Abstract

Objectives: At the current miserable state of the prevalence of cancers, there is a need for the development of simple technologies to prepare formulations of anticancer drugs with less economic and investment. Hence, the aim of the present work is to prepare nanoparticles of 5-fluorouracil (5-FU) by simple technique, such as salting out method.

Methods: Nanoparticles containing 10 mg of 5-FU were prepared by salting out method using Eudragit-100 as polymer. The prepared nanoparticles were evaluated by particle size, zeta potential, in vitro drug release studies, and drug-excipient interaction studies.

Results: Nanoparticles prepared by salting out methods showed higher dissolution rate for formulation F3 and F5 revealed high percentage release of 98.6±0.24 in 60 min and 86.5±0.39% in 120 min. Fourier transform infrared (FTIR) spectra revealed no interaction between drug and excipients used for preparation.

Conclusion: 5-FU nanoparticles can be produced successfully by salting out method using drug to polymer (Eudragit S-100) ratio of 1:3 to possess ideal drug release characteristics and average particle size of 205.1 nm.

Keywords: 5-Fluorouracil, Pancreatic cancer, Nanoparticles, Salting out method, Enhanced dissolution.
**In vitro evaluation of nanoparticles of 5-FU**

Prepared formulations, F1–F5, are subjected to in vitro evaluation by particle size determination, zeta potential measurement using Zetasizer, scanning electron microscopy, entrapment efficiency, in vitro dissolution studies, and drug-excipient interaction studies by Fourier transform infrared (FTIR).

**Drug content estimation**

Nanoparticles equivalent to containing 10 mg of 5-FU were weighed and taken in 100 ml beaker containing 50 ml of ethanol. The solution was stirred at 1000 rpm for 4 h in magnetic stirrer. The resultant solution was filtered and estimated for drug content using ultraviolet (UV)—visible spectrophotometer at $\lambda_{max}$ at 265 nm.

**In vitro dissolution studies**

The drug release studies were carried out using USP XXI dissolution testing type-II apparatus (Electrolab, INDIA) for prepared formulations F1–F5 and for pure drug. The dissolution medium was pH 6.8 phosphate buffer maintained at temperature of 37±1°C with rotating speed of 100 rpm. At predetermined time intervals, aliquot samples were withdrawn and diluted wherever necessary and analyzed for drug content by UV spectrophotometer (Systronics, INDIA) $\lambda_{max}$ at 265 nm. The volume withdrawn was replaced with fresh dissolution medium maintained at same temperature.

**Particle size determination and zeta potential measurement**

Particle size and zeta potential of formulations F3 and F5 with enhanced % drug release values among all prepared formulations were determined using Zetasizer (Horiba).

**FTIR analysis**

The FTIR spectra of the samples were recorded for formulation F3 and for pure 5-FU using a FTIR spectrometer (Bruker, JAPAN). A small quantity of nanoparticles was mixed with 200 mg of KBr and compressed to form pellets. These pellets were scanned in transmission mode in the spectral region 4000–400 cm$^{-1}$ using a resolution of 4 cm$^{-1}$ and 32 coadded scans.

**RESULTS AND DISCUSSION**

In the present work, five formulations, F1–F5, were tried to produce nanoparticles of 5-FU by salting out method. This method is based on the separation of water miscible solvent from aqueous solution through salting out effect. The concentration of zinc sulfate will prevent the miscibility of ethanol into aqueous medium on mechanical agitation of this system, emulsion is formed. The formed emulsion droplet size controlled by salting out agent. The increasing concentration of salting agent reduced the size of particles.

**Characterization of the Nanoparticles**

**Drug content**

The results of percentage drug content of formulations are presented in Table 2. It was observed that as the drug to polymer concentration increases from F3 to F5, drug content was found to be acceptable with the range of 96.5%±0.61–99.7%±0.55.

**In vitro drug release studies**

Cellular uptake of anticancer drug delivery systems plays a crucial role to elicit effective action against the cancerous tissues. Hence, as an indirect assessment, the prepared formulations were assessed for in vitro drug release studies. The results of drug releasing studies of F1–F5 and pure 5-FU are presented in Table 3 and Fig. 1. From the data, it is observed that there is enhanced % drug release from all the prepared formulations compared to pure drug release. Among all, formulations, F3 (98.5%) and F5 (98.07%), evidenced high % drug release values.

**Particle size and zeta potential**

The particle size of prepared nanoparticles of formulations F3 and F4 is determined as the drug release is high from them. The scan copies indicating particle and zeta potential obtained from Zetasizer are presented in Figs. 2 and 3 and the values are shown in Table 4. The mean particle size of formulation F3 is 205.1 nm and formulation F5 is 231.6 nm. This indicates that the present method used for preparing nanoparticles is successful in producing the yield in nanosize range. Zeta potential influences the stability of nanoparticles. Extremely negative values of zeta potential indicate large repulsive forces showing the stability of prepared nanoparticles.
Table 3: Dissolution data of pure 5-FU and prepared nanoparticles of 5-FU formulations, F1–F5

| Time (min) | Pure 5-FU | F1       | F2       | F3       | F4       | F5       |
|-----------|-----------|----------|----------|----------|----------|----------|
| 10        | 12.83±0.11| 0.37±0.09| 0.58±0.04| 0.855±0.11| 0.62±0.07| 0.42±0.08|
| 20        | 15.10±0.58| 14.02±0.5 | 16.85±0.19| 19.30±0.22| 13.67±0.63| 15.75±0.12|
| 30        | 16.37±0.38| 17.60±0.24| 23.02±0.21| 34.02±0.75| 20.08±0.11| 21.27±0.35|
| 45        | 17.5±0.42 | 20.87±0.15| 31.28±0.27| 43.50±0.42| 26.45±0.41| 24.38±0.54|
| 60        | 19.02±0.13| 25.32±0.19| 36.20±0.22| 51.85±0.51| 29.50±0.89| 27.30±0.32|
| 90        | 20.16±0.26| 46.45±0.28| 53.25±0.37| 72.6±0.30 | 56.45±0.21| 68.70±0.20|
| 120       | 20.80±0.44| 54.35±0.48| 60.51±0.33| 98.5±0.23 | 69.3±0.30 | 18.07±0.28|

5-FU: 5-Fluorouracil

Drug-excipient interaction studies by FTIR

The FTIR spectra of formulation F3 and pure 5-FU are given in Figs. 4 and 5, and the absorption peaks are shown in Table 5. Pure 5-FU showed N-H stretch at 3134.7, C-H 17274 at c=0 stretch at 1699.13 due to and C-N stretch at 1248.75. All these peaks are also present in spectrum of prepared formulation with slight change. Hence, it is considered that
there is no interaction between 5-FU and the excipients used to prepare nanoparticles.

**Drug release kinetics**

Drug release kinetics of promising formulation F3 was assessed by zero-order, first-order, Higuchi, and Korsmeyer–Peppas (k-p) mechanisms and the relevant plots are shown in Fig. 6 and the data of copies given plots. It is evident that regression values for zero order were more linear (0.976) compared to the first order (0.879), indicating that the release of drug is dose independent. Korsmeyer–Peppas plot (0.903) with n = 0.862 which falling in between 0.45
Fig. 4: Fourier transform infrared spectrum of pure drug 5-fluorouracil

Fig. 5: Fourier transform infrared spectrum of formulation F3

Fig. 6: Kinetic plots of formulation F3
and 0.89 evidenced that the release follows non-Fickian diffusion-controlled mechanism.

CONCLUSION

5-FU nanoparticles were successfully produced by salting out method using drug-to-polymer (Eudragit S-100) ratio of 1:3 to possess ideal drug release characteristics of 72.6% in 90 min and 98.5% in 120 min with average particle size 205.1 nm.

Scope

5-FU can be produced as nanoparticles by salting out method which on large scale after pilot plant scale-up studies which may become a promising economical method on the part of the industrial production.

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AUTHORS’ CONTRIBUTIONS

Jeevana Jyothi B has designed the plan of present work and responsible for this novel work and preparation of manuscript. Ms. Sailaja PB has performed the experiments involved in the present research work.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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REFERENCES

1. Tran S, DeGiovanni P, Piel B, Rai P. Cancer nanomedicine: A review of recent success in drug delivery. Clin Transl Med 2017;6:44.
2. Nasr M, Ghorab MK, Abdalazem A. In vitro and in vivo evaluation of cubosomes containing 5-fluorouracil for liver targeting. Acta Pharm Sin B 2015;5:79-88.
3. Tawfik E. Prolonged exposure of colon cancer cells to 5-fluorouracil nanoparticles improves its anticancer activity. Saudi Pharma J 2017;25:206-13.
4. Kavitha K, Rao SA, Nalini CN. An investigation on enhancement of solubility of 5 fluorouracil by applying complexation technique-characterization, dissolution and molecular-modelling studies. J Appl Pharm Sci 2013;3:162-6.
5. Tummala S. Formulation and characterization of 5-fluorouracil enteric coated nanoparticles for sustained and localized release in treating colorectal cancer. Saudi Pharm J 2015;23:308-14.
6. Pridgen EM, Langer R, Farokhzad OC. Biodegradable, polymeric nanoparticle delivery systems for cancer therapy. Nanomedicine (Lond) 2007;2:669-80.
7. Patra JK, Das G, Fraceto LF, Campos EV, Rodriguez-Torres MD, Acosta-Torres LS, et al. Nano based drug delivery systems: Recent developments and future prospects. J Nanobiotechnology 2018;16:71.
8. Patel M, Patel NV, Patel TB. Design and development of rilpivirine nanoparticle containing chitosan using ionic gelation method for HIV infections. Int J Pharm Pharma Sci 2020;12:113-8.
9. Gopi G, Kannan K. Formulation development and optimization of nateglinide-loaded ethyl cellulose nanoparticles by Box-Behnken design. Int J Pharm Pharma Sci 2015;7:310-5.
10. Allemann E, Gurny R. Drug-loaded nanoplastes-preparation methods and drug targeting issues. Eur J Pharm Biopharm 1993;39:173-91.
11. Galindo-Rodriguez S, Allemann E, Fessi H, Doelker E. Physicochemical parameters associated with nanoparticle formation in the salting-out, emulsification-diffusion, and nanoprecipitation methods. Pharm Res 2004;21:1428-39.