Original Article

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Preparation and *in vitro* evaluation of controlled release granules of mesalazine for colon targeted drug delivery system

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Abstract

Targeted drug delivery systems into the colon to cure different local diseases like ulcerative colitis, cancer, and irritable bowel diseases have gained attention. These drug delivery systems are more effective for local inflammation and have limited side effects. The purpose of the present study is to formulate a controlled-release system of mesalazine, an anti-inflammatory agent by fluidized bed coating. The formulation was prepared using hydroxyl propyl methylcellulose as sustained delivery and cellulose acetate phthalate for enteric-coated behavior. The prepared granules were evaluated for particles size, moisture content, friability, dissolution test. The granules made with wet granulation had a suitable size and free flowability with carr's index lower than 20. It was concluded that the prepared granules could be successfully formulated with the use of release retarding polymers. The formulation showed appropriate release retardation of the drug, indicating the potential of a delivery system. A further investigation like capsule preparation and microbial count examination is needed for better evaluation of the formulation.

Keywords: Cellulose Acetate Phthalate, Controlled Release, Inflammation, Hydroxyl Propyl Methylcellulose, Mesalazine

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1. Introduction

Oral drug delivery is the preferred route for drug administration due to patient compliance, formulation flexibility, and ease of administration (1). However, this method is insufficient for drugs intending to cure local colon disease since most drugs are absorbed before reaching the colon (2). In recent years, specific drug delivery to the colon for local diseases like irritable bowel disease (IBD), Ulcerative colitis (UC), and Crohn's disease (CD) has gained considerable attention. The beneficial effects of targeted colon drug delivery systems include lower doses consumption, reduced gastric mucosal damage, reduced drug side effects, and inhibited the first pass effect (FPE) (3, 4).

Mesalazine (5-aminosalicylic acid (5-ASA)) acts as a local anti-inflammatory compound by inhibiting cyclooxygenase, reducing prostaglandins production in the colon for mild to moderate UC patients, and maintaining treatment during a recovery phase. Absorption of the drug at the upper part of the gastric intestinal tract reduces the therapeutic effects, and the anti-inflammatory effect of mesalazine would be increased when the drug primarily targets the mucosa of the colon or terminal ileum (5). Therefore, a modified release drug delivery system to control or delay the release of the drug can provide stable delivery of the drug to the colon (6).

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Many controlled-release formulations have a pH-dependent manner and are developed with enteric-coated polymers like cellulose acetate phthalate, polyvinyl acetate phthalate, and hydroxypropyl methylcellulose phthalate (7, 8). Granulation methods include a dry method using mechanical compression-compaction to produce sludge and a wet method that utilizes liquid-like binder or solvent to form a wet mass, which assigns powder to fine granules with free flowability (9). Wet granulation is the most widespread technique used despite the limitations involving multiple unit processes like wet massing preparation, drying, and screening, which are complex, time-consuming, and expensive, requiring ample space and different types of equipment. Different granulation techniques such as roller compaction, spray dryer, supercritical fluid, low or high shear mixing, fluid bed granulation, extrusion/spheronization have been successfully developed in the preparation of various pharmaceutical dosage forms (10-12).

This study aimed to prepare controlled release mesalazine granules with a wet granulation method. Two polymers were used for this formulation include cellulose acetate (CAP) for an enteric coat (EC), and hydroxypropyl methylcellulose (HPMC) was used for extended-release behavior of granules.

2. Materials and methods

2.1. Materials

Exir Pharmaceutical Company kindly donated Mesalazine. (Borujerd, Iran). All chemical compounds and materials needed for the present study had been prepared locally, and all solvents were HPLC grade.

2.2. Drug assay

This study used the high-performance liquid chromatography (HPLC) method to determine the mesalazine amount. The applied HPLC instrument (KNAUER, Azura made in Germany) consists of a pump controller unit (P 6.1 L, FBE 182300004, KENAUVER), Reodyne injector with 20 ml loop for sample delivery with a flow rate of 1 ml/min, and ultraviolet detector (UVD2.1 L, FOA182300006, KENAUVER) for drug detection, which set at 258 nm. C18 column used as stationary phase, and acetonitrile and acetate buffer (pH =6.8) with a ratio of 60: 40 were used as mobile phase (13). Chromatogram analysis was performed by clarity® software. In this drug assay method, validation tests were performed completely. Standard sample solutions in an aqueous solution were prepared. Samples concentrations (0.5, 1, 5, 10, 25, 100 µg/ml) with serial dilution was prepared from the stock solution (1mg/ml). Each sample was prepared three times and injected into the HPLC system on three different days.

2.2.1. Method validation test

The developed method was validated by evaluation of selectivity, specificity, linearity, the limit of quantification (LOQ), precision, accuracy, limit of detection(LOD) according to the US Food and Drug Administration (FDA) guidelines (14).

2.2.2. Linearity

Serial samples (0.5, 1, 5, 10, 25, 100 µg/ml) from drug concentrations were injected into the HPLC in three separate runs. In each case, the linear regression analysis was obtained on the known added concentrations of mesalazine against the corresponding peak AUC. Then the regression coefficient (r), slope, and intercept of the resulting calibration curves were determined.

2.2.3. Within day variation

Each concentration (0.5, 1, 5, 10, 25, 100 µg/ml) was prepared three times, and each of them was injected into HPLC on the same day. The coefficient of variation (CV %) for all samples was measured.

2.2.4. Between day variation

On three different days, three same samples were used for the construction of the standard curve. The same concentrations used in the within-day variations test were prepared and analyzed by the HPLC method. The CV% was obtained in each case.

2.2.5. Limit of detection and limit of quantification

The limit of quantitation (LOQ) of the method was determined as the lowest mesalazine

concentration capable of being quantitated with acceptable accuracy (80-120%) and precision (20%). Limit of detection (LOD) was determined as the mesalazine concentration that produced a signal/noise ratio of 3.

2.2.6. System suitability tests

The following parameters were calculated as system suitability factors that indicated the suitability of the developed method (Equation 1-3).

$$\text{Number of theoretical plates}\,(N) = 5.54\left(\frac{T_R}{W_{h/2}}\right)^2 \qquad \text{(Eq. 1)}$$

$$\text{Peak symmetry}\,(ps) = \frac{w}{2f} \qquad \text{(Eq. 2)}$$

$$\text{Retainability}\,(K') = \left(\frac{Rt}{ta}\right) - 1 \qquad \text{(Eq. 3)}$$

Where "Rt" is the peak retention time, "W" is the peak width at 0.05 peak height, "Wh/2" is the peak width at 0.5 peak height, "f" is the front half-width of the peak at 0.05 peak height, and "ta" is the retention time of non-retained peak (solvent front) (15).

2.3. Preparation of mesalazine granules

2.3.1. Preparation of core

For core preparation, the wet granulation method was used with fluidized bed dryer bottom spray Glat®. For this purpose, microcrystalline cellulose (avicell) as a filler, starch paste 10% as a wet binder, and starch as a disintegrant was used. In brief, 50% of mesalazine, 16.67% of starch, and 27.5% of Avicell were mixed geometrically. The 30 mL of starch paste 10% was added drop-wise in five steps and mixed with other substances with fluidized bed dryer bottom spray Glat®. The obtained granules were left at room temperature for 24 hours (16).

2.3.2. Granule coating

Two layers for specific delivery of mesalazine to the colon were used. The inner layer was hydroxyl propyl methylcellulose (HPMC) as sustained delivery, and the outer one was cellulose acetate phthalate (CAP) for the enteric coat of mesalazine (17).

HPMC 5% in water was prepared, and mesalazine granules were coated with HPMC solution by fluidized bed dryer bottom spray Glat®. After that, 24 hours later, enteric-coated granules were coated with CAP in the mixture of dichloromethane, propyl glycol, and ethanol.

2.4. In vitro characterization tests

2.4.1. Particle size and size distribution

Particle size is an important indicator for determining the bio-fate of particles. After the drying process, particle size and size distribution were evaluated with passed the granules through sieves NO 25 and 8.

2.4.2. Moisture content evaluation

The moisture content of prepared granules was determined by comparison of the initial weight (wi) of prepared granules with the final weight (wf) of granules according to the following formula (18) (Equation 4).

$$Wi - Wf \;/\; Wi \times 100 \qquad \text{(Eq. 4)}$$

2.4.3. Flowability of granules evaluation

Tapped density tester was used for obtained obtaining tapped and bulk density of granules. Bulk density was obtained after a 100 ml glass cylinder filled with granules, whereas tap density obtained after 1250 taps were applied to the granules. By calculating these parameters, carr's index (CI) according to the following equation was obtained. This parameter provides good information for granules flowability (Equation 5).

$$CI = \frac{\tilde{n}T - \tilde{n}B}{\tilde{n}T} \times 100 \qquad \text{(Eq. 5)}$$

The carr's index is higher than 25 indicates poor flowability of the granules, and CI lower than 20 indicates good flowability (19).

2.4.4. Dissolution test

In vitro dissolution test was performed for coated granules using USP dissolution apparatus II (paddle type). In brief, 900 ml of HCl 0.1 N with pH 1.2 was transferred to the apparatus and allowed to reach the temperature of 37°C. 120 mg of coated granules were rapidly placed into the apparatus, and the test was started. Sampling was carried out at times 1 and 2 hours, and the solution was

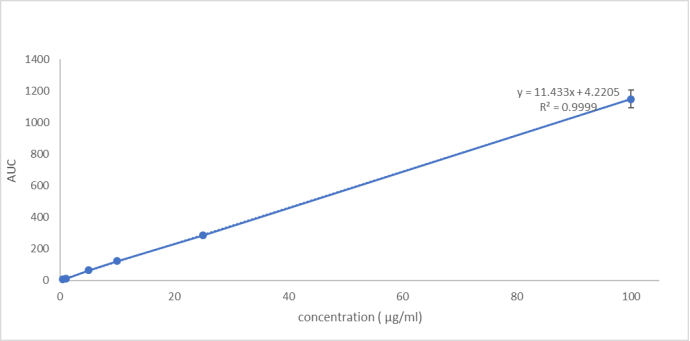

Figure 1. Calibration curve of mesalazine (n=3).

filtered through a sieve with the mesh number 80. Then granules were placed in the phosphate buffer with pH 6.8 in the apparatus with a temperature of 37°C. Sampling was performed at times 1, 2, 3, and 4 hours. Then the solution was filtered through a sieve with number 80, and granules were placed in phosphate buffer with pH 7.5. The sample was withdrawn at a time of 90 minutes.

In all steps, the temperature was kept at 37 °C with 100 rpm. All samples were kept at -20°c for further evaluations (20).

3. Results and discussion

3.1. Drug assay

In this study, the HPLC method was used to analyze the mesalazine amount. The retention time was 3 minutes at 258 nm. Figure 1. demonstrated a linear correlation between concentration and AUC in Mesalazine concentration 0.5 to 100 mg/ml. Linear regression with R2 (0.9999), slope (11.433), and intercept (4.2205) were acquired. In statistical regression analysis, the correlation between R square and adjusted R square was determined. With p-Value < 0.05, the R square and adjusted R-square are 0.9999 and 0.9999, respectively. Additionally, the hypothesis test was deliberated for intercept and slope with p-value<0.05 and p-value>0.05, respectively.

3.2. Method validation test

The LOD was 200 ng/ml, and LOQ was 0.5 µg/ml. The number of theoretical plates (N), peak symmetry (Ps), and K' were 1929.0036, 0.3, and 2, respectively. The values obtained by between and within day variations and given in Table 1.

3.3. In vitro Characterization tests

3.3.1. Particle size and size distribution

In order to unify the granule's size and size distribution, the granules were passed through the sieves. The granules were placed at the surface of sieve 25 were selected as optimal size. Results from particle size and size distribution indicated uni-dispersed particles larger than 710 mm. Due to their uniformed size and smooth surface was desirable candidates for designing different types of specific colon delivery systems.

3.3.2. Moisture content

The moisture content of obtained granules was less than 5%. The low amount of moisture content and the absence of fine particles in final granules indicated that binder (microcrystalline

Table 1. The accuracy of within and between day variations of mesalazine.

conc. (µg/ml)	Within day variations Accuracy % (Mean±SD)	Between day variations Accuracy % (Mean±SD)
0.5	78.47±0.05	111.27±0.16
1	78.87±0.002	78.27±0.08
5	107.04±0.26	110.80±0.53
10	113.80±0.13	109.07±1.07
25	101.88 ±1.07	105.08 ±0.91
100	105.25±4.93	104.83±10.28

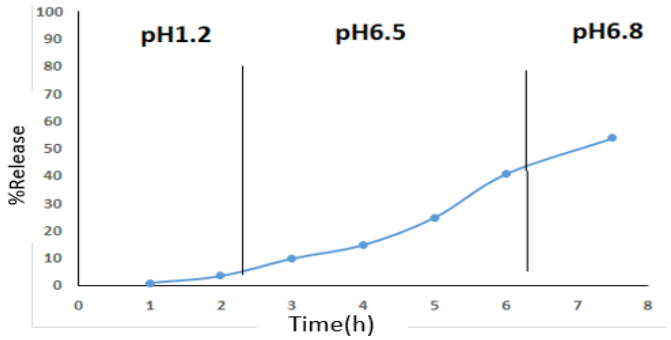

Figure 2. The results of drug released from granules.

cellulose) and filler (starch paste 10%) have a good performance in granule preparation.

3.3.3. Flow ability of granules

The CI of prepared granules was calculated 18, and this parameter lower than 20 indicated good flowability of granules.

3.3.4. Dissolution analysis

The results of drugs release (Figure 2.) in different media from granules with different pH indicated extended-release and enteric-coated behavior of granules in acidic media.

4. Conclusion

A colon-targeted drug delivery-based novel drug delivery system has been developed to provide controlled release systems for oral delivery of mesalazine. The formulation showed appropriate release retardation of the drug, indicating the potential of the delivery system. A further investigation like capsule preparation and microbial count examination is needed for better evaluation of the formulation.

Conflict of Interest

The authors have no conflict of interest.

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