength. An assembly was created by hanging the pan with strong thread and attaching the patch to the other end of the thread. Weights were kept on the pan and the whole assembly was held like a beam balance. Based on this formula, the tensile strength was calculated: The following formula was used to calculate the tensile strength:

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).

Moisture Content: After weighing the patches individually, they were placed in a desiccator containing calcium chloride and kept at room temperature for 24 hours. After a specified interval, the patches were weighed again until they showed a constant weight. Using the following formula, we calculated the percent moisture content[18].

$$\% \text{ Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

***In Vitro* Drug Release**

A kinetic study was conducted using USP Apparatus-I with 50 rpm and 600 ml of PBS maintained at 37°C and pH 3.4, 6.4, and 7.4. We placed 10 mg (conc.2 mg/ml) of pure drug and Pectin - Eudragit 100 mucoadhesive patches separately in each dialysis tube and immersed them in PBS at the pH of 7.2. An aliquot of 4 ml of release medium was withdrawn at predetermined times (0, 15, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 8 hours) and its concentration was determined by UV spectroscopy at 325 nm. Rather than replacing the dissolution medium with fresh buffer, the total volume was kept constant by adding fresh buffer (4ml)[19] .

RESULTS AND DISCUSSION

Surface pH

As study was done to investigate the effects of pH on mucosa by determining the surface pH of mucoadhesive patches[11]. 6MP mucoadhesive patch surfaces tested were 6.8 $\pm$0.1, which seemed to be almost neutral and did not cause irritation to the mucosa lining.

Thickness

The mucoadhesive patches were evaluated for thickness using Vernier calipers and mean value was found to be 0.08±0.003 mm which indicates the uniformity of the mucoadhesive patches[20].

Folding Endurance

Mucoadhesive patches' mechanical strength is determined by their folding endurance. A folded structure having a higher folding endurance will have a greater mechanical strength[21] (Semalty et al., 2008). The folding endurance of the drug loaded mucoadhesive patches was 374 which signify that these patches were strong enough to handle.

Swelling Percentage (S %)

Solvent penetration into the polymer matrix increases the weight of the films and facilitates the diffusion of drug molecules from the matrix bulk to external environments[22] (Brazel and Peppas, 2000). The mean swelling percentage was found to be 30.63%. Water absorption by these films for drug release would appear to be significant based on these results.

Drug Content

The drug content shows that the amount of drug loaded in the path which was analyzed by spectrophotometrically at 325 nm using methanol as a blank[15] (Senthil et al., 2010).

Tensile Strength: The tensile strength of the patches was measured using an assembly designed for measuring tensile strength. An assembly was created by hanging the pan with strong thread and attaching the patch to the other end of the thread. Weights were kept on the pan and the whole assembly was held like a beam balance. Based on this formula, the tensile strength was calculated: The following formula was used to calculate the tensile strength.

Moisture Content: After weighing the patches individually, they were placed in a desiccator containing calcium chloride and kept at room temperature for 24 hours. After a specified interval, the patches were weighed again until they showed a constant weight. Using the following formula, we calculated the percent moisture content.

In Vitro Drug Release Studies

In order to investigate 6-MP release behavior of Pectin - Eudragit 100 patch, were incubated in different release media (phosphate buffer pH: 3.4, 6.4 and 7.4) and assessed by double beam UV-visible spectrophotometer. Figure 4 demonstrates 6-MP release profiles up to 48h of incubation period. As shown in Figure 4, Pectin - Eudragit 100 patch showed an initial burst release of 6-MP in a period of 4-6 h for all incubation media (Aydin and Pulat, 2012). After this initial burst effect, a slower sustained and controlled release occurred throughout the incubation period. Cumulative drug release profiles of 6-MP mucoadhesive patch at pH 3.4 (62%), 6.4 (72%) and 7.4 (82%); which were better than release profiles of pure drug (46%, 48% and 45%) (Fig 4a, 4b and 4c). We could also observe significant fall in concentration of 6-MP pure drug (pH 3.4) at 16 hrs. onwards, when compared to 6-MP thin film, whereas at pH 6.4 and 7.4 there was no sudden decline in concentration. Release profiles supported that 6-MP molecules were encapsulated among the positively charged hydrophilic chains.

CONCLUSIONS

In this study, we prepared 6-MP loaded Pectin - Eudragit 100 patch for enhanced drug delivery. The preparation of drug loaded Pectin - Eudragit 100 patch is a simple technique that can be easily scaled up. In vitro drug release of 6-MP from the thin film has enhanced to a considerable extent. So these formulations can be an alternative for delivering 6-MP, which could enhance solubility, bioavailability with its sustaining drug release process.

Table 1: Evaluation of drug loaded patch Evaluation of drug loaded patch [Thickness, Folding

FC	Surface pH	Thickness	Folding Endurance	Weight variation (mg)	Water vapour transmission rate (gms/cm^2)	Tensile strength (dynes/cm2)	% Drug release
F1	6.3	0.08mm	435	56.3	0.132	3.1	70.41±1.99%
F2	6.4	0.10mm	469	43.6	0.139	4.2	83.45±1.44%
F3	6.3	0.10mm	378	44.8	0.110	2.8	74.48±0.56%
F4	6.4	0.10mm	476	46.3	0.189	2.9	93.25±2.10%
F5	6.6	0.14mm	385	48.2	0.199	3.5	89.05±0.38%
F6	6.5	0.14mm	472	46.2	0.129	3.3	82.35±0.46%
F7	6.8	0.08mm	374	71.5	1.12	3.8	67.28±0.84%
F8	6.5	0.11mm	385	64.4	0.112	4.4	91.02±0.55%
F9	6.4	0.10mm	386	63.3	0.121	4.3	90.01±0.54%

Factorial Design

Table 2: Experimental Design Employed with two Independent Variable at three level:

Formulation	Variable	
	X1	**X2**
F1	+1	+1
F2	+1	0
F3	+1	-1
F4	0	+1
F5	0	0

F6	0	-1
F7	-1	+1
F8	-1	0
F9	-1	-1

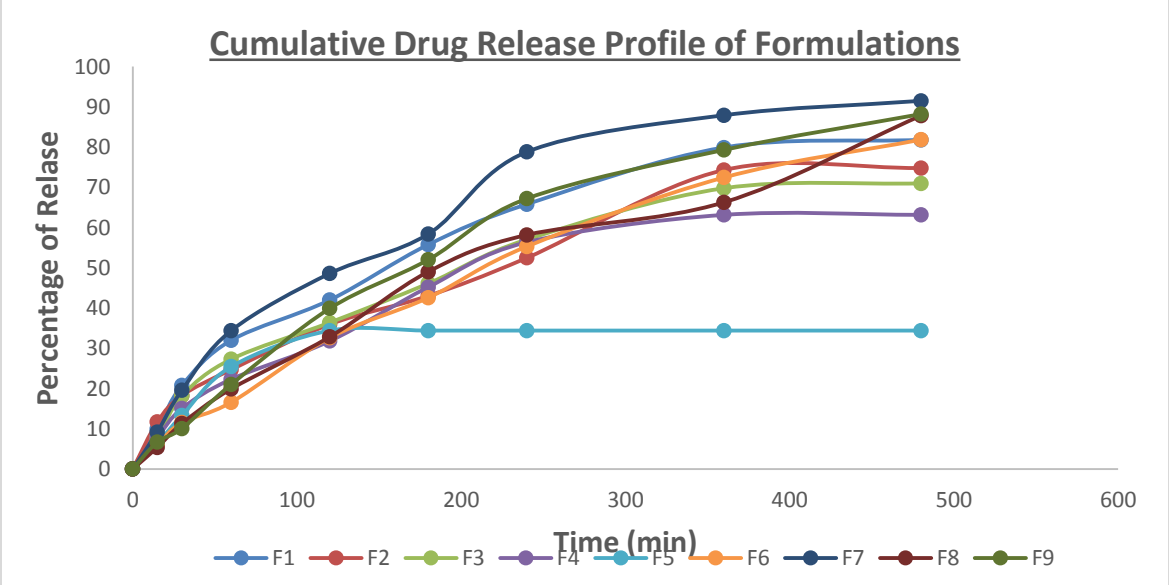
Coded Variables:

***X1 (Pectin Concentration):-** +1 = (2%), 0 = (4%), -1 = (6%)

***X2 (Eudragit L Concentration):-** +1 = (2%), 0 = (1.5%), -1 = (1%)

Table 3: Cumulative drug release

Time (Min)	Cumulative drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
15	10.00± 0.36	11.7 ± 0.34	7.88 ± 0.37	7.88 ± 0.38	6.36 ± 0.17	6.08 ± 0.05	9.21±0.07	5.39 ± 0.15	6.72±0.36
30	20.8 ± 0.76	18.27±0.10	18.20 ±0.28	15.06 ±0.12	13.31±0.11	11.59±0.23	19.6 ± 0.34	1.31 ± 0.14	10.08±0.30
60	32.0 ± 0.07	24.7 ± 0.31	27.30±0.12	22.32±0.15	25.45±0.16	16.59±0.28	34.41±0.15	19.93±0.38	21.01±0.55
120	42.0 ± 0.08	35.96±0.46	36.4±0.72	31.80±0.40	34.41±0.08	32.63±0.24	48.6±0.31	32.86±0.47	39.91±0.33
180	55.77±0.32	43.04±0.18	46.11±0.07	45.20±0.10	41.66±0.28	42.58±0.31	58.4±0.10	49.03±0.20	52.02±0.12
240	65.8±0.42	52.47±0.16	57.03±0.24	56.36±0.14	50.90±0.51	55.30±0.14	78.80±0.40	58.18±0.46	67.20±0.25
360	79.90±0.55	74.29±0.13	69.78±0.32	63.16±010	78.12±0.08	72.45±0.20	87.91±0.27	66.27±0.13	79.30±0.25
480	81.80±0.38	74.80±0.37	70.99±0.38	63.16±0.14	79.28±0.13	81.85±0.35	91.49±0.15	87.82±0.37	88.20±0.53



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