

# Thermosensitive nanohydrogel of 5-fluorouracil for head and neck cancer: preparation, characterization and cytotoxicity assay

Chintan Dalwadi  
Gayatri Patel

Department of Pharmaceutics and  
Pharmaceutical Technology, Ramanbhai  
Patel College of Pharmacy, Charotar  
University of Science and Technology,  
Changa, Gujarat, India

**Abstract:** Systemic chemotherapy has been shown to produce side effects. A small fraction of the drug reaches the tumor site; other healthy organs or normal tissues get affected or damaged due to the nonspecific action of these cytotoxic agents. Furthermore, due to their short period of activity, repeat injections are often required, which can lead to the exacerbation of side effects and inconvenience. To overcome these obstacles, in this study, we developed controlled and targeted intratumoral injection. Hydrogel was prepared by physical cross-linking method; however, nanohydrogel was prepared using tip probe-sonicator method. Our results revealed that biodegradable and thermosensitive 5-fluorouracil-loaded methylcellulose nanohydrogel synthesized by physical cross-linking method may be a beneficial approach in targeting the therapeutic agent to the tumor site.

**Keywords:** biodegradable, intra tumor, targeted drug delivery

## Introduction

The primary treatment for solid tumors is surgery followed by irradiation and/or systemic chemotherapy to kill malignant cells, which may remain even after surgery. Systemic chemotherapy has been shown to cause side effects upon administration as they are extensively transported to the whole body; a small fraction of it reaches the tumor site, whereas the rest is taken up by other healthy organs or normal tissues thereby getting damaged due to the nonspecific action of the cytotoxic agents. Due to their short period of activity, repeated injections are often required, which can lead to exacerbation of side effects and inconvenience to the patients. To overcome these obstacles, in this study, we developed controlled and targeted intratumoral injection. Such targeted delivery may be potentially more effective in maintaining therapeutic concentrations of drug, while reducing systemic drug levels and decreasing the side effects in healthy normal tissues. In recent years, an injectable in situ thermosensitive nanohydrogel has attracted considerable attention because it achieves targeted drug delivery and prolonged activity.<sup>1,2</sup> This in situ nanohydrogel exhibits sol phase to gel phase transition in response to temperature. At room temperature, various cytotoxic drugs can be easily incorporated into the solution by simple mixing, and this forms a gel in situ after administration under physiological conditions.

Among the many cytotoxic drugs used in clinics, 5-fluorouracil (5-FU) is an effective chemotherapeutic agent that is extensively employed in systemic chemotherapy for solid tumors such as head and neck cancer, breast cancer, colorectal cancer and brain tumor. After administration, 5-FU is rapidly metabolized in the body with an apparent terminal half-life of approximately 8–20 minutes as it is metabolized by

Correspondence: Gayatri Patel  
Department of Pharmaceutics and  
Pharmaceutical Technology, Ramanbhai  
Patel College of Pharmacy, Charotar  
University of Science and Technology,  
CHARUSAT Campus, Changa 388421,  
Gujarat, India  
Tel +91 99 2505 4142  
Fax +91 2697 247 100  
Email gayatripatel.ph@charusat.ac.in

**Table 1** Three-level two-factor full factorial design with observed response of methylcellulose (MC) hydrogel

Batch no	Independent parameters		Dependent parameters		
	Concentration of MC (% w/v) $X_1$	Concentration of trisodium citrate (% w/v) $X_2$	Gelling temperature ( $^{\circ}$ C) $Y_1$	Gelling time (seconds) $Y_2$	Viscosity (cP) at 25 $^{\circ}$ C $Y_3$
F-1	1 (-1)	1 (-1)	56 $\pm$ 0.180	84 $\pm$ 0.026	31 $\pm$ 0.227
F-2	1.5 (0)	1 (-1)	54 $\pm$ 0.161	74 $\pm$ 0.001	66 $\pm$ 0.041
F-3	2 (+1)	1 (-1)	50 $\pm$ 0.004	66 $\pm$ 0.032	149 $\pm$ 0.021
F-4	1 (-1)	3 (0)	52 $\pm$ 0.002	76 $\pm$ 0.011	36 $\pm$ 0.036
F-5	1.5 (0)	3 (0)	48 $\pm$ 0.235	68 $\pm$ 0.127	69 $\pm$ 0.218
F-6	2 (+1)	3 (0)	44 $\pm$ 0.186	58 $\pm$ 0.006	156 $\pm$ 0.06
F-7	1 (-1)	5 (+1)	40 $\pm$ 0.041	70 $\pm$ 0.003	38 $\pm$ 0.016
F-8	1.5 (0)	5 (+1)	38 $\pm$ 0.069	62 $\pm$ 0.084	80 $\pm$ 0.113
F-9	2 (+1)	5 (+1)	36 $\pm$ 0.029	54 $\pm$ 0.059	176 $\pm$ 0.210
F-10	1.5 (0)	3 (0)	48 $\pm$ 0.192	65 $\pm$ 0.006	72 $\pm$ 0.007
F-11	1.5 (0)	3 (0)	48 $\pm$ 0.231	68 $\pm$ 0.142	73 $\pm$ 0.004

**Note:** Results are shown as mean (n=3)  $\pm$  standard deviation.

dihydropyrimidine dehydrogenase.<sup>3</sup> Therefore, continuous administration of 5-FU is required to maintain its therapeutic activity, which can be achieved by thermosensitive nanohydrogel.

## Materials and methods

Methylcellulose (MC-METHOCEL A4C) was a gift from the Colorcon Asia Pvt. Ltd., Verna, Goa, India. Viscosity of 2% aqueous solution at 20 $^{\circ}$ C was  $\sim$ 400 cP. 5-FU was purchased from the Zydus Cadila Healthcare Ltd., Ahmedabad, India. Trisodium citrate was purchased from the Merck Limited, Mumbai, India. Deionized water was used for the experiments.

Hydrogel was prepared by physical cross-linking method and three-level two-factor full factorial experimental design. Design Expert 9.0 was employed (Table 1). In this method, MC at 1%–2% concentration by weight of water was dissolved in deionized water by stirring gently at room temperature. A clear solution was achieved by storing it overnight in a refrigerator. Then, trisodium citrate (1%–5% w/v) was added to the MC solution.<sup>1</sup> This was mixed gently using magnetic stirrer until a clear solution was obtained, and its gelling time, temperature and viscosity were noted. Then, 5-FU was added and dissolved in MC hydrogel by stirring gently at room temperature. Nanohydrogel was prepared using tip probe-sonicator (Vibracell VCX-500; Sonics, Newtown, CT, USA). Different parameters of 5-FU-loaded nanohydrogel were checked (Table 2).

Cytotoxicity of 5-FU-loaded nanohydrogel, drug solution and MC polymer against KB oral cancer cell line (purchased from National Centre for Cell Science (NCCS), Pune, Maharashtra, India) was investigated via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide blue-indicator dye

assay. Briefly, KB cells were seeded in 96-well plates and incubated for 24 hours for cell attachment. Following attachment, 5-FU drug solution, 5-FU nanohydrogel and MC polymer at a concentration ranging from 1 to 2,000  $\mu$ g/mL were added.<sup>4,5</sup> Optical density was determined at 570 nm using ELISA plate reader (Bio-Tek, Winooski, VT, USA).

## Results and discussion

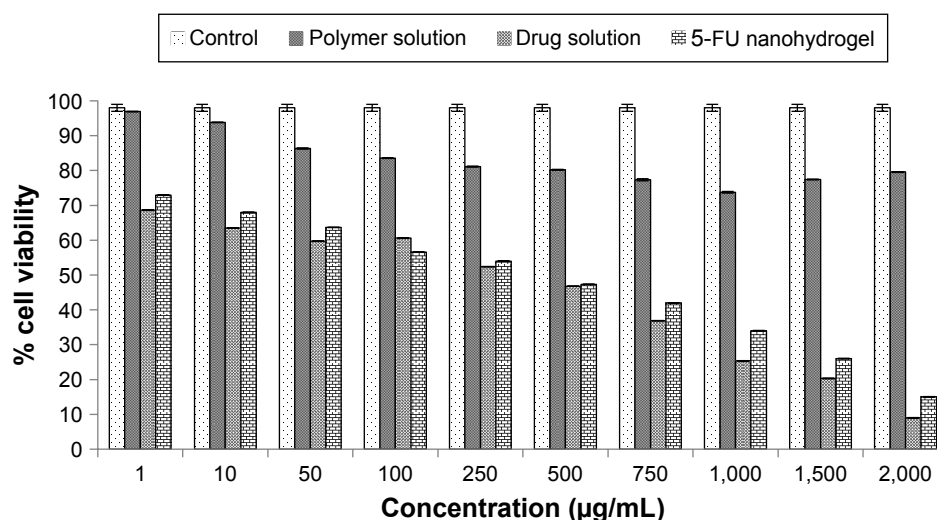
Polymeric MC solution has a gelling temperature of 65 $^{\circ}$ C–70 $^{\circ}$ C. Addition of trisodium citrate reduces the gelling temperature to physiological temperature. This may be due to the interaction of trisodium citrate with water that ultimately breaks the intermolecular hydrogen bonding between water and MC molecules. This permits higher intramolecular cross-linking between MC chains resulting into gelation.

Results of MC hydrogel as per factorial design are given in Table 1. Based on the desirability value 0.96, batch F-8 was selected as optimized batch (Table 1). Results of 5-FU-loaded

**Table 2** Characteristics of 5-FU-loaded nanohydrogel

Sr no	Parameters	Results
1	Gelling time	70 $\pm$ 0.08 seconds
2	Gelling temperature	37 $^{\circ}$ C–42 $^{\circ}$ C
3	Viscosity of solution	37.27 $\pm$ 0.32 cP
4	Syringeability (using 26G needle)	234 g (WFI: 149 g)
5	Viscosity of gel	2,293 $\pm$ 0.38 cP
6	Gel strength	66 g
7	Swelling index	19.40% $\pm$ 0.86%
8	In vitro biodegradation	78% and 95% in 24 and 48 hours, respectively
9	% drug release	98.6% in 24 hours
10	Mechanism of drug release	Diffusion mechanism (Korsmeyer–Peppas model n=0.79)

**Abbreviations:** 5-FU, 5-fluorouracil; WFI, water for injection.



**Figure 1** Cytotoxicity assay of 5-FU-loaded nanohydrogel, MC polymer and drug solution. Results are shown as mean (n=3) ± standard deviation. **Abbreviations:** 5-FU, 5-fluorouracil; MC, methylcellulose.

nanohydrogel are given in Table 2. It shows the sheer thinning viscosity of  $37.27 \pm 0.32$  cP which degrades within 2 days by the activity of cellular enzymes. Drug release by nanohydrogel indicates that it reduces the burst release and also releases the drug up to 24 hours. Sustained drug release of the formulation may be attributed to the pore size of nanohydrogel, which is also supported by lower swelling ratio of the formulation. It follows the Higuchi kinetic model for drug release. Korsmeyer–Peppas model n value (0.79) indicates a coupling of diffusion and erosion mechanisms, called anomalous diffusion. Results of cytotoxicity assay (Figure 1) indicate that  $IC_{50}$  value of 5-FU drug solution and 5-FU nanohydrogel was found to be 250 µg/mL. Cytotoxicity assay shows that drug efficacy against cancer cells remained unchanged after incorporation in the nanohydrogel.

## Conclusion

Biodegradable and thermosensitive 5-FU-loaded MC nanohydrogel synthesized by physical cross-linking method may be beneficial in efficiently targeting tumor site. Further in vivo pharmacokinetic and pharmacodynamic studies are needed to confirm this findings.

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## Disclosure

The authors report no conflicts of interest in this work.

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