**Supplementary Data**

**2.3.1 Determination of Thiol Group Content (Ellman’s Method)**

In 1959 Ellman introduced 5,5'-dithio-*bis*-(2-nitrobenzoic acid), also known as DTNB, as a versatile water-soluble compound for quantitating free sulfhydryl groups in solution ([Ellman 1959](#_bookmark0)). DTNB or Ellman's reagent can be used to quantitate thiols in proteins, cells and plasma by absorption measurements. The quantitative determination was performed by standard protocol provided along with product by thermofisher, India [1]. Sulfhydryl groups can be estimated in a sample by comparison to a standard curve composed of known concentrations of a sulfhydryl-containing compound such as cysteine. Alternatively, sulfhydryl groups may be quantitated by reference to the extinction coefficient of TNB [2, 3].

**2.3.2 Mucoadhesion Study**

After estimating the quatitiative concentration of sulfahydril group after transesterification, in reaction with GO. The *ex-vivo* mucoadhesion of synthesized GO and TrGO formulation was determined by wash-off test [4]. A freshly excised goat intestine was obtained from a local butcher house (Shirpur, India) within an hour of slaughter and transported to laboratory in isotonic saline solution. It was cleaned by washing with isotonic saline solution. The intestinal mucosal membrane was tied on glass slide. About 100 mg of GO and TrGO were adhered to intestinal mucosal tissue by applying light force with fingertip for 30 sec. The glass slide was hung on to arm of USP tablet disintegrating machine which was suspended in 900 mL of phosphate buffer (pH 7.4) at 37±0.5 0C and tissue specimen was given slow, regular up and down movement by operating the machine. The adhering particles to tissue were analyzed visually.

**2.3.3 *In-Vitro* Release Study**

*In vitro* release of methotrexate from TrGO-MTX was studied using dialysis method [5]. Nanoparticulate suspension (5 mL) containing TrGO-MTX (equivalent to 10 mg MTX) and MTX (10 mg) filled in dialysis bag sealed at both ends. The dialysis bag was dispersed into dissolution medium (150 mL) phosphate buffer saline pH 7.4 at 37 0C (100 rpm). The aliquots of 3 mL was withdrawn at predetermined interval of 1 h each upto 24 hr. The sink condition was maintained by replacing the equivalent of 3 mL of fresh aliquots containing 7.4 pH phosphate buffer saline dissolution medium. The quantitative estimate of MTX from the aliquote was analyzed using Uv-Vis spectrophotometer (258 nm). The *Invitro* dissolution profile was drawn on percent drug release Vs time points from MTX and TrGO-MTX.

**2.3.4 *In-Vitro* Cytotoxicity (MTT Assay)**

The MTT assay was performed to evaluate the cytotoxicity of developed TrGO-MTX nanoparticles on U-373 MG cell line. U-373 MG cells were seeded into 96-well plates and incubated at 37°C for 24 h. The developed formulations were incubated with the cells in the concentration range of 1μg/mL, 5μg/mL, 10μg/mL, 25μg/mL, and 50μg/mL along with the culture medium. After incubation, MTT dye was added into the formulation. Cell viability was determined for the cells by recording the absorbance for the compound seeded in the 96-well plate. Obtained absorbance was compared with that of the control (Jain, Bajaj et al. 2016). To study the cellular uptake of TrGO-MTX, Fluorescein isothiocyanate (FITC) labelled TrGO-MTX (TrGO-MTX-FITC) was then incubated with U-373 MG-CELLS at 37 °C for 24 h, and the cells were observed by confocal fluorescence microscopy [6].

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