Preparation and *In Vitro* Characterization of Poly(sebacic acid-*co*-ricinoleic acid)-Based Tamoxifen Citrate-Loaded Microparticles for Breast Cancer

J. G. Hiremath,[1] C.G. Rudani,[1] A. J. Domb,[2] R. V. Suthar,[1] N. S. Khamar[1]

[1]*Department of Pharmaceutics, East West College of Pharmacy, Bangalore 560 091, Karnataka, India*
[2]*Institute for Drug Research, Medicinal Chemistry, School of Pharmacy, Faculty of Medicine, Hebrew University of Jerusalem 91120, Israel*

Received 25 March 2011; accepted 25 August 2011
DOI 10.1002/app.35529
Published online 6 December 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: This study was aimed to develop an injectable polymeric drug delivery system for tamoxifen citrate (TC) using poly(sebacic acid-*co*-ricinoleic acid) [poly(SA-RA) 70 : 30 w/w] as a drug carrier for the treatment of estrogen receptor positive breast cancer. Injectable biodegradable microparticles of TC were produced by solvent displacement technique of microencapsulation and were characterized by surface morphology (scanning electron microscopy), particle size, size distribution, physical and chemical interaction (Fourier transform infrared), nature and physical state of drug [DSC and X-ray diffraction (XRD)], and *in vitro* release studies. TC loading over different concentrations was analyzed by high performance liquid chromatography (HPLC) technique. Polyanhydride microparticles obtained after lyophilization were nearly spherical in shape with smooth surface and size less than 2.5 μm. TC was dispersed in the form of amorphous state, and TC remains intact and stable during the process of microencapsulation. *In vitro* drug release studies demonstrated prolonged controlled release of TC with zero-order kinetics. Stability studies revealed that the production process of microparticles itself did not affect the chemical stability of the drug and polymer forming the particle matrix. Significant difference in drug release capacity was observed in microparticles with different drug loadings, and the drug release was more sustained in microparticles prepared with high TC. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 4747–4754, 2012

Key words: tamoxifen citrate; poly(SA-RA) 70 : 30 w/w; injectable; microparticles; breast cancer

INTRODUCTION

Present scenario reveals that breast cancer is the most common cancer in women representing 16% of all female cancers worldwide. The United States have the highest annual incidents rate of breast cancer in the world. In year 2009–2010, 192,370 postmenopausal women were diagnosed with invasive breast cancer and 62,280 with *in situ* breast cancer.[1] Nonsteroidal antiestrogen such as tamoxifen citrate (TC) is the treatment of choice for the patients with all stages of estrogen receptor (ER) positive breast cancer.[2] TC, trans isomer of triphenylethylene derivative, is an oral selective ER modulator. TC is used as an adjuvant therapy in the ER positive breast cancer.[3] Oral TC undergoes extensive hepatic metabolism and the subsequent biliary excretion of metabolites. TC can lead to harmful long-term side effects such as the development of endometrial cancer or an acquired TC resistance leading to further tumor progression.[4] Several novel approaches have been reported with the aim of increasing effectiveness and bioavailability of TC, such as nanoparticles of tamoxifen using poly(caprolactone) (PCL)[5], tamoxifen-loaded injectable microspheres of PCL,[6] and alginate/chitosan microparticles of tamoxifen.[7]

To avoid undesirable effects of TC and provide better means of drug delivery at tumor site, microparticles of TC have been developed using polyanhydride polymer, poly(sebacic acid-*co*-ricinoleic acid) [poly(SA-RA)]. Polyanhydrides are versatile class of biomaterials, composed of anhydride bonds that undergo hydrolysis in the presence of water.[8] They get eroded primarily by surface erosion and provide linear release profile of drugs and proteins.[8,9] In this study, biodegradable poly(SA-RA) microparticles of TC have been prepared and *in vitro* characterization has been carried out.

MATERIALS AND METHODS

Materials

Microparticles were prepared by using poly(SA-RA) (sebacic acid to ricinoleic acid in the ratio of 70 : 30;

Correspondence to: J. G. Hiremath (jagadeeshgcp@gmail.com).

Contract grant sponsors: East West Group of Institution (Ravikiran C. N., Secretary); East West College of Pharmacy (K. A. Sridhar, Department of Pharmacology).

TABLE I
Composition of Biodegradable TC-Loaded Poly(SA-RA) 70 : 30 w/w Microparticles

Formulation	Drug Tamoxifen citrate (mg)	Polymer Poly(SA-RA) (70 : 30 w/w) (mg)	Cryoprotectant Glucose (mg)	Mannitol (mg)
F_1	40	160	14	14
F_2	60	140	14	14

weight-average molecular weight = 21,000; number-average molecular weight = 10,000], was previously synthesized and reported.[10] Briefly, poly(SA-RA) was prepared in a one-pot reaction in which poly(sebacic anhydride) was reacted with ricinoleic acid (7 : 3 w/w ratio) at 120°C for 2 h, followed by anhydride polycondensation at 130°C under vacuum (0.1 mmHg) using acetic anhydride for activation of the carboxylic acid end groups. The formed polymers were used without further purification. TC was obtained as a gift sample from Khandelwal Laboratories (Mumbai, India). Mannitol was obtained from the Pharmatrans Sanaq AG, Basel (Switzerland). Glucose, sodium chloride, disodium hydrogen orthophosphate, and potassium dihydrogen orthophosphate were supplied by SD Fine Chem (Mumbai, India). High performance liquid chromatography (HPLC) grade acetone, methanol, and ethanol were purchased from Thermo Fisher Scientific India (Mumbai, India). All other chemicals were of analytical grade and used without further purification.

Preparation of microparticles

TC-loaded poly(SA-RA) microparticles were prepared by solvent displacement method.[11] Drug was loaded in the concentration of 20 and 30% w/w of polymer. In brief, 40-mL solution containing ethanol and water mixture in 1 : 1 ratio was introduced into the 10 mL of clear solution containing varying quantity of poly(SA-RA) and TC in acetone. The system was stabilized for 30 min with stirring at 1000 ± 5 rpm using magnetic stirrer. The above preparation procedure is carried out at ~ 25°C under dark light. The obtained triphasic system was subjected to rotary flash evaporator (Superfit, Bangalore) at 40 ± 2°C with 100 ± 5 rpm to remove organic solvent which also controls the particle size. Finally, for microparticles, aqueous phase containing drug and polymer was lyophilized with cryoprotectants, glucose and mannitol (7% w/w of drug and polymer) using Christ alpha 1–4 LD plus lyophilizer (−52 ± 1°C and 0.10 mbar). The particles were then stored in dry, cool, and dark place until used. Drug-free microparticles were prepared in the same manner without adding the drug. The coded formulations with their compositions are shown in Table I.

Determination of percentage yield of the microspheres

The percentage yield of produced microparticles was determined for each batch by dividing the whole weight of product (M) by the total expected weight of drug, polymer, and cryoprotectants (M_0).

$$\text{Percentage yield } = \frac{M}{M_0} \times 100$$

Drug loading

The average drug content was estimated by extracting the drug with methanol and phosphate buffer saline (PBS) pH 7.4 (9 : 1) solvent. Accurately weighed 10.0 mg of microparticles were dissolved in 25.0 mL of methanol and PBS pH 7.4 (9 : 1) solvent. The solution was filtered through 0.22 μm nylon membrane filter (Millipore, India); clear solution was suitably diluted with methanol: PBS pH 7.4 (9 : 1); and TC concentration was determined by HPLC analysis: HPLC system consists of a Shimadzu SPD-10ATVP, binary pump equipped with a normal sample injector SPD-10AVP variable wavelength UV detector and Spincotech station for data analysis: 50 μL was injected into Phenomenex C-8 column, (4.6 × 250 mm, 5 μm) and Phenomenex C-8 guard column cartridge (KJ0-4282, 4.0 × 3.0 mm, 5 μm). Mobile phase used was methanol: PBS pH 7.4 (9 : 1) with flow rate of 1 mL min^{-1}. The effluent was detected at 277 nm. To account for the drug, which could be lost throughout the above procedure, the recovery efficiency of the procedure was determined by dissolving a known quantity of TC in methanol: PBS pH 7.4 (9 : 1) and subjected according to the same procedure as described above. The drug loading was calculated using below mentioned equation.

$$\text{Drug loading (\%)} = \frac{\text{Wt of drug in microparticles}}{\text{Wt of microparticles taken} \times 100}$$

Entrapment efficiency

Entrapment efficiency of poly(SA-RA) microparticles was determined by analyzing the free drug content. Known quantity of microparticles (10 mg) were suspended in 10.0 mL PBS pH 7.4 followed by cold centrifugation at ~ 4°C with 14,000 rpm for 15 min.

TABLE II
Results of Microparticles Yield, Drug Loading, Entrapment Efficiency, Mean Particle Size, and Polydispersity Index

Formulations	Yield (% w/w)	Drug loading (% w/w ± SD)	Entrapment efficiency (%± SD)	Mean particle size (μm ± SD)	PdI[a]
F_1	68.4	18.44 ± 2.84	87.82 ± 2.30	1.915 ± 0.084	0.130 ± 0.014
F_2	73.2	27.97 ± 0.86	91.43 ± 1.72	2.391 ± 0.157	0.068 ± 0.013

Data are expressed as mean ± SD, n=3.
[a] Polydispersity index.

After being suitably diluted with methanol, free drug concentration in the supernatant solution was analyzed by HPLC method. The entrapment efficiency was calculated by using below mentioned equation.

$$\text{Entrapment efficiency (\%)} = \left[1 - \frac{\text{Free drug}}{\text{Theoretical drug loaded}}\right] \times 100$$

Surface morphology studies

Scanning electron microscopy (SEM) was used to characterize the internal and external morphology of the microparticles. The microparticles were first dried under vacuum. Samples were glued to aluminum stab and gold coated under argon atmosphere (JFC-1100E, Tokoyo, Japan). The coated samples were finally analyzed by using SEM (JSM-848 SEM, Jeol, Japan) under suitable magnification.

Particle size analysis

Particle size analysis was performed by using laser scattering light (Malvern Laser Analyzer, Malvern Instruments, UK). Drug-loaded lyophilized microparticles were dispersed in deionzed water, vortexed for 10 min, and sonicated for 5 min before sampling. Polydispersity was determined according to a previously reported method.[12] Each sample was measured in triplicate.

Fourier transform infrared

Infrared spectroscopy (Thermo Nicolet Avtar 370, Japan) was performed for pure TC, poly(SA-RA), physical mixtures of TC and poly(SA-RA), and TC-loaded microparticles F_1 and F_2. Samples were mixed with KBr and vacuum packed to obtain pellets of the material, which were analyzed at ambient temperature. All the spectra acquired scans between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1}.

Thermal studies (DSC)

Thermal analysis was performed on pure TC, poly(SA-RA), physical mixtures of drug and polymer, and TC-loaded microparticulate formulations. Thermograms were recorded on differential scanning calorimeter (Mettler Toledo DSC 822e). Lyophilized TC microparticles (4.00–6.00 ± 0.1 mg) samples were heated in crimped aluminum pan from 10 to 170°C at a scanning rate of 10°C/min using nitrogen flow as coolant.

XRD studies

The X-ray diffraction (XRD) patterns of pure TC, poly (SA-RA), physical mixtures of drug and polymer, and TC loaded microparticles were recorded using a Bruker AXS D8 Advance diffractometer with Cu K_α radiation generated at 40 kV and 30 mA at room temperature. The data were collected within the diffraction angle range of 0–70° (2θ) at a step size of 0.02° and a scanning speed of 0.04° min^{-1}.

In vitro drug release studies

TC release from poly(SA-AR) microparticles was performed under sink condition in PBS pH 7.4 using horizontal water bath shaker (Remi, Mumbai, India). Amber-colored screw-capped bottles (30 mL capacity) containing accurately weighed microparticles (F_1 = 12 mg and F_2 = 18 mg) were suspended in 20-mL PBS pH 7.4 that were placed in holders on platform inside the water bath shaker, maintained at a constant under mechanical stirring (75 rpm) at 37 ± 2°C. At predetermined intervals, 1 mL of samples were withdrawn (replaced with fresh medium). The samples were further diluted with methanol up to 10.0 mL, filtered (0.22 μm Nylon membrane filter, Milipore, India), and analyzed by HPLC method mentioned earlier for the estimation of TC released from microparticles. The cumulative percentage drug release was calculated to establish the drug release profile of the TC-loaded microparticles. To determine the order of drug release, drug release profile of all the formulations evaluated were fitted into zero-order, first-order, Higuchi, and Korsmeyer-Peppas models.[13]

Stability studies

As per ICH guidelines, TC-loaded microparticles were stored at elevated temperatures with relative humidity (25 ± 2°C/60 ± 5% RH, 40 ± 2°C/75 ±

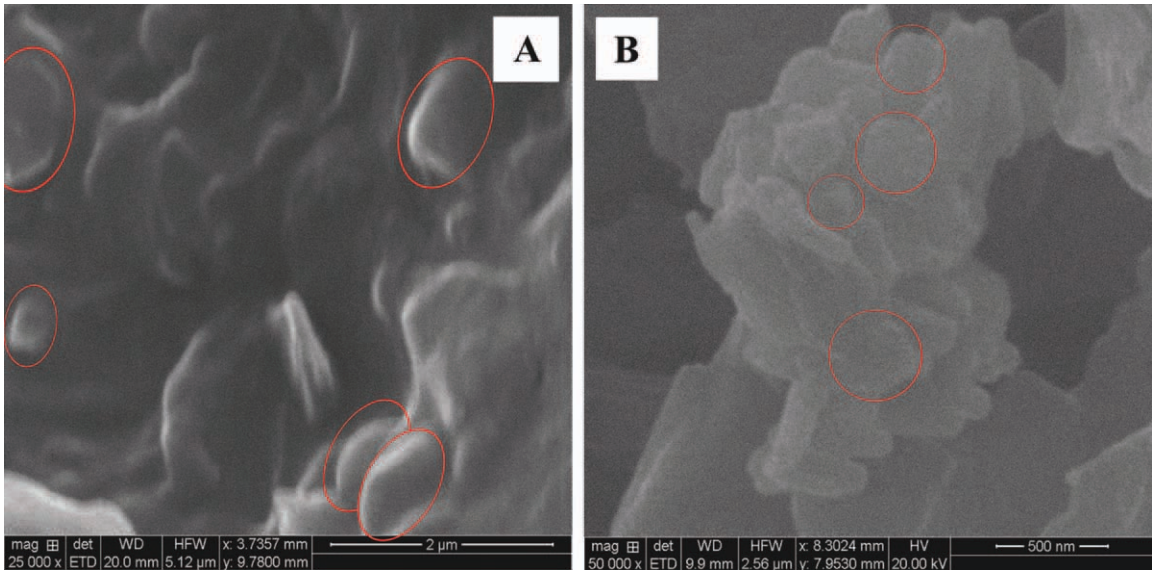

Figure 1 Scanning electron micrographs of the (A) blank microparticles (magnification 25,000× and scale bar distance 2 µm) and (B) drug-loaded poly(SA-RA) microparticles (magnification 50,000× and scale bar distance 500 nm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

5% RH) in a stability analysis chamber (GMG, India) for a period of 3 months.[14] Freshly prepared TC-loaded poly(SA-RA) microparticles, stored at 5 ± 3°C were used as control. Samples were kept for 3 months for stability analysis and at interval of 15, 30, 60, and 90 days. TC content of microparticles was analyzed by mentioned HPLC method and compared with those of the control formulations.

RESULTS AND DISCUSSION

TC-loaded poly(SA-RA) were prepared by solvent displacement method of microencapsulation. Evaporation of organic solvent was done at 40 ± 2°C to prevent the degradation of drug and polymer during the process. Glucose and mannitol used as cryoprotectants prevent the shrinkage of particles during lyophilization. Hydrophobic nature of poly(SA-RA) enables it to be used as a carrier for both hydrophobic and hydrophilic drugs.[15]

The qualification of HPLC method

HPLC method developed in the present study for the estimation of TC provided excellent sensitivity, accuracy, and precision. The retention time (t_R) of TC was 6.989 min. The detection limit was 25 ng/mL. There was a good linearity over the concentration range of 25–5000 ng/mL. The typical equation describing the calibration curve is $y = 0.0223x + 0.011$, with mean correlation coefficient (R^2) of 0.9999. The recovery range of pure TC was found to be 95–101%. The RSD of interday and intraday (consecutive 5 days) precision was 0.81–1.40 and 1.6–2.2%, respectively. The recovery efficiency of pure TC when compared to drug recovery from a mixture of drug and polymer was found have a deviation of less than ±5%.

Characterization of microparticles

Microparticles with two different drug-polymer ratios were prepared with 20 and 30% w/w of the polymer to determine the effect of change in drug-polymer

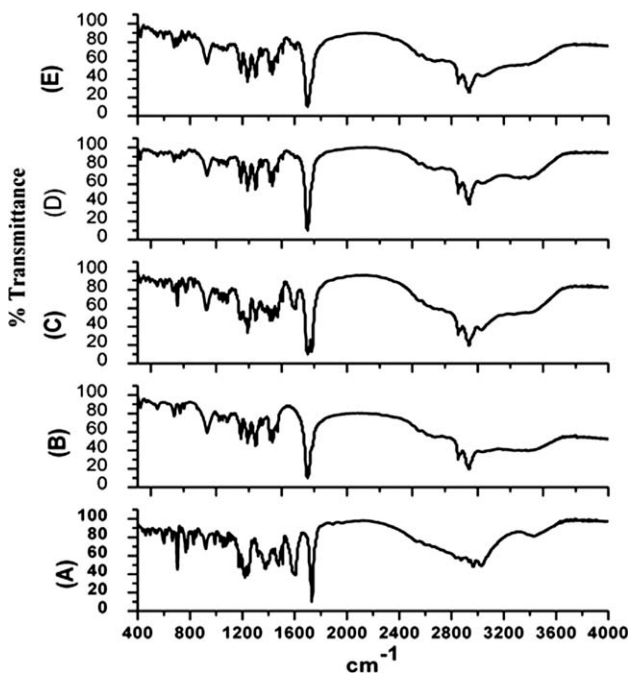

Figure 2 Transmission FTIR spectra of (A) pure TC, (B) poly(SA-RA), (C) physical mixture of drug and polymer, (D) F_1 (20% TC), and (E) F_2 (30% TC).

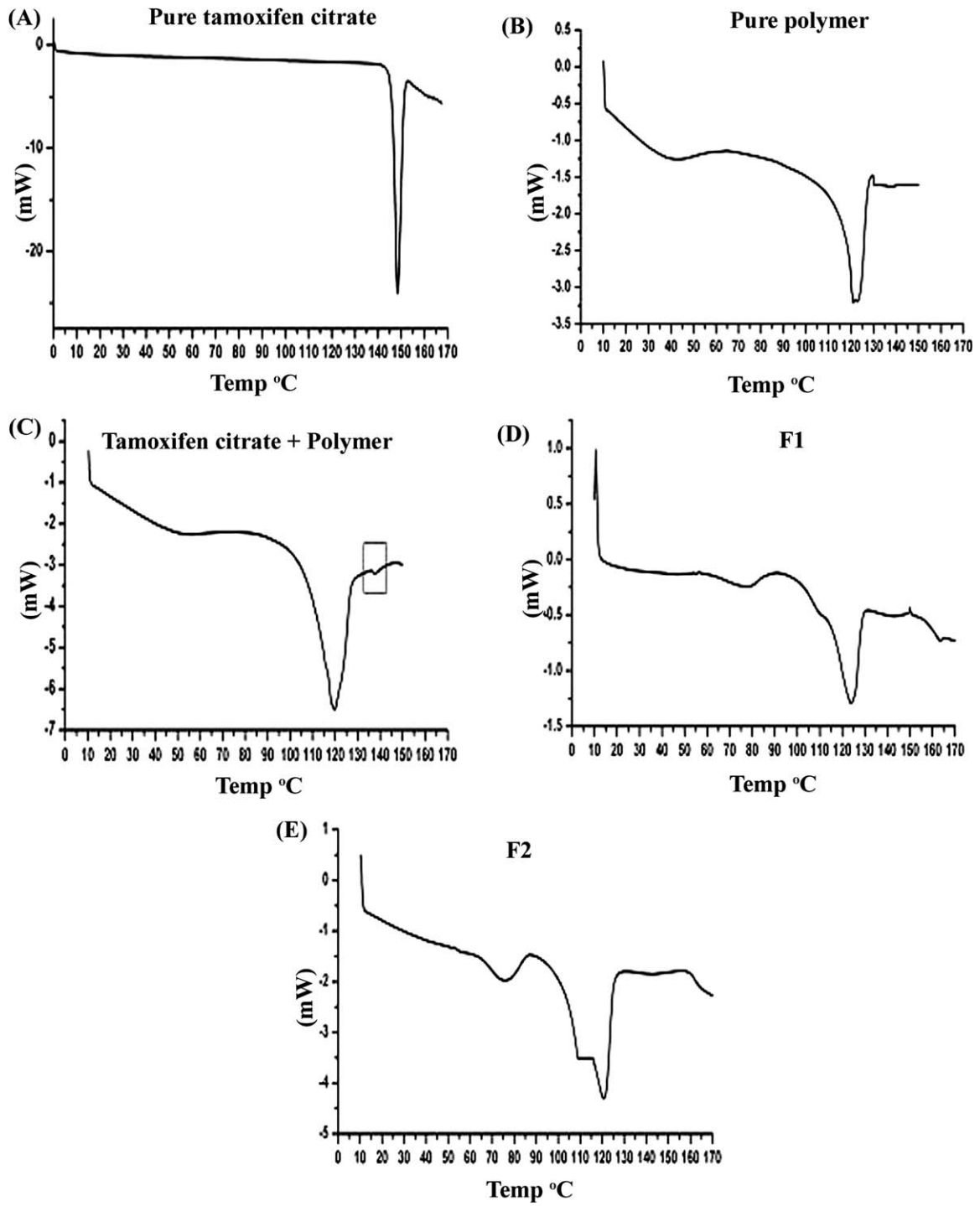

Figure 3 DSC thermograms of (A) pure TC, (B) poly(SA-RA), (C) physical mixture of drug and polymer, (D) F_1 (20% TC), and (E) F_2 (30% TC). The experiment was carried with crimped aluminum pans; the samples were scanned at rate of 10°C/min from 10 to 170°C.

concentration on physicochemical properties such as yield, particle size, entrapment efficiency, and *in vitro* release of TC from poly(SA-RA) microparticles.

Yield, drug loading, and entrapment efficiency

Table II illustrates the results obtained for the percentage yield drug loading and entrapment efficiency of prepared TC microparticles. Yield was obtained in the range of 68.4–73.2% w/w. The loss of yield might be due to recovery problem and adherence of formulation due to sticky nature of polymer. The entrapment efficiency and drug loading of poly (SA-RA)-based microparticles increased with increase in TC content. The large entrapment efficiency and drug loading can be explained by the TC

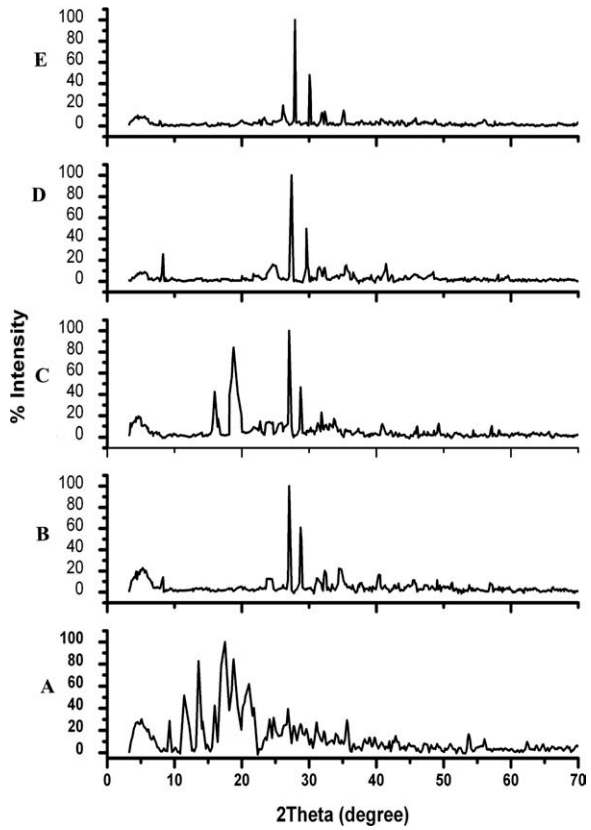

Figure 4 X-ray diffraction studies of (A) pure TC, (B) poly(SA-RA), (C) physical mixture of drug and polymer, (D) F_1 (20% TC), and (E) F_2 (30% TC).

hydrophobicity and insolubility in water, which minimizes its loss into external water phase. Polyanhydrides are relatively hydrophobic polymers; therefore, improved incorporation of drugs having low water solubility is expected.[13]

Surface morphology and particle size analysis

Lyophilization process of poly(SA-RA) and TC aqueous solutions lead to the microparticles with nearly spherical shape with smooth surface (Fig. 1) and size less than 2.5 μm with the population in the range between 1.8 and 2.5 μm with narrow size distribution (Table II). The particles were observed aggregated probably due to the fatty acid nature of polymer.[13] Temperature and rate of solvent evaporation controls the particle size at the stage of removal of organic solvent. Particle size was increased slightly with increasing TC content. Distribution of particle size range was observed narrow for F_2 compared to F_1. Increase in particle size with decreased in polydispersity index was observed. The particle size distribution (mean diameter) and polydispersity index variations observed in the TC-loaded poly (SA-RA) microparticles might be due to the variations in drug and polymer concentration and also the method of preparation.

FTIR analysis

Figure 2 shows the typical spectra of pure TC, poly(SA-RA), physical mixture, and a drug-loaded microparticles. The spectrum of TC shows characteristic absorption bands at 3028.2 cm^{-1} (C—H sp^3 stretching), 1476.3 cm^{-1} (C=C ring stretching), and 1605.7 cm^{-1} (—NH bending).[16] Poly(SA-RA) displays a characteristic absorption strong bands such as the carbonyl stretching mode around 1698.1 cm^{-1} (C=O), asymmetric stretching 2951.1 cm^{-1} (CH_2), and anhydride bands 1303.0 cm^{-1} (C—O bending). In comparison to pure TC and poly(SA-RA), spectrum was not sharp in microparticulates. A shift in wave number and decrease peak intensity owing to C=C stretching at 1476 cm^{-1} of TC to 1466 occurred, might be due to the presence of drug moiety in amorphous form but absence of degradation peak revealed that drug remains intact and stable within polymeric microparticles.

DSC and XRD studies

DSC analysis of drug, polymer materials, and produced microparticles, the nature of the drug inside the polymer matrix can be assessed, which may emerge in solid solution, metastable molecular dispersion, or crystallization.[17,18] Figure 3 shows a sharp endothermic peak of pure TC at 148.5°C. A broadened endothermic peaks of poly(SA-RA) was observed at 122.5. DSC thermogram of physical mixture shown melting peaks at 120.0 and 138.5°C. Shift in drug-melting peak in physical mixture owing to impure form of drug was observed. While the drug peak was absent in the microparticulate formulations, which indicated that the drug was dispersed in the microparticles as an amorphous form.

This phenomenon was further confirmed by X-ray diffraction analysis of TC, poly(SA-RA), physical mixture, and microparticles preparation in the range

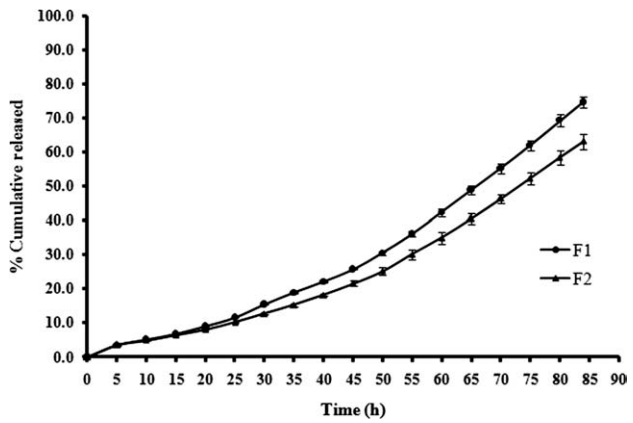

Figure 5 *In vitro* drug release profile of TC-loaded microparticles, F_1 (20% TC) (●) and F_2 (30% TC) (▲).

TABLE III
***In Vitro* Release Kinetic Equation Data Treatment of TC from the Poly(SA-RA) 70 : 30 w/w Microparticles**

	Regression coefficient (R^2)			
Formulations	Zero order	First order	Higuchi	Korsmeyer Peppas
F_1	0.971	0.888	0.828	0.923 ($n = 0.723$)
F_2	0.988	0.906	0.915	0.964 ($n = 0.772$)

of 0–70° (2θ). The diffraction pattern of TC showed peaks at 9.26, 11.42, 13.57, 15.97, 17.49, 18.77, and 21.07° (2θ) [Fig. 4(A)]. In case of pure polymer, diffraction peaks were observed at 8.30, 24.15, 27.04, and 28.74° (2θ) [Fig. 4(B)]. Physical mixture of drug and polymer when subjected for XRD, same prominent diffraction peaks of drug and polymer were observed in mixture at 15.97, 18.77, 24.15, 27.04, and 28.74° (2θ) [Fig. 4(C)]. The drug peaks did not appear in the formulations F_1 and F_2 while only polymer characteristic peaks were appeared at 8.30, 24.61, and 27.40° [Fig. 4(D)] and 7.80, 26.12, and 27.89° [Fig. 4(E)], respectively. However, drug peak did not appear which probably may be due to conversion of TC from crystalline state to amorphous or dissolution. This amorphous nature of the drug may have pronounced pharmaceutical significance as it could lead to increased solubility and finally to an improved biological activity.[17,18]

Drug release kinetics

Figure 5 shows the release behavior of TC from poly(SA-RA) microparticles, which indicates the sustained release pattern for up to 84 h. The percentage cumulative drug release versus time plots showed small burst phase followed by slow and constant drug release mimicking zero-order release. The percentage cumulative drug release at the end of 84 h period from F_1 and F_2 formulations was 74.82 ± 1.57% and 63.26 ± 2.23%, respectively. Initial fast release or small burst effect phase is considered to be the result of rapid diffusion/dissolution of drug particles at the surface. The magnitude of burst effect was dependent on the proportion of TC on the outer surface of the microparticles and also probably due to the low permeability of water in poly(SA-RA), a hydrophobic polyanhydride polymer.[15] At the later stage, the drug release was slow and sustained, whose rate was determined by the diffusion/erosion of polymer matrix. Polyanhydrides preferentially degrades by erosion mechanism; thus, the release of drug from the microparticles was by erosion of polymer. TC has very low water solubility and resists diffusion from the hydrophobic polymer to the aqueous medium. The high affinity of the drug for the hydrophobic matrix and the low solubility of the drug in the aqueous medium have lead to the slow and controlled release of the drug from the polyanhydride microparticles. The formulation becomes more hydrophobic with increase in TC content, which does not allow water to penetrate and dissolve the drug and degrade the polymer.

Zero-order and Korsmeyer Peppas model gave a good fit for the drug release profiles of both the formulations with greater regression coefficients in comparison to other models (Table III). The fitting of these data to the Korsmeyer-Peppas model demonstrated that drug release occurs mainly through diffusion and erosion process. As the value of n was in between 0.723 and 0.772, it indicates anomalous transport mechanism.[13] Hence, sustained and controlled release phenomenon was obtained in developed formulations when subjected for *in vitro* release study.

Stability studies

Drug stability data (Table IV) illustrates no effective change in the TC content in the formulations stored

TABLE IV
Stability Studies Data of TC Microparticles

		Drug loading (% w/w)		
Time (days)	Formulations	Control 5 ± 3°C	25 ± 2°C/60 ± 5% RH	40 ± 2°C/75 ± 5% RH
Initial	F_1	18.44	18.44	18.44
	F_2	27.94	27.94	27.94
15	F_1	18.43	18.41	17.89
	F_2	27.97	27.74	27.09
30	F_1	18.42	18.34	17.07
	F_2	27.95	27.59	26.77
60	F_1	18.39	18.19	16.17
	F_2	27.94	27.34	25.04
90	F_1	18.39	18.04	14.57
	F_2	27.91	27.09	22.79

at 25 ± 2°C/60 ± 5% RH at the end of 90 days. However, the samples kept at 40 ± 2°C/75 ± 5% RH showed a significant reduction in amount of TC at the end of 90 days in each formulation. This might be due to the degradation of both drug and polymer having a low glass transition temperature[19] and low melting point was concluded.

CONCLUSIONS

TC-loaded poly(SA-RA) microparticles have been successfully prepared by solvent displacement technique of microencapsulation. The polyanhydride microparticulates showed sustained prolong release of drug which is required for the effective treatment of diseases as breast cancer. Thus, poly(SA-RA) could be used as a potential vehicle in delivery of antineoplastic agents. Optimizations of formulations with *in vitro–in vivo* corelations are future subjects for the assessment of these formulations as effective dosage form for TC.

The authors thank Indian Institute of Science, Bangalore, India, for assistance in characterization and Khandelwal Laboratories, Pvt. Ltd., Mumbai, India, for providing as a gift sample of tamoxifen citrate.

References

1. American Cancer Society. Breast Cancer Facts and Figure 2009-2010. American Cancer Society: Atlanta, GA, 2010.
2. Macgregor, J. I.; Jordan, V. C. Pharmacol Rev 1998, 50, 151.
3. Shi, S. J.; Li, Z. F.; Xu, S. Q.; Chen, H. T.; Zeng, F. D. Asian J Pharmacodyn Pharmacokinet 2007, 7, 233.
4. Jordan, V. C.; Collins, M. M.; Rowsby, L.; Prestwich, G. J Endocrinol 1977, 75, 305.
5. Chawla, S. J.; Amiji, M. M. Int J Pharm 2002, 249, 127.
6. Hiremath, J. G.; Kusum, D. V. Int J Pharm Sci 2010, 2, 189.
7. Coppi, G.; Iannuccelli, V. Int J Pharm 2009, 367, 127.
8. Petersen, L. K.; Sackett, C. K.; Narasimhan, B. Acta Biomater 2010, 6, 3873.
9. Berkland, C.; Kipper, M. J.; Narasimhan, B.; Kim, K.; Pack, D. W. J Controlled Release 2004, 94, 129.
10. Krasko, M. Y.; Shikanov, A.; Ezra, A.; Domb, A. J. J Polymer Sci Part A: Polym Chem 2003, 41, 1059.
11. Agueros, M.; Ruiz-Gaton, L.; Vauthier, C.; Bouchemal, K.; Espuelas, S.; Ponchel, G. Eur J Pharm Sci 2009, 38, 405.
12. Zili, Z.; Sfar, S.; Fessi, H. Int J Pharm 2005, 294, 261.
13. Costa, P.; Lobo, J. M. S. Eur J Pharm Sci 2001, 13, 123.
14. Sahana, B.; Santra, K.; Basu, S.; Mukherjee, B. Int J Nanomed 2010, 5, 621.
15. Shikanov, A.; Ezra, A.; Domb, A. J. J Controlled Release 2005, 105, 52.
16. Hiremath, J. G.; Kusum, D. V.; Kshama, D.; Domb, A. J. J Appl Polym Sci 2007, 107, 2745.
17. Mu, L.; Feng, S. S. J Controlled Release 2001, 76, 239.
18. Dubernet, C. Thermo Chim Acta 1995, 248, 259.
19. Kumar, N.; Langer, R. S.; Domb, A. J. Adv Drug Deliv Rev 2002, 54, 889.