BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902020000419147

Article

# Sodium alginate-guar gum and carbopol based methotrexate loaded mucoadhesive microparticles for colon delivery: An *in vitro* evaluation

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Methotrexate (MTX) is famous for its therapeutic potential against different cancers including colorectal cancer. Goal of the present investigation was to formulate MTX loaded mucoadhesive microparticles for colon targeting. The optimized formulation (MTX-MS2) was composed of mucoadhesive polymers (sodium alginate, guar gum and carbopol 940) in an appropriate ratio. MTX-MS2 was developed by ionic-gelation method. The suitable particle size and zeta potential were found to be 21.10 ± 0.18 µm and 3.01 ± 0.16 mV for MTX-MS2 respectively. The % yield (98.60 ± 2.12), % entrapment efficiency (97.98 ± 1.22) and % drug loading (1.04 ± 0.03) were estimated for MTX-MS2. The swelling index (0.99 ± 0.04 θ) and mucoadhesion (97.29 ± 4.61%) were significantly (*** $P < 0.01$) achieved with MTX-MS2 as compared to other formulations. The optimum drug release (96.07 ± 4.52%) was significantly achieved with MTX-MS2 at simulated gastric fluid (pH 7.4) for 36 h in a sustained manner. This profile may be attributed towards excellent mucoadhesivness of the polymers used in the formulation. Therefore, the current investigation suggests that mucoadhesive carrier system could be promising approach for colon delivery. Thus, the proposed work would be helpful for the treatment of colorectal cancer.

**Keywords:** Methotrexate. Mucoadhesive microparticles. Colorectal cancer. Sodium alginate. Guar gum. Colon.

## INTRODUCTION

In the last few decades, a continuous quest has been raised towards colonic delivery of drugs to enhance their therapeutic efficacy at target sites and minimize related side effects. Although, merely a few approaches like colon targeted drug delivery system that has the ability to work in a complex environment of the gastrointestinal tract (GIT) of the human (Anande, Jain, Jain, 2008). From patient compliance point of view, the oral route has been considered as a most preferred route for delivery of the dosage forms. An effective level of drug absorption is depended on the physico-chemical properties of GI fluids. But this route has certain limitations of poor bioavailability due to gastric degradation at acidic (pH ~1.5) surrounding of the stomach. Hence, to get a therapeutic response required dose of the drug is increased. Dose frequency and related side effects also persists (Chen *et al.*, 2018; Teruel *et al.*, 2018). Therefore, the targeted oral drug delivery approaches have been popularized with biodegradable and biocompatible polymers. They offer specific advantages over the conventional drug delivery systems like drug protection during site specific targeting; improved drug release at the target site by smart carrier system, stabilization of drug in stomach fluid, minimized side effects and prolonged drug release to maintain the blood-plasma drug concentration in a sustain manner. The colon targeted drug delivery system is highly required for site specific therapy against colon disease, particularly colorectal cancer (Akala *et al.*, 2003; Choudhury *et al.*, 2012; Ma *et al.*, 2016; Kang *et al.*, 2018).

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Colorectal cancer is one of the fourth common deadly diseases in the world that generally occurs in cecum, rectum or colon part of the large intestine. According to the International Agency for Research on Cancer (IARC) and Globocan reports 2018, the annual mortality rate of colorectal cancer is reported to be 8,80,792 (9.2%) worldwide (Data source: Globocan 2018; http://gco.iarc.fr/today). It has been associated with numerous risk factors such as ulcerative colitis, diet, modern life-style, genetic, viral and bacterial infection. It has been characterized by the malignant development in epithelium tissues of the colon through a series of histopathological and clinical consequences like adenomatous polyps. It results from the accrual of mutation in oncogenes and tumor suppressor genes. Molecular pathway of colorectal cancer is related to the activation and deactivation of WNT, and transforming growth factor (TGF) -β signaling pathway respectively, which results in MYC regulator gene activity and transcription factor (Wong, Colombo, Sonvico, 2011; Bak, Ashford, Brayden, 2018).

Methotrexate (MTX) is a potent chemotherapeutic agent used in the treatment of colorectal cancer, breast cancer, osteosarcoma, acute lymphocytic leukemia, lymphoma etc. (Oliveira *et al*., 2015).

Sodium alginate (SA) is a sodium salt of alginic acid (anionic polysaccharide) obtained from seaweed (*Laminaria hyperborea*, *Macrocystis pyrifera* and *Ascophyllum nodosum*) and bacteria (Pseudomonas sp. and Azotobacter sp.). SA consists of α-1,4-$_{L}$-guluronic acid and β-1,4-$_{D}$-manurunic acid unit which has the good gelling ability in aqueous media. The specific feature of SA includes mucoadhesiveness, pH-sensitivity, cross-linking capability, low toxicity and biodegradability that offer more suitability for colon drug targeting (Prajapati, Bansal, Sharma, 2012; Agüero *et al*., 2017).

Guar gum (GG) is a seed gum obtained from the plant, *Cyamopsis tetragonolobus*. GG is a reverse type polysaccharide containing mannose [(1→4)-β-$_{D}$-mannopyranosyl] and galactose [α-$_{D}$-galactopyranosyl] unit linked by (1→6) linkage. GG has been well explored as a polymer for colon targeting due to its extraordinary gelling efficiency, mucoadhesiveness, biodegradability and sustained release property (Gaba *et al*., 2011). GG is a pH responsive polymer act by chemical modifications of functional groups like -$CH_3$, -COOH, $SO_3H$, -$CONH_2$ which makes it more suitable for delivery of bioactive molecules to the colon (George, Shah, Shrivastav, 2019).

Carbopol 940 (CP) is a high molar mass cross-linked polyacrylic acid polymer and generally regarded as safe (GRAS) polymer by FDA. It has extremely wetting and colloidal viscosity property (1000 times more viscous) which forms a sparkling clear gel in water and alcohol. CP has been explored as a mucoadhesive, biodegradable and biocompatible polymer for colon delivery (Singla, Chawla, Singh, 2000; Guo, 2003; Andrews, Laverty, Jones, 2009; Amit *et al*., 2012; Guzmán *et al*., 2018).

Nowadays, the mucoadhesive microparticle is being used as a promising carrier for drug delivery to colon, especially in case of colorectal cancer. The mucoadhesive drug delivery system offers enhanced drug absorption through the colonic mucosal surface by intimate contact with longer retention time. It makes possible to 'site and specific' targeting of drug loaded mucoadhesive microparticles for improved therapeutic efficacy (Tao *et al*., 2009; Ahmad *et al*., 2012; Jelvehgari, Mobaraki, Montazam, 2014; Preisig *et al*., 2016; Mansuri *et al*., 2016). The aim of the present study was to prepare methotrexate loaded microparticles by using a blend of mucoadhesive polymers for the treatment of colorectal cancer.

## MATERIAL AND METHODS

### Material

Methotrexate (assay 99%) was procured from Sigma Chemical Co., USA. Folitrax tablet (IPCA Laboratories Ltd., Mumbai, India) was bought from a local medical store. Sodium alginate was purchased from Finar Chemicals Ltd., Ahmedabad, India. Guar gum, carbopol 940 and calcium chloride dihydrate were obtained from Qualikems Fine Chem Pvt. Ltd., Vadodara, India. Glacial acetic acid was purchased from Merck, Mumbai, India. The other analytical grade chemicals and distilled water (DW) was used throughout the experiment.

### Preparation of calibration curve

UV-study of MTX in various buffer solutions has been performed as per the procedure described by Ayyappan *et al.* (2010) and Oliveira *et al.* (2015). Briefly, MTX (10 mg) was accurately weighed and dissolved in 100 mL volumetric flask containing hydrochloric acid buffer (0.1 N HCl, pH 1.5) and simulated gastric fluid (SGF, pH 4.5, pH 7.4) individually. Then volume was adjusted up

to mark to get a 100 µg/mL concentration in the buffer medium of pH 1.5, pH 4.5, and pH 7.4 separately.

Different volumes 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mL were taken from various stock buffers and volume made up to 10 mL with each buffer pH 1.5, pH 4.5, and pH 7.4 individually in a volumetric flask to produce 2, 4, 6, 8, 10, 12, 14, 16 18 and 20 µg/mL respectively. Prior measurement, the resultant sample solutions were passed through the through 0.45 µm syringe filter. Then the absorbance of each sample was recorded at 256 nm by UV-spectrophotometer, Shimadzu 1700, Japan (Lanjhiyana *et al.,* 2010). The MTX standard curve was plotted between absorbance and concentration for each media [0.1 N HCl pH 1.5 ($R^2$ = 0.9980), SGF pH 4.5 ($R^2$ = 0.9988) and pH 7.4 ($R^2$ = 0.9978)].

### Preparation of mucoadhesive microparticles

MTX loaded mucoadhesive microparticles were formulated by modified ionic gelation process according to the method described by Amin, Ahmed, Mannan (2016). Briefly, sodium alginate (SA 3%, w/v) was dissolved in distilled water (DW). Carbopol 940 (CP; 250, 500, 750, 350 and 150 mg) and guar gum (GG; 500, 500, 250, 150 and 100 mg) were suspended in 25 mL DW separately and kept at room temperature for 24 h to swell completely. The CP and GG dispersion were mixed together at equal ratio (1:2, 1:1, 3:1, 2.33:1 and 1.5:1 w/w) under digital magnetic stirrer (Remi, India) at specific stirring rate for 1 h in order to get a homogeneous mass. MTX (25 mg) was dissolved in a weak acidic solution and then added into gum slurry. Further, this slurry was added in SA solution and mixed properly at an appropriate stirring rate by using magnetic stirrer as shown in Table I. Crosslinking solution was prepared by dissolving calcium chloride (CC, 5% w/v) in DW containing glacial acetic acid (GAA, 10%, v/v).

The drug and gum mixture should be free from air bubbles before use. This mixture was added drop-wise into the cross-linking solution (CC) through a disposable syringe needle (24 G size). The drug loaded micro beads were formed immediately and kept aside for 30 min to complete the reaction. The MTX loaded beads were collected by filtration and washed with a DW 4-5 times to remove the $CaCl_2$ residues from the beads. Beads were dried under hot air oven at medium temperature (55 ºC) for 3 h. The spherically dried MTX-mucoadhesive microparticles were packed into airtight vials and stored in desiccator for further studies. Similarly the placebo (without drug) mucoadhesive microparticles were also prepared. The experimental data was demonstrated as a mean ± standard deviation (SD). All the tests were conducted in triplicate (n = 3).

**TABLE I –** Formulation of MTX loaded mucoadhesive microparticles

| Formulation Code | Drug: Polymer ratio (mg, w/w) | Polymer ratio [CP : GG] (mg, w/w) | Stirring rate (rpm) |
|---|---|---|---|
| MTX-MS1 | 1:30 (25:750) | 1:2 (250:500) | 500 |
| MTX-MS2 | 1:40 (25:1000) | 1:1 (500:500) | 1000 |
| MTX-MS3 | 1:40 (25:1000) | 3:1 (750:250) | 1500 |
| MTX-MS4 | 1:20 (25:500) | 2.33:1 (350:150) | 2000 |
| MTX-MS5 | 1:10 (25:250) | 1.5:1 (150:100) | 2200 |

Where, CP: Carbopol 940; GG: Guar gum

## EVALUATION OF MTX LOADED MUCOADHESIVE MICROPARTICLES

### Analysis of zeta potential, particle size and polydispersity index (PDI)

The zeta potential, average particle size and PDI of distilled water-suspended mucoadhesive microparticles (MTX-MS1-5, 10 mg/mL) were evaluated by using Zetasizer, Nano ZS90, Malvern instruments Ltd., UK with a laser (50 mV). The experiments were performed in a controlled environment of 25 ± 0.5 ºC.

### % Yield, drug loading (% DL) and drug entrapment efficiency (% EE) study

% Yield, % DL and % EE of the MTX loaded mucoadhesive microparticles (MTX-MS1-5) were determined according to the method suggested by Tao *et al.* (2009) and Anande, Jain, Jain (2008). Briefly,

100 mg microparticles were accurately weighed and grounded into a fine powder. Then the powders were suspended in 90 mL DW containing 2 mL of basic media (0.4% NaOH) and ultrasonicated for 2 h, final volume was adjusted up to 100 mL. Before UV-spectrophotometer analysis at 256 nm, the samples were passed through a membrane filter (0.45 µm), exactly 1 mL filtrate taken out and 10 times diluted with DW. Analysis was performed in triplicate (n = 3). The % EE, % DL and % yield were calculated according to the following equations:

$$\%EE = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100$$

$$\%\ DL = \frac{\text{Total amount of drug in microparticles - Amount of free drug}}{\text{Total weight of microparticles}} \times 100$$

$$\%\ Yield = \frac{\text{Total weight of microparticles}}{\text{Total weight of drug, polymer and other non-volatile solids (if added)}} \times 100$$

## Swelling index

Swelling index was determined by measuring the extent of swelling of microparticle formulations (MTX-MS1-5) in SGF (Chaurasia *et al.*, 2008; Gaba *et al.*, 2011). Briefly, microparticles (100 mg) were accurately weighed and poured in SGF (pH 7.4) at 37 ± 0.1 ºC for 24 h to swell completely. The filter paper was used to soak the surface adhered excess SGF drops and weights of the swollen microparticles were measured. The swelling index (α) of the formulations were determined according to the following equation:

$$\%\ Swelling\ index\ (\alpha) = \frac{W2 - W1}{W1}$$

Where, W2 = Total weight of microparticles at equilibrium swelling in the medium, W1 = Initial weight of microparticles.

## Mucoadhesion by *in vitro* wash-off method

*In vitro* wash-off technique was used to determine the mucoadhesive properties of MTX-loaded microparticle formulations (MTX-MS1-5) (Malik *et al.*, 2013). Briefly, intestinal mucosa of the goat was received from the local slaughter house. The specific size (1 × 1 cm) of intestinal mucosa was applied over the glass slide (7.5 × 2.5 cm) and tied with the thread which was used for measurement of mucoadhesion efficiency of the MTX-MS1-5. Tablet disintegration test apparatus was applied for evaluation; each formulation (~50 microparticles) was sprinkled on the mucosal surface separately which was connected to an arm of apparatus. The samples were dipped in 900 mL beaker containing SGF pH 7.4 at 37 ± 0.5 ºC with regular movement (up and down). The microparticles were observed time to time for adherence with membrane and adhered microparticles were recorded for a period of 12 h. The microparticles mucoadhesion percentage was calculated by the following formula:

$$\%\ Mucoadhesion = \frac{\text{Number of adhered of microparticles}}{\text{Total number of applied of microparticles}} \times 100$$

## *In vitro* drug release study

SGF is a commonly used dissolution medium intended to represent stomach acid. SGF (pH 1.2) consist of NaCl (2 g), pepsin (3.2 g) and HCl (7.0 mL). These components were dissolved in DW and volume was adjusted up to 1 L (Anande, Jain, Jain, 2008). *In vitro* drug release study from MTX loaded formulations (MTX-MS1-5) and marketed MTX tablet (Folitrax 10 mg, IPCA Laboratories Ltd., Mumbai, India) was performed according to the modified procedure as reported by Anande, Jain, Jain (2008). Multiple dissolution rate test (six paddles) apparatus (Vankel Vk-7010 Dissolution Apparatus with Vk 750d Heater Circulator, Varian, Inc., Cary, North Carolina) was used for performing the release profile of the formulations. SGF was used as the dissolution medium. The MTX-MS1-5 formulations (100 mg) were suspended into 1 L dissolution beaker containing SGF (900 mL) and this dispersion medium was agitated at 100 rpm speed at a temperature (37 ± 0.5 ºC) to attain the sink condition. The different SGF pH and gastrointestinal transit conditions were obtained through changing the SGF medium at specified time intervals. Initially, SGF (pH 1.5) was kept for 2 h with the help of 0.1 N HCl. Then accurately weighed quantity of potassium di-hydrogen phosphate (1.7 g) and di-sodium-hydrogen-phosphate-dihydrate (2.2 g) were incorporated into the SGF medium. SGF pH was adjusted to 4.5 with NaOH (1 M)

for studying the release rate of the formulations for 2 h period, which was further studied at pH 7.4, maintained by 1 M NaOH for prolonged periods. A 5 mL sample was pipette out and transferred into pre-cleaned test tube. The sink condition of the SGF medium was maintained by replenished with an equal amount of fresh medium. Before measurement, the collected test samples were passed through 0.45 µm syringe filter, NYL. Then all the samples were analyzed using UV-spectrophotometer (Shimadzu 1700, Japan) at 256 nm. The concentration of MTX in the test samples was obtained from the calibration curve. The experiments were performed in triplicate (n = 3).

### FTIR analysis

FTIR spectrophotometer, Perkin Elmer, USA was used to analyze the MTX and its formulations. The spectrum investigation was carried out to confirm the compatibility of pure drug (MTX) with different excipients which were used for the preparation of optimized MTX loaded mucoadhesive microparticles (MTX-MS2) and placebo formulation (PL-MS2, without MTX-mucoadhesive microparticles). The KBr discs of individual ingredients i.e. pure MTX, MTX-MS2 and PL-MS2 were prepared and spectrum of the sample was recorded in the instrument at 4000-500 $cm^{-1}$ wave number.

### Surface electron microscopy (SEM) study

The surface behavior of MTX-MS2 was studied by using scanning electron microscopy (Jeol JSM-1T300LV, Delhi, India). Prior to analysis, the formulation was spread on the aluminium stub containing double adhesive tape and scanned for their surface, internal matrix and shape properties. Suitable photomicrographs of the formulations were recorded.

### Statistical analysis

A one-way analysis of variance (ANOVA), Dunnet's post hoc test was applied for analysis of the experimental data. Graph Pad Prism software-5, San Diego, CA, USA was used for statistical analysis of the data. All the data were determined by the mean ± standard deviation (SD) and mean variations were considered to be significant at $P < 0.05$.

## RESULTS AND DISCUSSION

### Development of MTX-mucoadhesive microparticles

MTX loaded different mucoadhesive microparticles (MTX-MS1-5) were developed by an appropriate ionic-gelation at a various drug: polymer ratios (w/w). Time of cross linking between $CaCl_2$ (10% GAA) and sodium alginate based CP/GG slurry was a key point for the formation of spherical micro-beads at specific stirring rates under magnetic stirrer. The optimized stirring rate was found to be 1000 rpm for fabricating MTX loaded appropriate mucoadhesive microparticles.

### Particle size and zeta potential

The particle size, PDI and zeta potential of the MTX loaded mucoadhesive microparticles (MTX-MS1-5) were analyzed significantly. Details have been explained in Table II. The particle size (21.10 ± 0.18 µm), PDI (0.89 ± 0.06) and zeta potential (3.01 ± 0.16 mV) were observed for optimized formulation, MTX-MS2 (Figure 1).

The particle size of any dosage form plays a major role in drug dissolution and absorption through the biological membrane. As we know that if the particle size is fine (micron size) so its surface area is also higher, which may facilitate the drug absorption from the mucosal membrane. The size of particle and surface area are inversely proportional to each other which affects the dissolution as well as the absorption rate of the formulation (Muramatsu, Kondo, 1995). Hence, the optimized formulation has a suitable particle size for drug dissolution and release. The low PDI value indicates the homogeneity of the formulation.

The positive zeta potential value indicates toward the better stability of the formulation. This phenomenon would be appropriate for the interaction of mucoadhesive microparticles with the negatively charged contents of intestinal mucosa. The +Ve charged microparticles may interact with the -Ve charged colonic mucosal contents (fucose residue of mucin, sulfate, and sialic acid) via electrostatic interactions which may prolong the colonic or intestinal residence time for the formulation. Intestinal mucoadhesion can be beneficial for colon delivery with improved mucosal surface contacts that facilitates site of action by cellular uptake (Anande, Jain, Jain, 2008; Hua *et al.*, 2015).

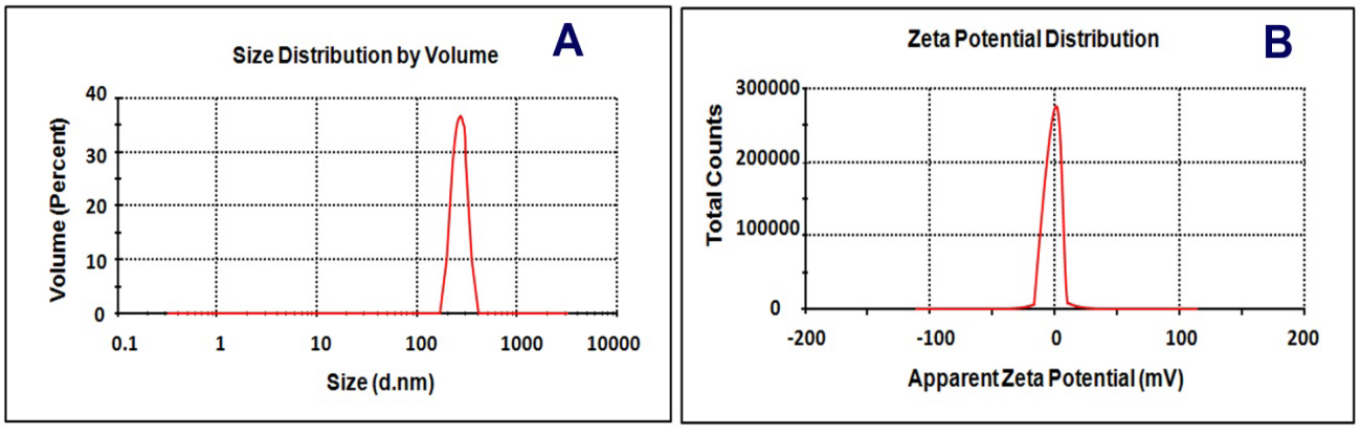


**FIGURE 1 –** Particle size (A) and zeta potential (B) optimized formulation, MTX-MS2.

### % Yield, % EE and % DL

% Yield, % EE and % DL of the different formulations, MTX-MS1-5 were calculated and the results were represented in Table II. Optimized formulation, MTX-MS2 exhibited 98.60 ± 2.12% yield, 97.98 ± 1.22% EE and 1.04 ± 0.03% DL. It may be due to an appropriate ratio of drug: polymer (1:1 w/w) for development of microparticles at constant 1000 rpm. At this ratio, % EE and % DL for MTX was found to be optimum, hence % yield was also good for MTX-MS2.

### Swelling index

Swelling properties of different formulations were performed in simulated intestinal fluid (pH 7.4) and results were represented in Figure 2 (A). MTX-MS1-5 showed SI ($\theta$) in between 0.78 ± 0.24 to 0.99 ± 0.04 while MTX-MS2 exhibited 0.99 ± 0.04 SI ($\theta$) for longer periods (24 h), which may be effective for colon targeting. As result stated that the SI value was higher for optimized MTX-MS2 due to cross-linked polymer, CP and GG with sodium alginate. Crosslinking of polymer with calcium chloride extended the swelling process in intestinal fluid and *in vitro* digestion of MTX-microparticles. It could be effective for sustained drug release from microparticles.

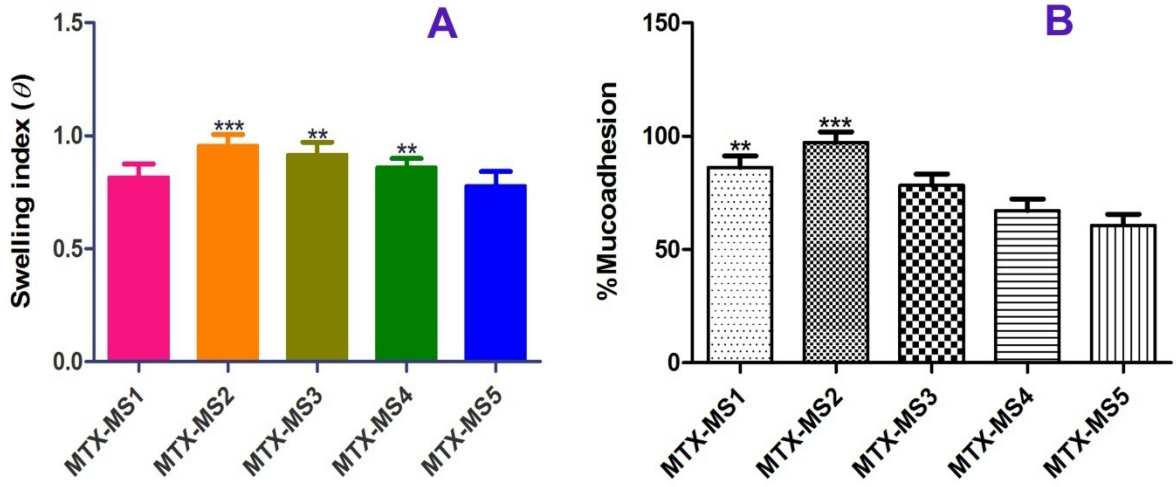


**FIGURE 2 –** Swelling index (A) and mucoadhesion (B) of different formulations, MTX-MS1-5. Values were mean ± SD (n = 3). $^{**}P < 0.05$, $^{***}P < 0.01$.

## Mucoadhesion

*In vitro* wash-off method was used to test mucoadhesive property of MTX-MS1-5 in the goat intestinal mucosa and results were represented in Figure 2 (B). % Mucoadhesion of various formulations, MTX-MS1-5 was performed to check out the adhesion efficiency of microparticles with the epithelium tissues (mucosa) of the colon. Mucoadhesion (60.65 ± 4.95 to 97.29 ± 4.61%) were observed for MTX-MS1-5. The MTX-MS2 showed significant mucoadhesion (97.29 ± 4.61%) ($P < 0.001$) compared to other formulations. This may be due to mucoadhesive affinity of the CP and GG gum towards colonic mucosal membrane containing glycoproteins.

**TABLE II –** Characterization of MTX loaded different mucoadhesive microparticles

| Formulation Code | Particle size (µm) | PDI | Zeta potential (mV) | % EE | % Yield | % DL |
|---|---|---|---|---|---|---|
| MTX-MS1 | 50.13 ± 0.12 | 0.76 ± 0.08 | 4.92 ± 0.28 | 86.12 ± 2.14 | 84.78 ± 3.16 | 0.87 ± 0.06 |
| MTX-MS2 | 21.10 ± 0.18 | 0.89 ± 0.06 | 3.01 ± 0.16 | 97.98 ± 1.22 | 98.60 ± 2.12 | 1.04 ± 0.03 |
| MTX-MS3 | 35.50 ± 0.20 | 0.81 ± 0.10 | 3.86 ± 0.26 | 92.43 ± 3.11 | 90.88 ± 4.18 | 0.89 ± 0.08 |
| MTX-MS4 | 55.02 ± 0.22 | 0.79 ± 0.18 | 4.14 ± 0.21 | 90.75 ± 4.19 | 89.68 ± 5.25 | 0.88 ± 0.14 |
| MTX-MS5 | 67.28 ± 0.36 | 0.75 ± 0.27 | 5.93 ± 0.30 | 77.84 ± 5.31 | 78.38 ± 6.41 | 0.77 ± 0.18 |

Where, values were represented as (mean ± SD, n = 3).

## Drug release

In different formulations, a remarkable drug (96.07 ± 4.52%) was released from MTX-MS2 compared to MTX-MS1 MTX-MS3-5 and Folitrax. The % cumulative drug release profile of MTX-MS1-5 was minute and insignificant at pH 1.5 and 4.5 for 2-4 h [Figure 3 (A & B)]. Only 12.52 ± 2.37% drug was released from the uncoated Folitrax tablet for 2 h at pH 1.5 but in case of MTX-MS2 very less amount (>1.80 ± 0.09%) of MTX was released for 2 h at pH 1.5. At pH 4.5, 21.01 ± 4.11% drug content was released from Folitrax, while 2.91 ± 0.08% MTX was released from MTX-MS2 after 4 h.

A few quantities of drugs were released from microparticles in an acidic environment (pH 1.5 and pH 4.5) due to drug diffusion. But in the case of pH 7.4, the good amount of drug was released which may be due to polymeric matrix erosion and drug diffusion process. MTX (96.49 ± 5.06%) was released from the Folitrax for a period of 12 h at pH 7.4 because of the uncoated tablet. MTX-MS2 exhibited remarkable drug release (96.07 ± 4.5%) for 36 h in a sustained order at pH 7.4. It may be due to the significant mucoadhesive ability of polymers used in the formulation. The hypothetical concept of mucoadhesive drug delivery system for colon targeting has been shown in Figure 4.

## FTIR

The drug-excipient interaction study of MTX-MS2 formulation was studied by FTIR spectroscopy. Details of the FTIR spectrum are shown in Figure 4 (A-C). Drug sample FTIR spectrum data were interpreted and matched with standard FTIR spectra of methotrexate, which confirms the authenticity of the sample drug by identifying peaks as similar as reference methotrexate.

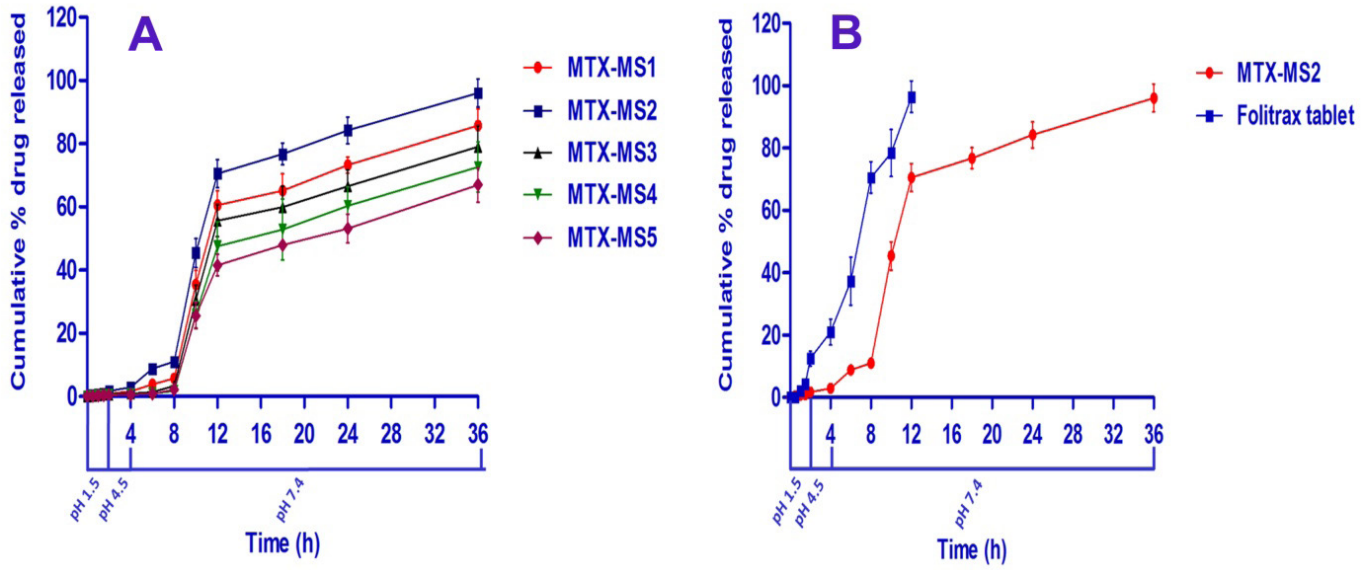


**FIGURE 3 –** *In vitro* drug release profile of methotrexate loaded mucoadhesive microparticles, MTX-MS1-5 (A) and optimized microparticles, MTX-MS2 and Folitrax (B) at pH 1.5, 4.5 and 7.4. The values were mean ± SD (n = 3).

FTIR spectrum of pure MTX represents bands located at 3323.35 $cm^{-1}$ (O-H, N-H stretching), 2993.52 $cm^{-1}$ (O-H stretching), 1683.86 $cm^{-1}$, 1645.28 $cm^{-1}$, 1544.98 $cm^{-1}$ (mixed, C-C stretching in aryl ring), 1496.76 $cm^{-1}$ (N-O asymmetric stretching in amide), 831.32 $cm^{-1}$, 767.67 $cm^{-1}$, 650.01 $cm^{-1}$ (mixed, C-H stretching in aromatic). In the case of MTX-MS2, slightly variation occurred in these bands which may be due to drug encapsulation into the polymer matrix. The spectrum proves that MTX was quite compatible with its polymer used in the development of mucoadhesive microparticles.

## SEM

Surface structure and morphology of the optimized formulation, MTX-MS2 has been shown in Figure 5 (A-C). Photomicrograph of the particle was seen as irregular in shape Figure 5 (A). A closed observation of the MTX-MS2 clearly shows the uniform distribution of polymer matrix [Figure 5 (B)]. Moreover, microparticles were broken down to visualize the internal morphology as shown in Figure 5 (C). The internal surface showed various layers of the polymers used in the formulation of the microparticles. Particle size was identical to that the size measured by Zetasizer.

## CONCLUSION

Microparticle played an important role in colon targeted drug delivery, which provides health benefits by producing local and systemic effects. It offers several advantages over the conventional dosage form by improvising the therapeutic effect, bioavailability, drug stability and minimizing the dose-related side effects. Mucoadhesive microparticles deliver the drug to the target sites with prolonged release profile for extended periods due to their adhesiveness property to the colonic mucosa. Therefore, this delivery system could be an efficient carrier for MTX delivery in the colon region for the treatment of colorectal cancer. Hence, in the present study an anticancer drug, MTX was selected to load in a blend of polymer (SA, GG and CP) matrix system of the microparticles for targeting to the colon. Schematic representation of the work has been shown in Figure 6.

Colon targeted mucoadhesive carrier system of MTX was successfully developed with GG, CP and SA by ionotropic gelation technique. Different parameters were tested significantly for MTX loaded mucoadhesive microparticles. The optimized formulation (MTX-MS2) showed a remarkable swelling and mucoadhesion property as evaluated by *in vitro* method. Cumulative % drug release profiles of MTX from various formulations were performed in SGF at different pH along with the marketed tablet, Folitrax.

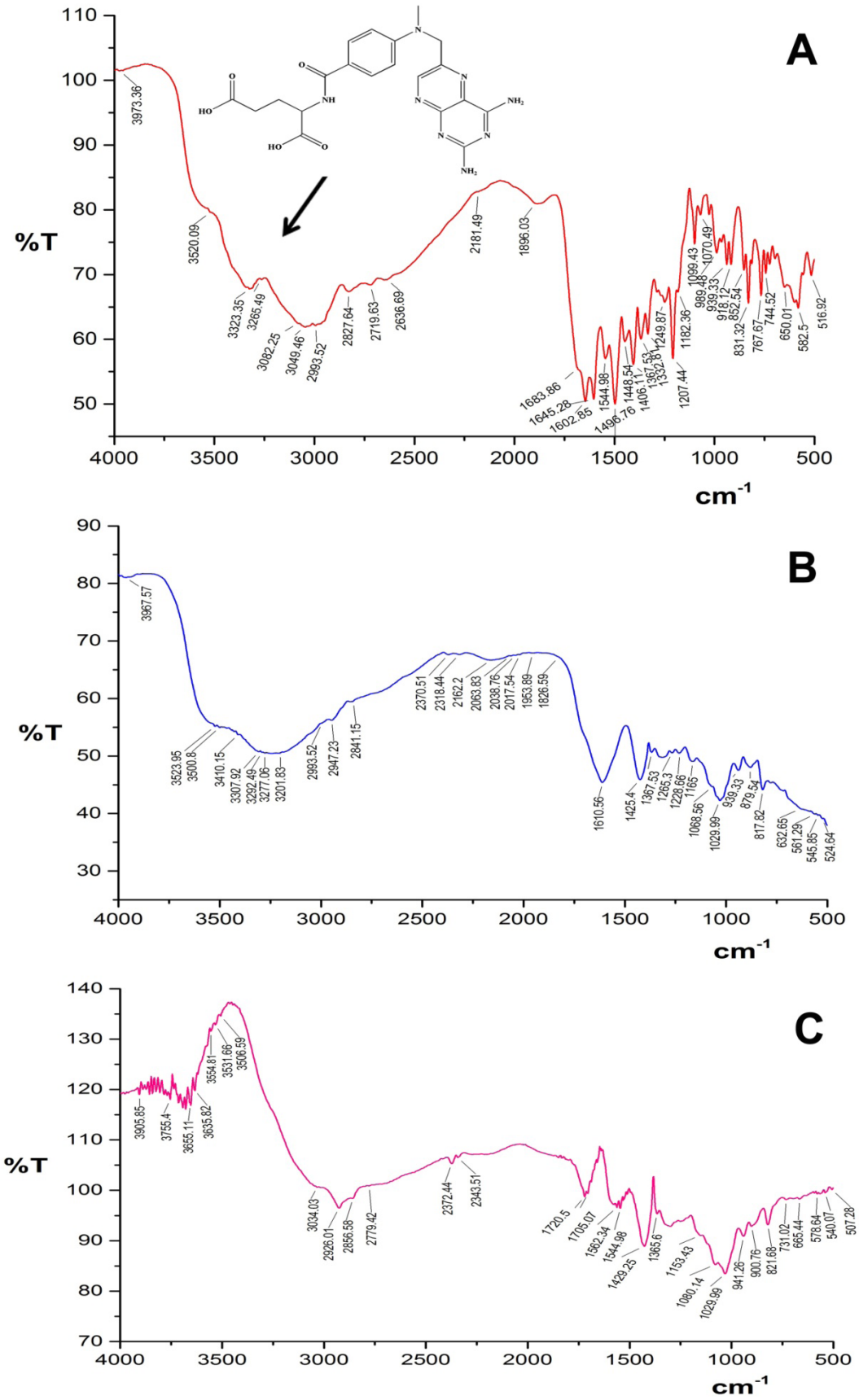


**FIGURE 4 –** FTIR spectrum of pure methotrexate (A), MTX-MS2 (B) and placebo formulation (C).

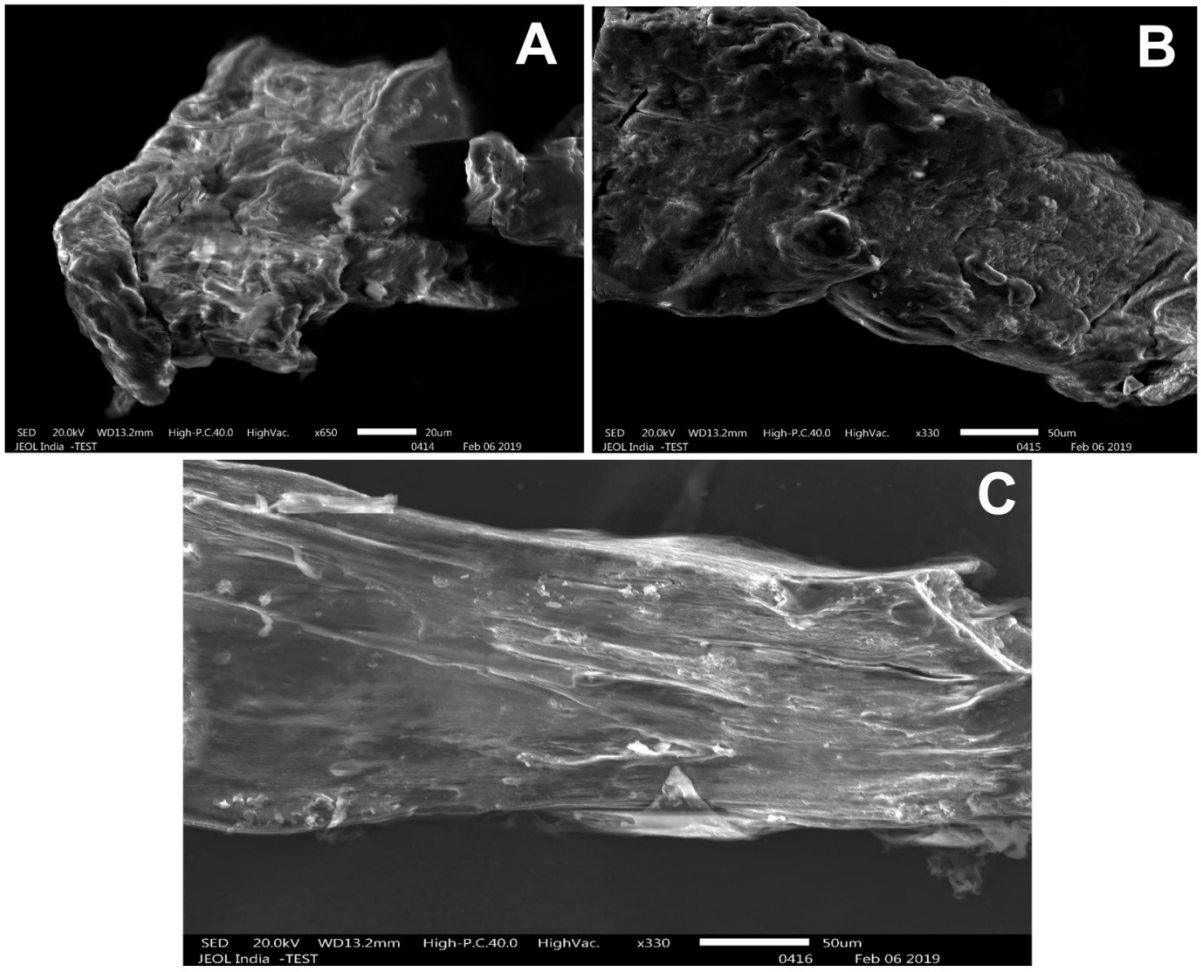


**FIGURE 5 –** SEM photomicrographs (A) irregular shape microparticle, (B) uniform distribution of polymer matrix and (C) internal morphology of MTX-MS2.

Result stated that MTX-MS2 was exhibited prolonged release profile for an extended period of 36 h at pH 7.4 while marketed tablet released drug content around 12 h. There was no interference of the acidic pH 1.5 and pH 4.5 for drug release from the different formulations. This sustained release activity of the MTX-MS2 could be due to adhesion of polymer (CP, SA and GG) to the environment of colonic mucosa for longer periods, it also favored by suitable particle size and zeta potential. Thus, mucoadhesive microparticles of methotrexate could be exploited as a novel carrier for the management of colorectal cancer.

## ACKNOWLEDGEMENT

Authors are highly thankful to the GLA University, Mathura, India for providing necessary facilities and support to accomplish this work.

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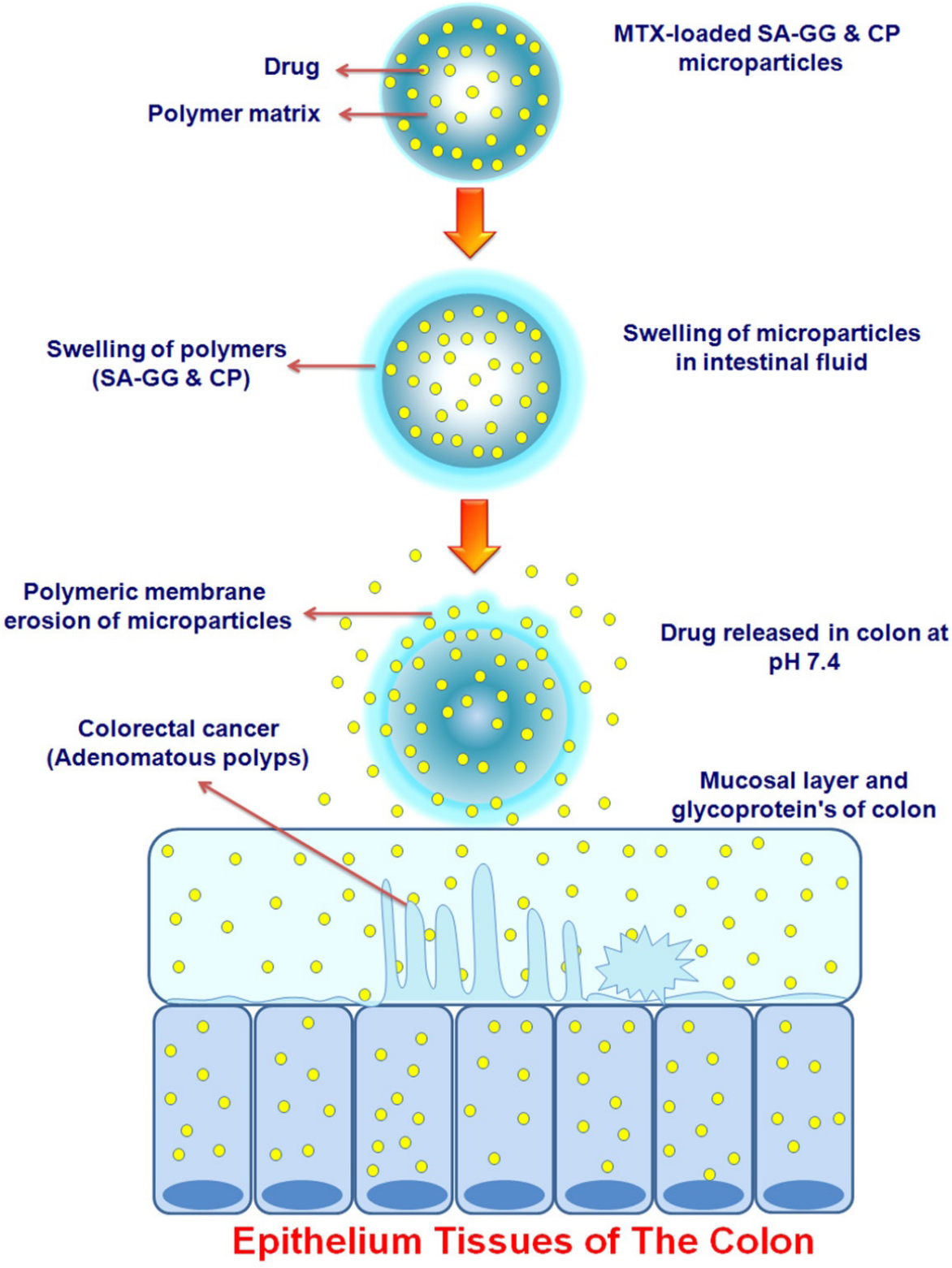


**FIGURE 6 –** Schematic of MTX loaded mucoadhesive microparticles for the treatment of colorectal cancer.

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Received for publication on 08th February 2019
Accepted for publication on 30th April 2019