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ORIGINAL ARTICLE

Preparation of cross-linked guar gum nanospheres containing tamoxifen citrate by single step emulsion in situ polymer cross-linking method

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Abstract In the present work, guar gum nanospheres containing tamoxifen citrate (TC) were prepared and characterized for using it as a carrier for targeted drug delivery. Tamoxifen is a non steroidal drug used in the treatment of breast cancer. The compound administered to patients is the citrate salt of the trans isomer, tamoxifen citrate. Single step emulsion in situ polymer crosslinking technique was employed to prepare polymer coated drug nanoparticles. Model polymer used in this study was guar gum, which is commonly used for colon specific drug delivery in the pharmaceutical industry. During preparation four-different drug loading solvents were tried and dichloromethane provided the best drug loading result. Briefly, 5 mg drug was dissolved in dichloromethane and emulsified with an aqueous solution of guar gum using span 80 as emulsifier. Cross-linking was made by the use of cross linker glutaraldehyde during the process. A core shell type particles were observed. Drug load was confirmed by FT-IR and quantitated by HPLC. Nanoparticles were further characterized for particle size and morphology. Particle size between 200 and 300 nm were obtained. Influence of process variables on the size of nanoparticles were studied. It was observed that the concentration of polymer and stabilizer determined the size of nanoparticles.

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Introduction

Tamoxifen has been the clinical choice for the antiestrogen treatment of advanced or metastatic breast cancer for more than 20 years. This non steroidal antiestrogen of the triphenylethylene type functions by competitively binding to estrogen receptors It is used as adjuvant or additional therapy following primary treatment for early stage breast cancer [1–3]. However, tamoxifen can act either as an antiestrogen or as an estrogen depending on the dose and tissue. Therefore, while tamoxifen is anti-estrogenic to the breast, it also acts as an estrogen to the uterus. Women treated with tamoxifen are at increased rate of developing endometrial cancer. Overall endometrial pathologies including hyperplasia, polyps, carcinoma and sarcoma have been identified up to 36% of postmenopausal breast cancer patients treated with tamoxifen [4, 5].

Other side effects include liver cancer, increased blood clotting and ocular side effects such as retinopathy and corneal opacities. These effects were reported to be dose dependent [6] suggesting the use of lower doses with colloidal delivery systems to be the key approach for the formulation of tamoxifen for long-term chemoprevention of breast cancer. Till date, tamoxifen has been formulated in nanoparticulate carrier systems in the form of nanospheres such as poly-q-caprolactone nanoparticles [7] and long-circulating PEG-coated poly (MePEGcyanoacrylate-cohexadecylcyanoacrylate) nanoparticles in the form of free base [8]. This approach was based on achieving required amount of drug at tumor site for a certain period of time and minimizing side effects on other organs of the

body. Citrate salt was preferred to tamoxifen free base due to its higher efficacy and the fact that commercially marketed products are manufactured with tamoxifen citrate.

Guar gum was used as the model polymer. The objective of the study was to develop and characterize in vitro nanoparticulate drug delivery systems in the form of nanospheres and nanocapsules based on cross-linked guargum [9] that are capable of incorporating a higher amount of tamoxifen citrate with a relatively slower in vitro release profile and to demonstrate the efficacy of these systems in female albino mice. Guar gum is a naturally occurring galactomannan polysaccharide. It is made up of a linear chain of β-D-mannopyranose joined by β-(1-4) linkage with α-D-galactopyranosyl units attached by 1,6-links in the ratio of 1:2. Guar gum has been extensively used for colon delivery [10, 11] due to its drug release retarding property and susceptibility to microbial degradation in the large intestine.

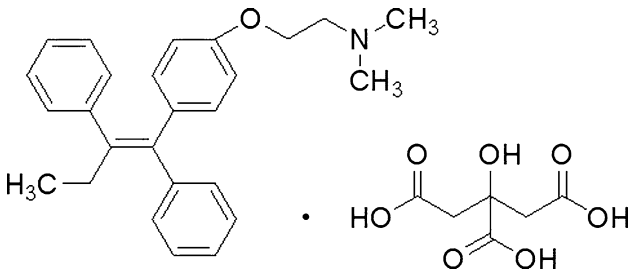

Fig. 1 Structure of tamoxifen citrate

Table 1 The basic recipe for the preparation of TC loaded guar gum nanoparticles

	Ingredients	Amount
Oil phase	Tamoxifen citrate	5 mg
	Solvents (DCM)	10 mL
	Span 80	3–4 mg
Aqueous phase	Millipore water	30 mL approx.
	Guar gum	Variables, 0.5, 1, 1.5, 2 (% w/v)

* Stabilizer concentration were varied from 10 to 25 mL

* Glutaraldehyde concentration were varied from 0.5 to 2 mL (25% solution)

Materials and methods

Materials

Tamoxifen Citrate was a gift from CDL, Kolkata, India. HPLC grade DCM, Span80, Glutaraldehyde (25–30% soln), guar gum powder and glycerol were purchased locally.

Preparation of guar gum nanoparticles

Tamoxifen citrate (Fig. 1) loaded guar gum nanoparticles were prepared by o/w emulsification and in situ polymer crosslinking method.

5 mg of the drug tamoxifen citrate was taken in 10 mL of different drug loading solvents, this forms the oil phase. To this added 4–5 mg of Span 80 under stirring. The oil phase was then added to a 0.5% aqueous guar gum solution under constant magnetic stirring. After mutual saturation of the oil and the continuous phase, the mixture was rapidly stirred at very high rpm. Glycerol (as stabilizer) was then added followed by addition of 25% glutaraldehyde solution to affect crosslinking. Nanosuspension was kept overnight for nanoparticle formation. Nanoparticles were obtained after centrifugation at 20,000 rpm at 0 °C for 30 min, washed with 15 mL HPLC grade water and recentrifuged. The yielded nanoparticles were lyophilized, harvested in micro centrifuge tubes and preserved in vacuum desicator. The basic recipe for the preparation of TC loaded guar gum nanoparticles is given in Table 1.

Particle size analysis

The mean particle diameter of the prepared nano particles was assessed by dynamic light scattering method (HORIBA, LB-550, Japan). The measuring range was from 1 nm to 6 μm, and the light source was 650 nm Laser diode of 5 mW. The samples of about 20.0 mL aqueous colloidal dispersions were measured directly without any pretreatment. Particle size was expressed as number weighted mean diameter in nanometers and was obtained from the measurements of three batches of nanoparticles.

Table 2 Formulation design and drug payload for tamoxifen citrate loaded guar gum nanoparticles

Solvent	Drug (mg)	Polymer solution (0.5% m/V, mL)	Emulsifier (mg)	Stabilizer (mL)	Loading (%)
Dichloromethane	5	30	4	25	15
1,2-Dichloroethane	5	30	4	25	5.8
Hexane	5	30	4	25	7.5
Chloroform	5	30	4	25	6.2

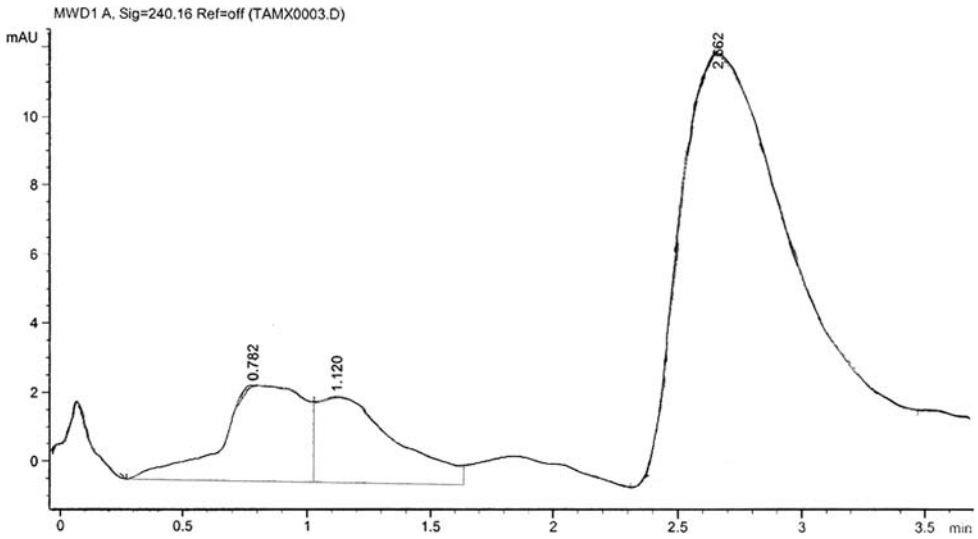

Fig. 2 HPLC chromatograph of tamoxifen citrate encapsulated in nanoparticles

Drug loading

Tamoxifen citrate content of nanoparticles was analysed by using HPLC, Shimadzu system with a UV-Visible detector and ODS column. A 20 μL sample was injected each time through rheodyne injectors and the HPLC peak areas were recorded from the chromatogram. A standard curve was prepared using known concentrations of Tamoxifen Citrate.

Prepared Tamoxifen citrate loaded nanoparticles were digested in 40 mL of 1% w/v sodium citrate solution and the final volume was made upto 50 mL with HPLC grade water. The mixture was sonicated at 20 KHz for 30 min and the solution was filtered using Centricon tubes in cold centrifuge (Shimadzu) at 0 °C, 20,000 rpm. Six samples from each batch were analysed in HPLC and the percentage drug loading was calculated.

Scanning electron microscope

The sample was dried properly and kept in a vacuum desiccator overnight prior to sample preparation. After drying, the sample in required size and shape is fixed on a carbon coated tap placed in an aluminium stub. The aluminium stub is then mounted in a Sputter coater (Polaron 760) unit for gold sputtering and run for 90 s. After coating is over the sample is mounted on the SEM (LEO 1430 VP) and scanning was performed in secondary electron mode at a voltage of 15 KV (Fig. 3a, b).

Fourier transform infrared spectroscopy

FTIR spectra of pure tamoxifen citrate, guar gum, tamoxifen citrate loaded guar gum nanoparticles were recorded in KBr pellets, using a Perkin Elmer 4200 spectrophotometer (Fig. 4a, b, c) to observe for any drug-polymer interaction.

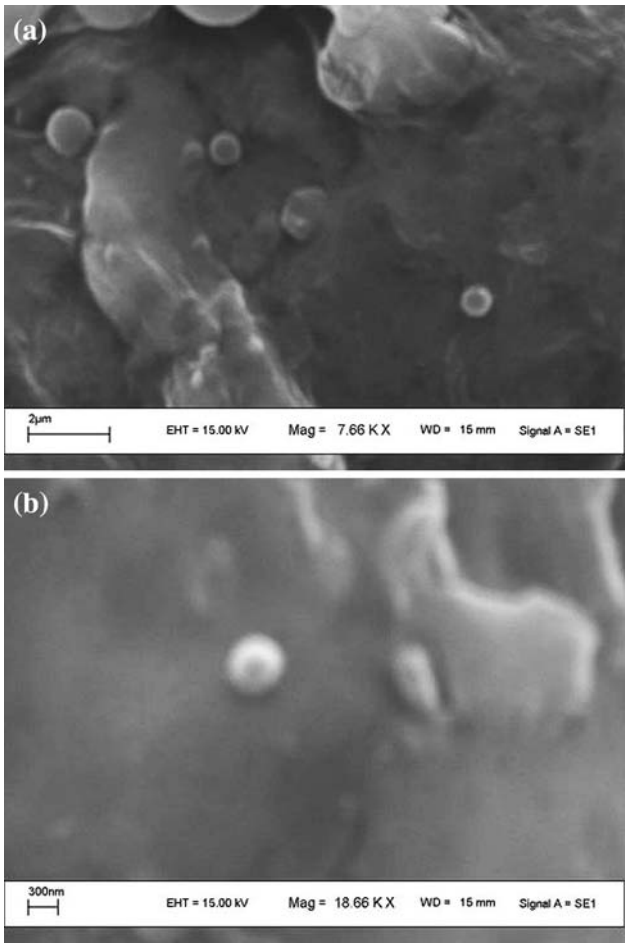

Fig. 3 **a** SEM micrograph of tamoxifen citrate loaded guar gum nanoparticles. **b** SEM micrograph (magnified) of tamoxifen citrate loaded guar gum nanoparticles

Results and discussion

Tamoxifen citrate loaded guar gum nanoparticles were prepared using nanoemulsification polymer crosslinking

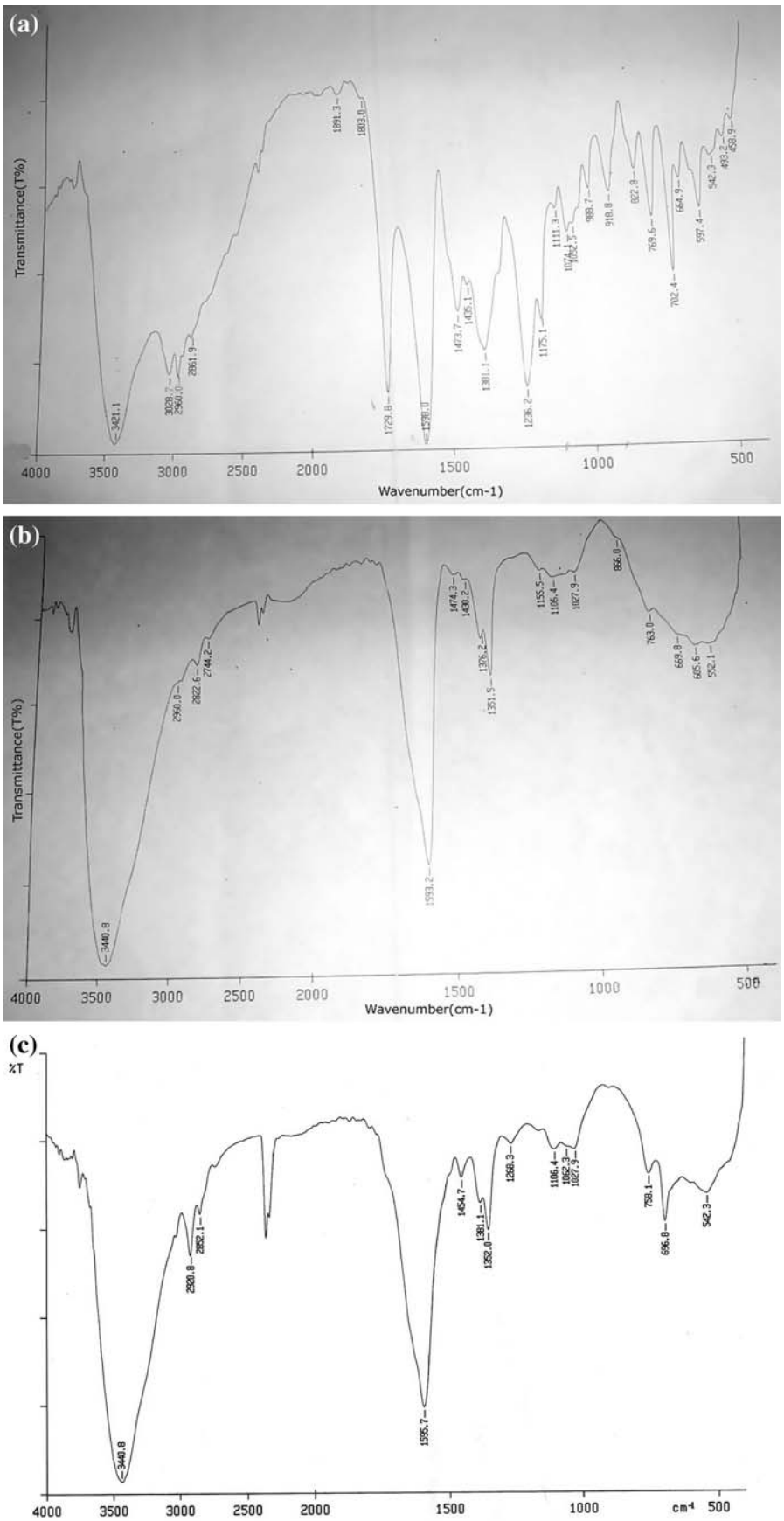

Fig. 4 **a** FTIR spectra of pure tamoxifen citrate. **b** FTIR spectra of guar gum. **c** FTIR spectra of tamoxifen citrate loaded guar gum nanoparticles

method. Four different formulations were designed (Table 2) using different drug loading solvents viz. dichloromethane, hexane, chloroform and 1,2- dichloroethane. Different drug loading solvents showed a distinct impact on drug payloading.

Formulation with DCM as drug loading solvent provided the best loading for tamoxifen citrate (Fig. 2). HPLC chromatograph showing peak is attributed to tamoxifen citrate encapsulated in the nanoparticles. Drug load was quantitated by HPLC, Shimadzu system with a UV-Visible detector and ODS column. In view of the drug pay load, formulation with DCM as drug loading solvent was chosen for further physicochemical studies and necessary evaluations to use it as a drug delivery device.

Guar gum concentration and stabilizer concentration were optimized to obtain the desired size of nanoparticles. Guar gum concentrations were varied from 0.5 to 2 (w/v) and it was observed that size of nanoparticles increase from 200 to 600 nm with increase in guar gum concentration. As the polymer concentration increases, the viscosity of the solution increases and the polymer solution dispersed into larger droplets. In the present investigation a 0.5% guar gum concentration was found to be optimal, ensuring relatively lower size of nanoparticles. It was found statistically significant ($p < 0.01$). Figure 3a and b exhibited spherical shape with a core–shell type nanoparticles in the size range of 200–300 nm in scanning electron microscopy (SEM).

Glycerol was used as stabilizer throughout the work. It was observed that the particle size of the nanoparticles increase sharply when concentration of stabilizer was decreased and polymer concentration was increased. It was found statistically significant ($p < 0.01$). Our work has been in good agreement with the work of Labhasetwar et al. [12].

FTIR experiments were carried out to determine for any drug—polymer interaction in the prepared nanoparticles. Figure 4a, b, c exhibited the FTIR spectra of Pure Tamoxifen Citrate, Guar gum and tamoxifen citrate loaded guar gum nanoparticles. When IR spectra of tamoxifen citrate in nanoparticles was compared with that of powder tamoxifen citrate, a clear loss of resolution of tamoxifen citrate is seen. Further disappearance of the peak at 1,729.8 cm^{-1} in tamoxifen citrate due to C=O stretching vibration was observed in the drug loaded nanoparticles. Peak at 2,960 cm^{-1} due to C–H stretching vibration of guargum has been shifted to 2,920 cm^{-1} in the nanoparticles due to presence of tamoxifen citrate. Disappearance of typical bands of tamoxifen citrate such as tertiary amine bands at 900–1,100 cm^{-1} is also seen in the nanoparticles.

In vitro release character and kinetics of tamoxifen citrate from the prepared nanospheres is under comparative investigation against conventional preparation available in the tablet form for evaluation of degree of release retardation obtained with the nanospheres. On finalizing the desired release rate the efficacy of the prepared drug delivery system will be investigated on an animal model experiment with female albino mice.

Conclusion

The present work has shown the drug containing nanoparticle formation by the emulsification in situ polymer cross-linking method. It demonstrates the potential process to encapsulate a comparatively higher amount of drug by guar gum with dichloromethane as a drug loading solvent. Choice of solvent is important to effect a higher drug payload. Dichloromethane was found to be the best choice as a drug loading solvent. The stability and the size of droplets formation during the preparation stages are important factors. Process parameters viz. Polymer concentration and stabilizer type and concentration are crucial in determining the size of the final nanoparticles.

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