# DOCETAXEL IN MUCOADHESIVE IN SITU GEL DELIVERY SYSTEM

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## ABSTRACT

The aim of this study was to develop an in situ gel delivery system containing Docetaxel (DTX) and mucoadhesives for sustained and targeted delivery of anticancer drugs. MUC1 gene encodes a transmembrane mucin glycoprotein that is overexpressed in human breast cancer and colon cancer. The delivery system consisted of chitosan and glyceryl monooleate (GMO) in 0.33M citric acid containing DTX. The in vitro release of DTX from the gel was performed in presence and absence of Tween 80 at drug loads of 0.18%, 0.30%, and 0.54% (wt/wt), in Sorensen's phosphate buffer (pH 7.4) at 37°C. Different mucin-producing cell lines (Calu-3 > Caco-2) were selected for DTX transport studies. Transport of DTX from solution and gel delivery system was performed in side by side diffusion chambers from apical to basal (A-B) and basal to apical (B-A) directions. In vitro release studies revealed that within 4 hours, only 7.61% ± 0.19%, 12.0% ± 0.98%, 31.7% ± 0.40% of DTX were released from 0.18%, 0.30%, and 0.54% drug loaded gel formulation, respectively, in absence of Tween 80. However, in presence of surfactant (0.05% wt/vol) in the dissolution medium, percentages of DTX released were 28.1% ± 4.35%, 44.2% ± 6.35%, and 97.1% ± 1.22%, respectively. Docetaxel has shown a polarized transport in all the cell monolayers with B-A transport 2 to 4 times higher than in the A-B direction. The highest mucin-producing cell line (Calu-3) has shown the lowest percentage of DTX transport from gels as compared with Caco-2 cells. Transport of DTX from mucoadhesive gels was shown to be influenced by the mucin-producing capability of cell.

**Keywords:** Docetaxel, Mucoadhesive, drug loaded gel.

## 1. INTRODUCTION

MUC1 is a tumor-associated antigen that is overexpressed in the majority of adenocarcinomas. It is overexpressed more than 10-fold in 90% of breast carcinoma and is being used as a target for active and passive cancer immunotherapy in many clinical trials (Gendler, 2001).MUC1is a heterodimer transmembrane glycoprotein consisting of mucin-like extracellular domain and a cytoplasmic tail containing docking sites for multiple oncogenic proteins including src, erbB receptors, and β catenin (Schroeder, 2001; Hamada, et al., 2004). The overproduction of mucin in these cancerous cells could be used as a targeting strategy for mucoadhesive drug delivery system for treatment of breast and colon cancer. Docetaxel blocks the G-2Mphase of the cell cycle of proliferating cell (Schiff, 1980) and stabilizes tubulin polymer formation by promoting microtubule assembly (Schiff, 1979). DTX is a very potent anticancer agent, but its efficacy is limited because of low solubility and oral bioavailability (Eiseman, et al., 1994). Some cancer cells have a high level of efflux pump, Pglycoprotein (Pgp), which results in removal of DTX from the cell during their transport through the cell (Brooks, et al., 2003). DTX is administered by IV infusion, and to enhance its solubility, cremphor EL is used as a solvent. This solvent causes severe hypersensitivity reactions and cytotoxicity and has shown incompatibility with polyvinyl chloride (PVC), commonly used in IV dosage forms (Trissel, 1977). Even though DTX is a highly effective anticancer agent, it cannot differentiate between cancer and normal cells, resulting in major toxicity to normal tissues. This toxicity can be fatal if not prevented. To minimize the cytotoxicity and adverse side effects associated with DTX, a localized drug delivery system needs to be developed. The underlying hypothesis of this investigation was that a mucoadhesive in situ gel delivery system containing DTX could be targeted to the cancer cells where MUC1 gene is overexpressed as compared with normal cells. This would substantially reduce toxicity to normal cells.

The primary objective of this investigation was to develop a safe, sustained, and more effective novel in situ gel delivery system containing DTX for the treatment

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of breast and colon cancer. When injected close to the site of tumor, the ionic polymer used in the delivery system will be deprotonated and will form an instant gel at the site of injection at the biological pH (7.4). This delivery system may provide a sustained release of DTX from the in situ gel at and around the site of cancer, while the systemic drug concentration will be negligible. The gel delivery system consisted of chitosan and glyceryl monooleate (GMO) in citric acid. Chitosan is a natural and immunogenic polymer with minimal toxicity (Chandy, 1990). The mucoadhesive properties of chitosan and its usefulness in drug delivery system design have been well documented (Fiebrig, 1995; He, 1990). Strong electrostatic interaction of positively charged chitosan with the negatively charged mucosal surface is the underlying mechanism for its mucoadhesive properties (Lueben, 1994).GMO, on the other hand, forms liquid crystal in the presence of water (Shah, 2001). GMO also possesses bioadhesive properties that can be used to enhance the therapeutic efficacy of the dosage forms by increasing the contact time at the site of action (Dash, 1999; Nielsen, 1998; Ganguly, 2004). The self-emulsifying property of GMO can further enhance the solubility of this highly hydrophobic compound in this formulation.

## 2. MATERIALS AND METHODS

### Materials

DTX with 98% purity, potassium phosphate monobasic, and sodium phosphate dibasic anhydrous was purchased from West Bengal Chemical Industries Limited, india. chitosan was purchased from Indian Research Products, Chennai, India. GMO, Anhydrous citric acid, Both Caco-2 and Calu-3 cells, Ammonium acetate, Acetonitrile, Tween80 and methanol were purchased from S.D.Fine chemicals.

### Analysis of Docetaxel

A highly sensitive high-performance liquid chromatography (HPLC) method was developed and validated for the quantitation of DTX. The chromatographic separation was achieved on a ZORBAX SB C-18 column (150 × 4.6 mm, 5 µm) with a flow rate of 0.75 ml/min with UV detection at 227 nm. Mobile phase consisted of acetonitrile:methanol:0.1M ammonium acetate (48.5%:16.5%:35% [vol/vol/vol]). Mobile phase was filtered and degassed prior to HPLC use. Standard curve was linear over the concentration range of 0.1 to 10 µg/mL with $R^2$>0.99. The relative standard deviations (RSD) for day-to-day and within-day precision for this assay were less than 10%. This method was sensitive enough to detect even less than 0.1 µg/mL of DTX in solution.

### Formulation of the Delivery System

Known weight of GMO was kept in an Erlenmeyer flask and heated in an oven at 45ºC for 30 minutes. Chitosan powder was added to molten GMO solution and stirred with a glass rod. A known amount of DTX was added directly to 0.33 M citric acid solution followed by sonication for 30 minutes. Citric acid solution containing DTX was added to GMO and chitosan mixture. The resulting mixture was sonicated again over a period of 45 minutes. The final concentration of GMO and chitosan was kept at 3% (wt/vol).

### In Vitro Release of Docetaxel From the Delivery System

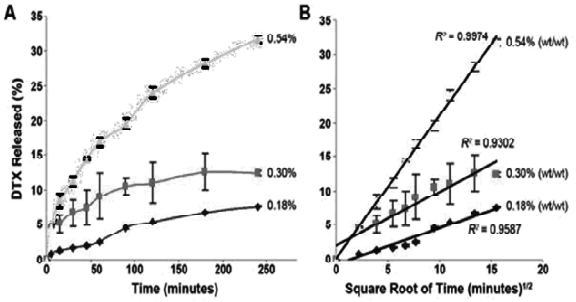
One milliliter of the delivery system containing different DTX loads (0.18%, 0.30%, and 0.54% (wt/wt) was added to 40 ml Sorensen's phosphate buffer with a syringe. The theoretical amount of DTX in this 1 ml of gel containing 0.18%, 0.30%, and 0.54% (wt/wt) of drug was 110, 180, and 330 µg, respectively. The insitu gel that formed when added to the Sorensen's buffer was shaken in a bath incubator at 80 rpm at 37$^0$C. At predetermined time intervals (5, 15, 30, 45, 60, 90, 120, 180, and 240 minutes) 200 µl of the release medium was collected via a filtered needle and replaced by same volume of fresh buffer. The drug concentration in the release medium was analyzed using HPLC as previously described. The data were expressed as percentage of DTX released from the gel delivery system.

### Cell Culturing

Caco-2 cells were obtained and used in the transport studies from passages 25 to 39. Caco-2 cells were seeded at 25 000 cells/cm2 density in 75cm$^2$ culture flasks in Dulbecco's modified Eagle's culture media (DMEM) supplemented with 10% fetal bovine serum with Penicillin G (100 U/mL) and streptomycin (10 µg/mL). The Caco-2 cells grown on the culture flasks were passaged using a trypsin/ethylenediaminetetraacetic acid solution when ~80% confluent. The passaged cells were either further propagated in culture flasks (1:3 splitting ratio) or seeded

onto Falcon polystrene membrane inserts. The density of the inserts was maintained as described earlier and used for permeability studies at 21 days. Calu-3 cells were obtained and maintained in a 1:1 mixture of Ham's, with 10% fetal bovine and supplemented with 100 µg/mL penicillin G and 100 µg/mL streptomycin sulfate. Cells were plated in 150 $cm^2$ cell culture flasks and subcultured before reaching confluency using a 0.25% trypsin solution in EDTA (Sigma). The culture medium was changed every 2 days. The cells were split 1:2 during each passage. Passaged cells were seeded ($5 \times 10^5$ cells/$cm^2$) onto Falcon polystyrene membrane inserts and used for permeability studies at 17 to 21 days.

**Figure 1. Effect of drug load on the in vitro release of DTX from the in situ gel.**




Mean ± SD (n = 4). The DTX load was 0.18%, 0.30% and 0.54% (wt/wt). (A) Plot of percentage DTX released versus time, and (B) plot of percentage of DTX released versus square root of time.

**Transport of DTX via In Vitro Cell Culture Models**

Caco-2 and Calu-3 cell monolayers were used as in vitro models for evaluation of the transport of free DTX from different dosage forms (solution and in situ gel). The cell monolayer integrity was evaluated by measuring Trans Epithelial Electrical Resistance (TEER) with a Millicell-ERS volt/ohmmeter prior to the transport studies. The cell monolayer integrity was considered ready for the transport studies when a minimum TEER value of 620 Ohm cm2 (TEER is reported in Ohms × surface area). Resistance measurement in Ohm with cell monolayer minus the blank reading with no cells times the total surface area was obtained. This TEER value is the mean of 3 readings taken from 3 different spots of cell inserts, expressed as Ohm × cm2 (TEER = [mean Ohms *"* 95 Ohms] × 4.76 cm2). Side by side diffusion chambers were used to study the transport across the cell monolayers as previously described (Dash 2001). In brief, fresh assay II buffer (3 mL) was placed in the receiver chamber. The donor chamber contained 3ml of either free DTX solution (25 µg/mL) or gel containing DTX (25 µg/mL). The polarity of drug transport was evaluated by investigating the differences in apical and basolateral transport. At predetermined time intervals (30, 60, 90, 120, and 180 minutes), 200 µL of the samples from the receiver side was collected and replaced with same volume of fresh buffer. The drug concentration in the sample was determined by HPLC.

## 3. RESULTS AND DISCUSSIONS

**Homogeneity of DTX in Gel Formulation**

During the preformulation development, homogeneity of the drug in the delivery system was a major challenge. Method of incorporation of DTX in the delivery system had an important influence in the formulation of this in situ gel delivery system. DTX was incorporated in the delivery system by 2 different ways. First method included addition of DTX directly to the delivery system containing 3% (wt/vol) GMO and 3% (wt/vol) chitosan in 0.33M citric acid. The experimental drug loads determined by HPLC analysis for 3 different batches of the above formulations resulted in a high relative standard deviation (46.4%) indicating a possible nonhomogeneity in the delivery systems. To address this homogeneity issue, a second method was introduced. In this method, DTX was first dispersed into the 0.33 M citric acid followed by its addition to the mixture consisting of 3% (wt/vol) GMO and 3% (wt/vol) chitosan. The relative standard deviation for this method of incorporation of DTX to the drug delivery system was found to be reduced dramatically to 5.2% (n = 3), indicating a better homogeneity in the formulation. Therefore, for the entire investigation the second method was adopted for the fabrication of the in situ gel delivery system containing DTX.

**Effect of Drug Load on the In Vitro Release of Docetaxel from the Gel**

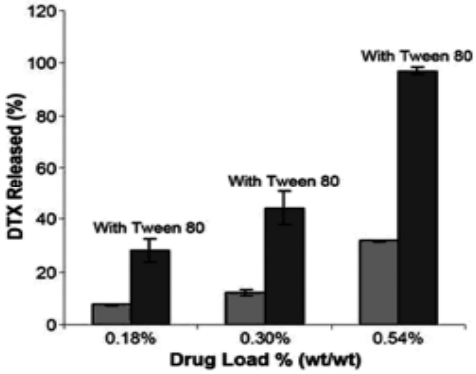
In vitro release of DTX from the mucoadhesive gel delivery system was performed in Sorensen's phosphate buffer (pH 7.4) at 37ºC. The in vitro release profiles are shown in Figure 1A. Three different DTX-loaded gels (0.18%, 0.30%, 0.54% [wt/wt]) were used to study the effect of drug load on the release of DTX from the gel. Within 4 hours, 7.61%, 12.0%, and 31.7% of DTX was released from 0.18%, 0.30%, and 0.54% (wt/wt) drug-loaded gels, respectively. The in vitro release mechanism of DTX from these gels was further evaluated. The percentage release of DTX from these 3 different drug-loaded gels was plotted against the square root of time and is shown in Figure 1B. The

linear relationship between DTX released versus square root of time with $r^2$>0.93 indicated that the drug release from such gel delivery system followed a matrix diffusion controlled mechanism as described by Higuchi.18 The slope of the lines in Figure 1B, which is a measure of the rate of drug release from the delivery system with 3 different drug loads, was then determined. The release rate of DTX from the gel formulation containing the highest drug load (0.54%, wt/wt) was found to be 4 times higher than the lowest drug-loaded gel (0.18%, wt/wt). However, this rate of release was only 3 times higher in the case of intermediate drug-loaded (0.30%, wt/wt) gels under similar test conditions (Higuchi, 1961).

**Effect of Tween 80 in the Release Medium on the Docetaxel Release**

As evidenced from the in vitro release studies, the release of DTX from the in situ gel delivery system was very low, as expected. We also investigated the effect of surfactant in the release medium and its effect on the DTX release. Addition of Tween 80 will not only increase the release of DTX from the gel matrix but also make the detection of this hydrophobic drug in the release medium feasible, especially at a very low drug load. The DTX release profile from the gel in the absence and presence of Tween 80 (0.05% wt/vol) in the release medium is shown in Figure 2. Addition of Tween 80 to the release medium significantly increased the percentage of DTX released from the gel. The DTX release was 4 to 6 times higher in the presence of Tween 80 as compared with in the absence of surfactant in the release medium. The enhanced release of DTX in the presence of surfactant can be explained by the increased solubility of this hydrophobic drug in the Sorensen's phosphate buffer. Similar results have already been reported elsewhere (Cho, 2004).

**Figure 2. Effect of 0.05% (wt/vol) of Tween 80 in the release media on the DTX release.**

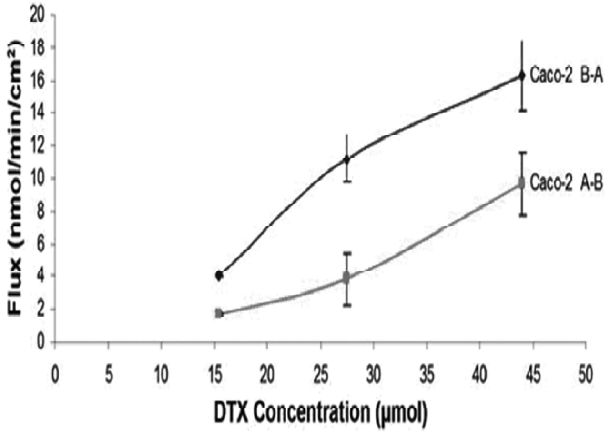



Drug load of DTX was 0.18%, 0.30%, and 0.54% (wt/wt). Mean ± SD (n = 4).

**Transport of DTX from Solution via Caco-2 Cells**

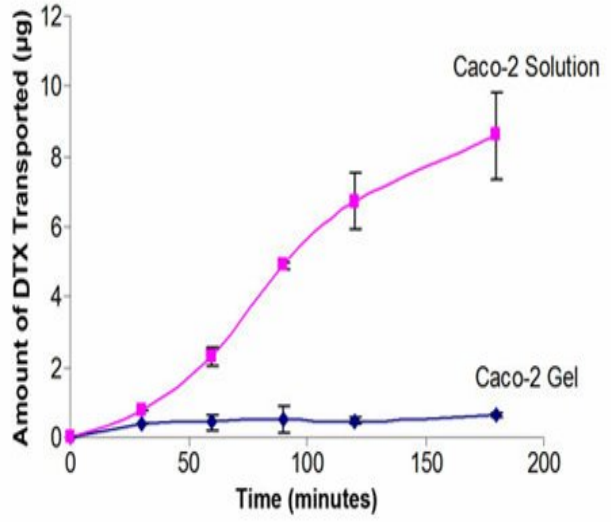
The transport and the transport polarity of DTX were evaluated in a Caco-2 cell line in this cell culture model. Transport of DTX across the monolayer was studied in both A-B and B-A directions in side-by-side diffusion chambers.

**Figure 3. Effect of DTX concentration on the flux.**




Three concentrations, 15.4 µM, 27.5 µM, and 44 µM, of DTX were used in this study. Mean ± SD (n = 3).

**Figure 4. Apical to basolateral transport across Caco-2 monolayer**.



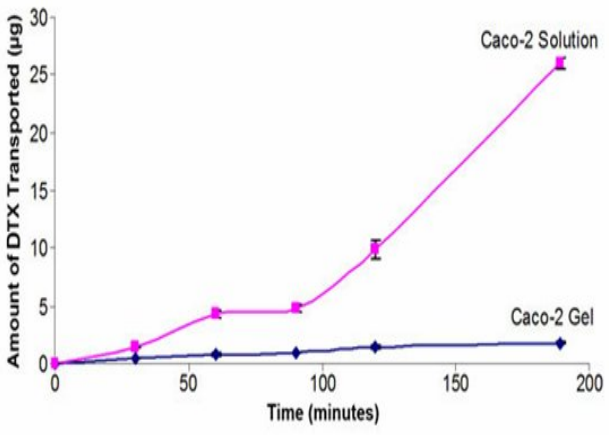

Concentration of DTX in donor side was 27.5 µM. DTX was present both in the solution and gel formulations. Mean ± SD (n = 3)

Caco-2 monolayers was determined at 3 different DTX concentrations in the donor side (15.4, 27.5, and 44 µM). As the concentration of the DTX increased, there was an increase in DTX flux. However, at a higher concentration, this increase was more prominent. DTX has been reported to be affected by Pgp glycoprotein efflux systems (Walle, 1998). We studied both the A-B and B-A flux of DTX at these 3 concentration levels. Results of this study indicated that flux across B-A direction is 2 to 4 times higher than A-B, as shown in Figure 3. This bipolar flux can be explained by Pgp efflux mechanism as reported by other authors.

**The Effects of a Gel Delivery System on the Transport of DTX**

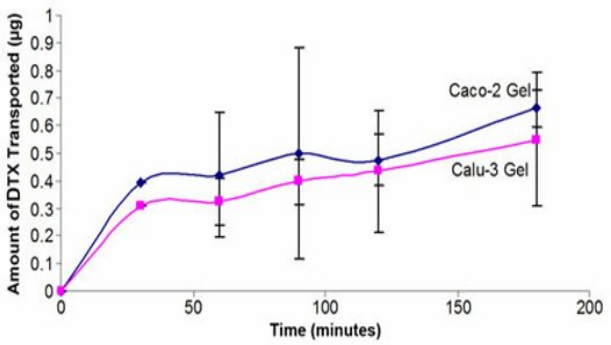
One of the objectives of this in situ gel delivery system was to provide a sustained release delivery of DTX. The DTX transport across Caco-2 monolayers was evaluated from both the free DTX in solution and the DTX-loaded gel delivery system (Figures 4 and 5). In brief, DTX (25 µg/mL) was added to the donor chamber in either free DTX in solution or loaded in the gel formulation, and samples (200 µL) were taken at various time points (30, 60, 90, 120, and 180 minutes) and replaced with fresh assay buffer. The concentration of DTX was determined by HPLC as previously described. Regardless of the cellular polarity, the transport of free DTX was significantly higher from the solution as compared with the DTX-gel delivery system (Figure 4). Certainly, the most reasonable explanation for the results displayed is the reduction of the free DTX available in the gel delivery system. The effect observed with the gel delivery system could be explained by the possible attraction and interactions between the cellular mucosal secretions and the gel delivery system. The adhesion of the gel delivery system to the mucosal secretions is a reasonable explanation for the reduced transport in the basolateral direction owing to decreasing the efflux surface area. However, further investigation would be required. To further investigate the possibility of these interactions other mucosal secreting cells should be evaluated. Caco-2 and Calu-3 cell lines were used to test the effect of mucin release on the transport of DTX from the mucoadhesive drug delivery systems. The concentration of DTX used in this transport study was kept at 25 µg/mL in the gel formulation. The results of the transport studies in both A-B and B-A directions are shown in Figures 6 and 7, respectively. The transport of DTX from both the A-B ($P < .01$) and B-A ($P < .03$) direction in case of Calu-3 cells was significantly lower than Caco-2 cells. Statistical significance was determined using 2-factor analysis of variance (ANOVA) without replication. Lower rate of transport of DTX across Calu-3 monolayer as compared with Caco-2 cells can be explained by the higher production of mucin in the Calu-3 cells. This overproduction of mucin possibly binds to the mucoadhesive delivery system, thereby providing extra barrier for diffusion of DTX and eventually affecting the transport of DTX. The transport of DTX from the gel in Calu-3 monolayers was then compared and shown in Figure 8. The A-B transport was lower than B-A transport. This difference in transport can be influenced by 2 factors. First, the production of mucin is much higher in the apical surface as compared with the basolateral surface. Second, this difference can also be explained by the presence of efflux pumps (Pgp glycoprotein) on the apical surface of the Calu-3 monolayer. However, previous studies have indicated that Calu-3 cells express lower levels of Pgp glycoprotein (Hamilton, 2001). Therefore, these 2 in vitro cell culture models clearly indicated that overproduction of mucin is the major contributing factor for the transport of DTX and can be used as a drug-targeting strategy and for sustained delivery of anticancer drug.

**Figure 5. Basolateral to apical transport across Caco-2 monolayer of DTX from solutions and gel formulations.**



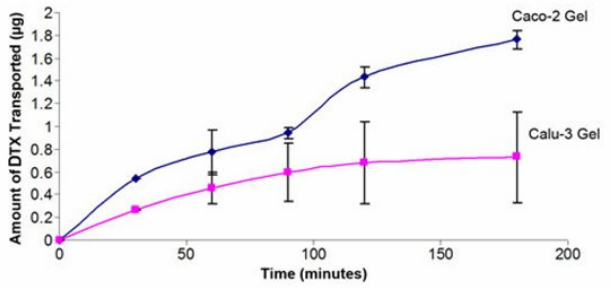

Concentration of DTX was 27.5 µM in both these formulations. Mean ± SD (n = 3).

**Figure 6. Apical to basolateral transport of DTX from gel formulation through Caco-2 and Calu-3 monolayers.**



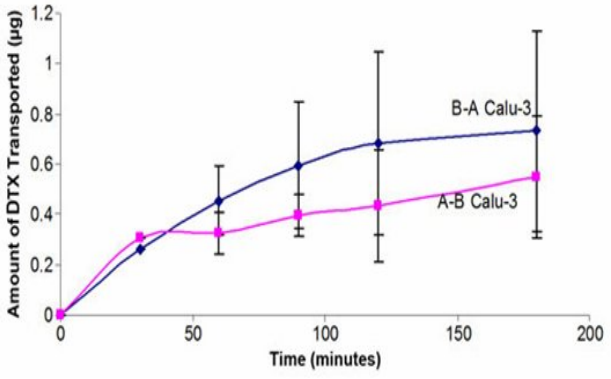

Concentration of DTX was 27.5 µM in both these formulations. Mean ± SD (n = 3).

**Figure 7. Basolateral to apical transport of DTX from gel formulation through Caco-2 and Calu-3 monolayers.**




Concentration of DTX was 27.5 µM in both of the formulations. Mean ± SD (n = 3).

**Figure 8. Comparison of apical to basolateral and basolateral to apical transport of DTX from gel formulation through Calu-3 monolayer.**




Concentration of DTX was 27.5 µM in the formulations. Mean ± SD (n = 3).

## 4. CONCLUSIONS

A mucoadhesive in situ gel delivery system containing chitosan, GMO, and DTX was developed for drug targeting and sustained delivery strategy for chemotherapy to mucin producing cancerous cells. The amount of DTX transported across cell lines was much lower in case of the gel as compared with DTX in solution indicating that this gel delivery system can be used to sustain the release of DTX. The decreased transport of DTX in case of Calu-3 cells as compared with Caco-2 cells was owing to its higher mucin producing capability and possible strong binding with the mucoadhesive gel.

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