Faraday Discussions
Cite this: DOI: 10.1039/c4fd00176a 177/4398

PAPER

Pluronic copolymer encapsulated SCR7 as a potential anticancer agent

Franklin John,[a] Jinu George,[a] Mrinal Srivastava,[b] P. A. Hassan,[c] V. K. Aswal,[d] Subhas. S. Karki[e] and Sathees. C. Raghavan[b]

Received 15th September 2014, Accepted 4th November 2014
DOI: 10.1039/c4fd00176a

Nonhomologous end joining (NHEJ) of DNA double strand breaks (DSBs) inside cells can be selectively inhibited by 5,6-bis-(benzylideneamino)-2-mercaptopyrimidin-4-ol (SCR7) which possesses anticancer properties. The hydrophobicity of SCR7 decreases its bioavailability which is a major setback in the utilization of this compound as a therapeutic agent. In order to circumvent the drawback of SCR7, we prepared a polymer encapsulated form of SCR7. The physical interaction of SCR7 and Pluronic® copolymer is evident from different analytical techniques. The *in vitro* cytotoxicity of the drug formulations is established using the MTT assay.

Introduction

Cancer drug targeting is challenged by problems like multi-drug resistance and low solubility of potential drug candidates. There are two general classes of resistance to anti-cancer drugs: those that impair delivery of anti-cancer drugs to tumor cells, and those that arise in the cancer cell itself due to genetic and epigenetic alterations that affect drug sensitivity. Impaired drug delivery can result from poor absorption of orally administered drugs, increased drug metabolism or increased excretion, resulting in lower levels of drug in the blood and reduced diffusion of drugs from the blood into the tumor.[1,2]

Cytotoxic therapy offers one prominent approach towards cancer treatment. Traditional cytotoxic therapy includes radiation and chemotherapeutic compounds such as platinum-based drugs.[3–6] Among the genetic damages, DNA double strand breaks (DSBs) are considered as the most lethal as they affect the integrity and continuity of the genome.[7–10] Inappropriate repair of DSBs may result in deletions, inversions, duplications and chromosomal translocations.[11–14]

[a]*Biotechnology Laboratory, PG and Research Department of Chemistry, Sacred Heart College, Kochi 682 013, India*
[b]*Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India*
[c]*Chemistry Division, Bhabha Atomic Research Centre, Mumbai 400 085, India*
[d]*Solid State Physics Division, Bhabha Atomic Research Centre, Mumbai 400 085, India*
[e]*Department of Pharmaceutical Chemistry, KLE University, Bangalore 560 010, India*

Nonhomologous DNA end joining (NHEJ) is one of the major DNA DSB repair pathways.[15] Recently, a novel inhibitor of NHEJ, 5,6-bis-(benzylideneamino)-2-mercaptopyrimidin-4-ol (SCR7) has been reported.[16] Inhibition of NHEJ by SCR7 in cancer cells results in the accumulation of unrepaired DNA double-strand breaks. Despite promising results of SCR7 as a good anticancer agent, it showed high IC_{50} which could be attributed to its high hydrophobicity.

Results and discussion

Although SCR 7 has shown promising results as a potential anticancer drug, its limited solubility in water may lead to poor bioavailability and requires the search for novel drug delivery systems. Traditional methods to improve the bioavailability of poorly soluble drugs include encapsulating them in nanosized carriers such as liposomes,[17] emulsions, polymer micelles, niosomes,[18] lipid particles *etc.* Poly Ethylene Glycol (PEG)[19] based block copolymers have the distinct advantage as compared to other delivery systems due to its ability to encapsulate large amounts of drug. One of the most widely studied classes of amphiphilic copolymers in this field is the Pluronic triblock copolymers. Thus, we investigated the role of Pluronic P123 in encapsulating and delivering SCR7 *via* hydrophobic interactions.

Fluorescence emission spectra of SCR7 showed a blue shift upon encapsulation with P123 copolymer in DMSO as solvent as shown in Fig. 1(a). The fluorescence spectra of SCR7 in DMSO exhibits an emission peak at ~474 nm while SCR7 in the presence of P123 show a maximum at ~442 nm with the same solvent (DMSO due to the high solubility of SCR7). The shift in the λ_{max} of fluorescence spectrum is an indication of interaction between SCR7 and the copolymer P123. Such shift in the fluorescence emission is reported for doxorubicin encapsulated with chitosan nano particles and curcumin casein micelles.[20,21]

Fig. 1(b) shows the UV-Vis absorption spectra of SCR7-P123 dissolved in a minimum amount of water. The hydrophobic PPO core of the copolymer effectively encapsulates the drug molecule and makes it dispersible in the aqueous layer. To reiterate the encapsulation and release of SCR7 from P123, the aqueous

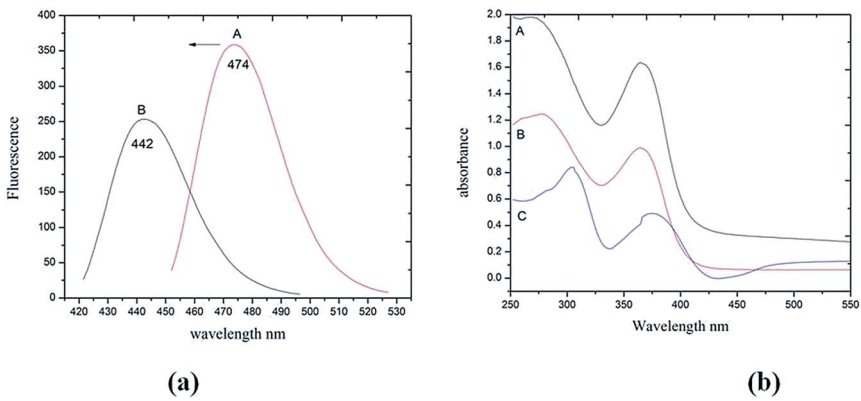


Fig. 1 (a) (A) Fluorescence emission spectra of SCR7 in DMSO (B) SCR7 loaded P123 in DMSO. (b) (A) Absorption spectra of SCR7 loaded P123 in water (B) chloroform extract (C) SCR7 alone in water.

layer was extracted with chloroform (3×). The chloroform layer is dried with Na_2SO_4. UV absorption spectra of the chloroform layer show absorption maxima at 267 nm and 365 nm. The optical absorption spectrum of the chloroform layer is compared with SCR7 in pure water and SCR7 encapsulated P123 in water (A and C of Fig. 1(b)). This indicates the ability of P123 in encapsulating the drug and its release upon treatment with hydrophobic solvents like chloroform.

The FTIR spectra of SCR7 and SCR7 encapsulated with P123 are depicted in Fig. 2. The C–S stretching vibrations occur at 3271 cm^{-1}, while the C–S bending occurs at 1047 cm^{-1} and 1082 cm^{-1}. The C–N stretching vibrations occur at 1026 cm^{-1}, whereas the N–H bending vibrations occur at 924 cm^{-1}. The absorptions at 1250 cm^{-1}, 1117.6 cm^{-1} and 1031.1 cm^{-1} are due to C–O stretching vibrations from the P123 matrix. The absorptions at 957 cm^{-1} and 817 cm^{-1} are arising from the aromatic ring of SCR7. As seen, the spectrum of SCR7-P123 did show the characteristic absorption band of SCR7 at 2965 cm^{-1} and 1107 cm^{-1}. FT-IR spectral analysis reveals that there is no appreciable shift in the IR signal of SCR7 when it changes from the free state to the polymer bound state. It can also be concluded that there is no chemical interaction taking place between P123 and SCR7. The result confirmed the presence of SCR7 in the encapsulated formulation.

Successful incorporation of SCR7 in P123 and its release in different solvents was elucidated by ^1H NMR spectroscopy. As is evident from Fig. 3, the ^1H NMR spectra of SCR7-P123 employing DMSO-d6 as the solvent showed characteristic aromatic signals from SCR7 at 7.1–7.5 ppm. In all the spectra, the aromatic proton signals corresponding to SCR7 are seen with lower intensity than compared with the Pluronic counterpart. The small signals from SCR7 are indicated by * in Fig. 2.

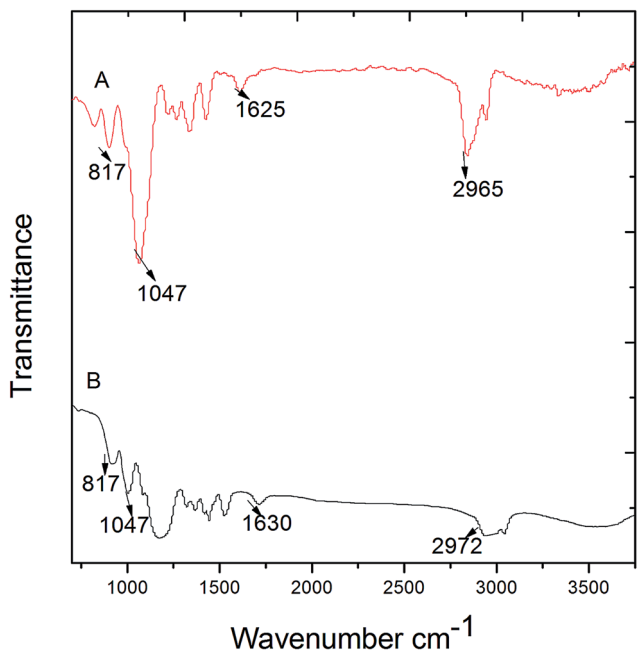
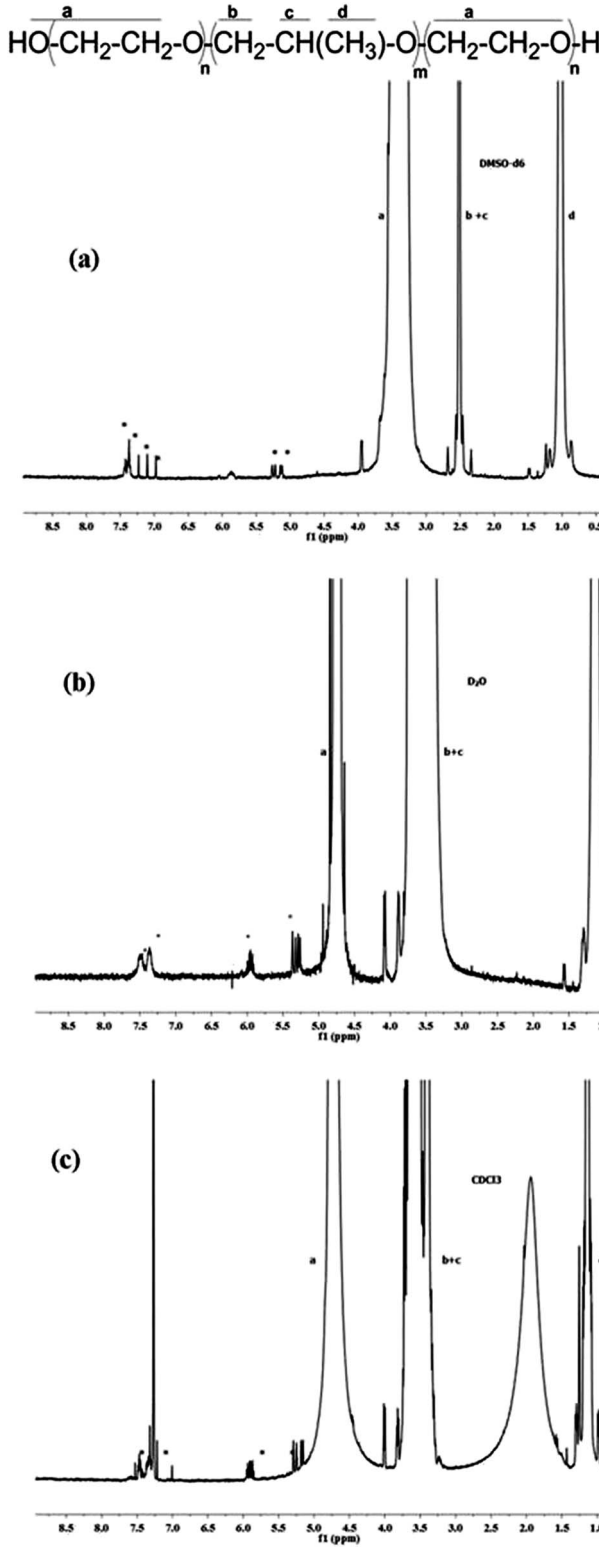


Fig. 2 FT-IR spectra of (A) SCR7 and (B) SCR7-P123.

ART ■ C4FD00176A



ART ■ C4FD00176A

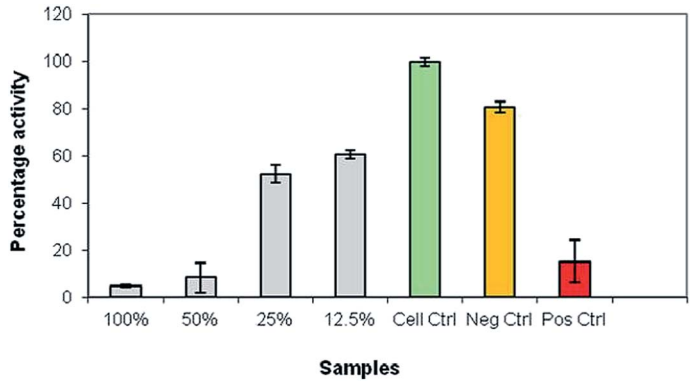


Fig. 4 MTT assay profile of SCR7P123.

In vitro cytotoxic assay

A cytotoxic assay was performed to measure the metabolic activity of cells. This was carried out by reducing yellow coloured tetrazolium salt 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide to purple coloured formazan. The test sample was prepared by dissolving 200 mg of SCR7-P123 in 0.5 mL of DMSO and made up to 5.8 mL with culture medium containing serum and sterilized filters. This was diluted with culture medium to 50%, 25% and 12.5%. Cells cultured in normal medium were considered as the cell control. An equal volume (100 μL) of various dilutions of test samples, extract of negative control, cell control and positive control were placed on subconfluent monolayer of L929 cells. After incubation of the cells with various concentrations of test sample and controls at 37 °C for 24 ± 2 h, the extract and control medium was replaced with 50 μL MTT solution (1 mg mL^{-1} in medium without supplements), wrapped with aluminium foil and incubated at 37 °C for 2 hours. After discarding the MTT solution 100 μL of isopropanol was added to all wells and the plates were shaken. The developed colour was quantified by measuring absorbance at 570 nm using a spectrophotometer. The data obtained for the test sample were compared with the cell control. As shown in Fig. 4, the MTT assay of L929 cells after 24 hour contact with samples 100%, 50%, 25% and 12.5% of test material SCR7P123 showed 4.72%, 8.23%, 52.29%, and 60.69% metabolic activity respectively. The positive control (diluted phenol with culture medium containing serum) showed 15.14% and the negative control (ultra high molecular weight poly ethylene with culture medium containing serum) showed 80.56% metabolic activity.

Experimental section

Pluronic P123 and D_2O were purchased from Aldrich (Bangalore, India). SCR7 was gifted from Indian Institute of Science, Bangalore. All the salts and solvents used in the study were purchased from Merck (Mumbai, India). All other reagents and buffer solution components were analytical grade. Distilled and deionized water was used in all experiments.

Fig. 3 ^{1}H NMR spectra of SCR7P123 in (a) DMSO-d6 (b) D_2O and (c) $CDCl_3$.

ART ■ C4FD00176A

Preparation of SCR7-loaded polymeric micelles

SCR7 loaded micelles were prepared by the thin-film hydration method. 30 8 mg of SCR7 and 440 mg of Pluronic block copolymer P123 were dissolved in 10 mL acetonitrile in a round-bottom flask. The solvent was evaporated by rotary evaporation at 50 °C for about 1 h to obtain a solid SCR7-P123 matrix. Residual acetonitrile remaining in the film was removed under vacuum overnight at room temperature. Then, the resultant thin film was hydrated with different amounts of water, while the hydration temperature was varied according to the experimental design. The mixture was stirred at 700 rpm for 30 min to obtain a micelle solution, which was then filtrated through 0.2 μm filter membrane to remove the unincorporated drug aggregates, followed by lyophilization.

Characterization

Fluorescence emissions were recorded on a F-2500 fluorescence spectrometer (Hitachi, Japan). UV measurements were performed on a Shimadzu (UV-2450) UV-visible double beam spectrophotometer with a matched pair of stoppered fused silica cells of 1 cm optical path length. Fourier transformed infrared (FT-IR) measurements were obtained using a SHIMADZU 8400 FT IR spectrometer. The drug loading and release characteristics of P123 encapsulated SCR7 was analyzed by ^{1}H NMR spectra which was recorded on a Varian 400 MHz spectrometer (Varian, Palo Alto, CA, USA) in deuterated dimethyl sulfoxide (DMSO-d6), chloroform ($CDCl_3$) and water (D_2O) at room temperature.

Thin layer chromatography (TLC) was adopted to study the physical interaction of SCR7 and P123. 10% methanol in chloroform was used as the mobile phase and silica gel G was used as the stationary phase. The spots were detected under UV light and stained using alkaline $KMnO_4$ solution and the R_f values were determined.[22] To evaluate the cell cytotoxic potential of SCR7-P123, a MTT assay was performed. The cell line was obtained from the biotechnology wing of SCTIMST Thiruvananthapuram. The source of the cell line is the ATCC strain and L-929. L-929 is an established and well characterized mammalian cell line that has demonstrated reproducible results. The cells were cultured in 10% FBS. Ultra high molecular weight poly ethylene was used as the negative control and it was prepared by incubating 3 cm^2 ultra high molecular weight poly ethylene with culture medium containing serum at 37 ± 1 °C for 24 ± 2 h. Diluted phenol was used as the positive control and was prepared by diluting phenol stock solution (13 mg mL^{-1}) to 1.3 mg mL^{-1} with culture medium containing serum.

Conclusions

Anti cancer agent SCR7 has the capability to inhibit NHEJ in a Ligase IV dependent manner within cells. Hydrophobicity of SCR7 is a major setback in the utilization of this compound. Polymer encapsulation of small molecules to enhance their physicochemical properties represents an alternative approach in recent drug discovery research. The major focus of the reported study is to formulate an aqueous soluble matrix for drug delivery. The thin film hydration method is confirmed to be one of the most suitable methods for the encapsulation of SCR7 within the copolymer. ^{1}H NMR spectra confirmed the entrapment of the drug within the Pluronic polymeric core, along with fluorescence, UV-Vis and

ART ■ C4FD00176A

FT-IR spectroscopic techniques. SCR7 is efficiently diffused from the polymeric core as evidenced from the chloroform extract. *In vitro* cytotoxic assays showed that the encapsulated form of SCR7 induced cytotoxicity in a concentration dependent manner. Biocompatibility of the polymeric matrix can serve as an ideal formulation for controlled drug delivery applications.

Acknowledgements

The authors thank Department of Biotechnology, Govt. of India (DBT, no. BT/PR7703/27/493/2013) for financial support. We are grateful to Sophisticated Instrumentation Facility, CUSAT, Kochi for the analytical instrumentation service. Fluorescence and TOF MS/MS spectra were recorded at School of Environmental Sciences, M.G University, Kottayam.

Notes and references

1 M. M. Gottesman, T. Fojo and S. E. Bates, *Nat. Rev. Cancer*, 2002, **2**, 48.
2 D. M. Molina, R. Jafari, M. Ignatushchenko, T. Seki, E. A. Larsson, C. Dan, L. Sreekumar, Y. Cao and P. Nordlund, *Science*, 2013, **341**, 84.
3 S. Aggarwal, *Nat. Rev. Drug Discovery*, 2010, **9**, 427.
4 H. A. Shih, J. S. Loeffler and N. J. Tarbell, Effects of CNS Radiation Therapy, *Late Effects of Treatment for Brain Tumors*, Springer, US, 2009, p. 23.
5 S. P. Jackson and J. Bartek, *Nature*, 2009, **461**, 1071.
6 T. Helleday, E. Petermann, C. Lundin, B. Hodgson and R. A. Sharma, *Nat. Rev. Cancer*, 2008, **8**, 193.
7 S. Sharma and S. C. Raghavan, *J. Nucleic Acids*, 2010, **1**, 2010.
8 M. R. Lieber, K. Yu and S. C. Raghavan, *DNA Repair*, 2006, **5**, 1234.
9 C. Wyman and R. Kanaar, *Annu. Rev. Genet.*, 2006, **40**, 363.
10 V. Gopalakrishnan and S. C. Raghavan, *Future Oncol.*, 2012, **8**, 1121.
11 M. Nambiar and S. C. Raghavan, *Cell. Mol. Life Sci.*, 2013, 1.
12 M. Nambiar and S. C. Raghavan, *Nucleic Acids Res.*, 2011, **39**, 5813.
13 S. C. Raghavan, P. C. Swanson, X. Wu, C. L. Hsieh and M. R. Lieber, *Nature*, 2004, **428**, 88.
14 F. W. Alt, Y. Zhang, F. L. Meng, C. Guo and B. Schwer, *Cell*, 2013, **152**, 417.
15 M. L. Hefferin and A. E. Tomkinson, *DNA Repair*, 2005, **4**, 639.
16 M. Srivastava, M. Nambiar, S. Sharma, S. S. Karki, G. Goldsmith, M. Hegde, S. Kumar, M. Pandey, R. K. Singh, P. Ray, R. Natarajan, M. Kelkar, A. De, B. Choudhary and S. C. Raghavan, *Cell*, 2012, **151**, 1474.
17 J. W. Lee, E. D. Jeong, E. J. Cho, J. A. Gardella Jr, W. Hicks Jr, R. Hard and F. V. Bright, *Appl. Surf. Sci.*, 2008, **255**, 2360.
18 W. Chen, Y. Shi, H. Feng, M. Du, J. Z. Zhang, J. Hu and D. J. Yang, *J. Phys. Chem. B*, 2012, **116**, 9231.
19 D. Qiu, C. Flood and T. A. Cosgrove, *Langmuir*, 2008, **24**, 2983.
20 S. Alexander, T. Cosgrove, T. C. Castle, I. Grillo and S. W. Prescott, *J. Phys. Chem. B*, 2012, **116**, 11545.
21 P. Alexandridis and L. Yang, *Macromolecules*, 2000, **33**, 5574.
22 M. J. Ansari, S. Ahmad, K. Kholi, J. Ali and R. K. Khar, *J. Pharm. Biomed. Anal.*, 2005, **39**, 132.