Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20181637

Original Research Article

In-vitro study of formulation and evaluation of nanosuspension of tamoxifen

Somasekhar M. Reddy[1], Navispaul N. Sriganth[2], Chandra S. Kumar[3], Santosh C. Gursale[4]*, Vijay V. Ragavan[5]

[1]Department of Pharmacology, Bhaskara Medical College, Moinabad, Rangaredy, Hyderabad, Telangana, India
[2]Department of EEE, Saveetha School of Engineering, Thandalam Chennai, Tamilnadu, India
[3]Department of Pathology, ESIC, Sanath Nagar, Hyderabad, Telangana, India
[4]Department of Pharmacology, B.K.L. Walawalkar Rural Medical College, Sawarde, Ratnagiri, Maharashtra, India
[5]Director, Saveetha University, Thandalam, Chennai, Tamilnadu, India

Received: 20 February 2018
Accepted: 28 March 2018

***Correspondence to:**
Dr. Santosh C. Gursale,
Email: drsantoshgursale@gmail.com

ABSTRACT

Background: Nanosuspension technology has been developed as a promising candidate for efficient delivery of hydrophobic drugs. It could maintain the required crystalline state of the drug with reduced particle size, leading to an increased reporting on dissolution rate and therefore improved bioavailability.
Methods: In this paper, we report on the preparation of Tamoxifen nanosuspension by high-pressure homogenization (HPH). The aim is to obtain a stable nanosuspension with an increased drug saturation solubility and dissolution velocity. The morphology and particle size distribution of the modified nanosuspensions were characterized by the means of several analyses that included: transmission electron microscopy (TEM), polarized light microscopy (PLM), scanning electron microscopy, differential scanning calorimetry (DSC) and powder X- ray diffractometry (XRD).
Results: HPH was employed to produce aqueous drug nanosuspensions with fine solubility and dissolution properties, which render the produced particles stable up to one month. In addition, the prepared nanosuspensions possessed a high drug-loading efficiency (10%). The recoded zeta potential values (≈ -27 mV) indicated that the prepared nanosuspensions possess a higher degree of long-term stability. TEM data showed narrow size distribution with average size 322.7 nm. Morphologically, as indicated from results, the produced nanosuspensions have a homogenous distribution even after redispersion, indicating the stability of the product.
Conclusions: It was possible to obtain Tamoxifen nanosuspensions with fine solubility and dissolution properties. Nanosuspensions possessed a high drug-loading (10%), which could reduce the dosage administration and gastrointestinal side effects. HPH can be employed to produce aqueous drug nanosuspensions that are stable up to one month. Aqueous nanosuspension can be converted to dry nanocrystals by lyophilization which offer superior physicochemical properties.

Keywords: Electron microscopy, Nanosuspension, Tamoxifen, Transmission

INTRODUCTION

Poorly water-soluble drugs are especially challenging, as they cannot achieve dissolution and therefore, they have a

very difficult pass through the dissolving fluid to contact the absorbing mucosa and to be absorbed. If the dissolution rate of the drug molecule is slow, due to the physicochemical properties of the drug molecules or formulation factors, the dissolution process will be the rate-limiting step in drug absorption and consequent bioavailability.[1,2] Tamoxifen citrate, an antiestrogenic compound, is the first choice for hormonal treatment of breast cancer in both post and premenopausal women for the last few decades. Depending upon the dose and tissue, Tamoxifen citrate can act as an antiestrogenic or as an estrogenic agent. For breast cancer it shows an antiestrogenic effect, and on the uterus it shows an estrogenic effect.[3-5] Tamoxifen, as a anti estrogen drug, has a poor water solubility characteristic, which is the reason for its poor bioavailability. For such kind of drugs, micronization, nanonization, inclusion complexation (e.g. cyclodextrins) have been proposed to increase the rate of dissolution; especially the drug stability and bioavailability after oral administration for systemic drug absorption.[6-12] An alternative and promising approach is the production of drug nanosuspensions, which has been utilized to improve the solubility and bioavailability.

A nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants. A pharmaceutical nanosuspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for either oral or topical use or for parenteral and pulmonary administration. The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200 to 600nm.[13,14]

In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability. An increase in the dissolution rate of micronized particles (particle size <10µm) is related to an increase in the surface area and consequently the dissolution velocity. Nanosized particles can increase solution velocity and saturation solubility because of the vapor pressure effect.[15]

METHODS

Table 1 shows chemicals and excipients were used in this in-vitro study.

Table 1: List of chemicals and excipients.

Material	Manufacturer
Tamoxifen	Pharmadeep Remedies, HYD
Tween® 20	Qualigens, Fischer Scientifics, India
Tween® 80	Qualigens, Fischer Scientifics, India
Span® 60	Qualigens, Fischer Scientifics, India
Cremophore RH40	Baris pharma Pvt Ltd, Hyderabad
Oleic acid	Qualigens, Fischer Scientifics, India
Soy bean oil	Qualigens, Fischer Scientifics, India
Ethyl alcohol	Qualigens, Fischer Scientifics, India
Cotton seed oil	Pharmadeep Remedies, HYD
Aerosil® 200	Pharmadeep Remedies, HYD
PEG 400	Qualigens, Fischer Scientifics, India
Span® 80	Qualigens, Fischer Scientifics, India

Table 2: List of equipments.

Equipment	Make	Model
Weighing balance	Shimadzu	AX200
Orbital Shaker	REMI Electrotech Ltd, India.	CL 24
Magnetic stirrer	REMI Equipment, Mumbai, India	5ml
UV-Visible Spectrophotometer	Labindia, India.	3200
Dissolution test apparatus II USP	Labindia, India	DS 8000
Zeta sizer	Horiba, Japan and Malvern Instruments, UK	Nano 90and Nano ZS
pH meter	Labindia, India	Phan
Conductivity meter	Labindia, India	Pico+
Ultrasonicator	Citizen, India	CD 4820
Viscometer	Brookfield, USA	LVDV-II+pro
FTIR	BRUKER, Germany	ALPHA T
Cyclo mixer	REMI Equipments, Mumbai, India	CM 101DX
DSC	SIIO, Japan	6300
HPLC-PDA	Shimadzu, Japan	SPD20A Detector, LC-20AD Pumps, DGU-20A3 Degasser
XRPD	Philips, Netherlands	PW 1729
TEM	Hitachi, Tokyo, Japan	H-7500

Equipments

Experimental work

Preparation of L-SNEDDS (Long time self nanoemulsifying drug delivery system) of Tamoxifen

10mg of Tamoxifen was dissolved in 1g of the mixture of oil and Smix respectively. The prepared mixture was vortexed using vertex mixer (Remi India) to obtain a clear homogeneous formulation. Various regions in phase systems at lower, medium and higher concentration of oil and Smix were selected to load the drugs in to plain nanosuspensions then the final drug content of the formulation was 1% and 0.2%w/w for Tamoxifen. The final formulations of suspension were examined for signs of turbidity and thermodynamic stability or phase separation after 72 hours prior to self-emulsification and droplet size determination studies (Table 3).

Table 3: Formulations of Tamoxifen selected from the region of phase diagram.

Sr.no.	Oil: Smix	Surfactant: Co-surfactant	Cotton seed oil (%)	Tween® 80 (%)	PEG 400 (%)
FC1	1:9.00	1:1	10.00	45.00	45.00
FC2	1:4.00	1:1	20.00	40.00	40.00
FC3	1:2.33	1:1	30.00	35.00	35.00
FC4	1:1.50	1:1	40.00	30.00	30.00
FC5	1:9.00	2:1	10.00	60.00	30.00
FC6	1:4.0	2:1	20.00	53.44	26.66
FC7	1:2.33	2:1	30.00	46.66	23.33
FC8	1:1.50	2:1	40.00	40.00	20.00
FC9	1:9.00	3:1	10.00	67.50	22.50
FC10	1:4.00	3:1	20.00	60.00	20.00
FC11	1:2.33	3:1	30.00	52.50	17.50
FC12	1:1.50	3:1	40.00	45.00	15.00

Screening of the suspension formulations for physical and Thermodynamic Stability

All the 12 formulations for Tamoxifen was employed to heating and cooling, centrifugation and freeze thaw analysis to observe thermodynamic stability.

Heating, cooling cycle

The 12 formulations for Tamoxifen was taken in microtubes and six cycle between freezing temperature 4^0 C and 45^0C with storage at each temperature of not less than 48 hours was studied. The formulations those are constant at both the temperature, were selected for centrifugation test.

Centrifugation test

The twelve formulations for Tamoxifen was taken in 2mL microtubes. All the formulations were centrifuged at 3000rpm at least for 30 minutes using micro centrifuge (Remi India). The formulations did not show any phase separation was taken for freez thaw stress test.

Freeze-thaw cycle

The formulations for Tamoxifen was taken in 2mL microtube and three freez thaw cycles between -4^0C and 40^0C for 24 hours was done for the formulations. The observations made for phase separation and drug precipitation. The formulations those are stable and passed thermodynamic stress test were further chosen for optimization of suspension.

Optimization of Tamoxifen suspension using droplet size and polydispersity index

The diameter of nanosuspensions globules and polydispersity index of the formulation selected was determined by dynamic light scattering particle size analyzer (Nano ZS, Malvern, UK) at 635 nm wavelength of 90^0 scattering angle at 25^0 C.0.1 mL formulation was added to 200mL beaker containing 100 mL of distilled water and shaken gently using magnetic stirrer to form fine and transparent nanosuspensions and kept at 25^0 C for 12 hours (47,61, 62 and 56). The z-average diameter was recorded. The z-average diameter also as the harmonic intensity weighed average hydrodynamic diameter of droplets. The z-average diameter of droplets obtained from cumulated examination by the auto measured software tool (Malvern Instruments, UK). The final and optimized formulations were shown in table 4 based on particle size and PDI.

Table 4: Optimized formulation of Tamoxifen.

Code	Drug	%OIL	% Surfactant	% Cosurfactant
FC 9	Tamoxifen	10	67.5	22.5

Characterization of optimized Tamoxifen nanosuspension

Transmission electron microscopy (TEM)

The structure and morphological examination of the oil droplets loaded with the drug in nanosuspensions were observed with transmission electron microscopy (56 and 75). TEM is also important to observe drug precipitation upon addition of aqueous phase. The optimized formulation (FC 9) was diluted with distilled water in 1:50 ratio and mixed gentle; a drop of diluted sample was placed over the formwar coated grid. The diluted sample droplets were stained negatively for 10 minutes with phosphotungstic acid (1% w/v) solution. Excess liquid is blotted with Whatmann filter paper. The samples were then examined with TEM (HITACHI, H-7500, Japan) operated at 80kV.

Determination of viscosity of formulation

Viscosity of the optimized suspension of Tamoxifen was investigated using LVDV II T Brook field viscometer using spindle number 64 attached with UL small sample adopter at 25^0 C.

Determination of Refractive index of nano suspension

A drop of the optimized suspension of Tamoxifen was placed in the lenses of the Abbe's type of Refractometer (Bellingam+ Stanley Ltd, USA).

Invitro release studies of nanosuspension

The drug release studies from optimized formulation were performed using USP type II dissolution test apparatus (LABINDIA, India) rotating speed of 100 rpm at 37^0C±0.5^0C. 5mg and 1mg equivalent amount of the suspension for Tamoxifen were placed into the 0 size hard gelatin capsule shells and sealed manually. The sealed capsules were dropped in to the 900mL of phosphate buffer pH 7.4 and 7.0 for Tamoxifen respectively. The 5 mL of the sample was withdrawn using cannula attached with 0.4 micron membrane filter at 0, 5, 10, 15, 30, 45 and 60 minutes time intervals. A 5mL quantity of the dissolution medium was replaced to maintain sink conditions of the dissolution study.

The samples withdrawn were diluted with fresh dissolution medium if necessary. The samples were analyzed using RP-HPLC attached with PDA (73).

Preparation of the S-SNEDDS (Short time self nanoemulsifying drug delivery system) from optimized nanosuspension

The nanosuspension of Tamoxifen formulated, adsorbed onto Aerosil® 200 (1:1 ratio) by physical mixing in a small motor and pestle for 5 minutes to form a free flowing and dry homogenous mass (47 and 48). The free flowing powder was passed through a sieve number 30 and the S-SNEDDS powder placed into the 0 size hard gelatin capsule shells and sealed manually. The resulting S-SNEDDS was a free flowing powder that was subsequently subjected to solid state characterization and dissolution studies (Table 5).

Table 5: Composition of an optimized nano suspension.

Formula	Components in S-SNEDDS	Proportions in mg	% Drug in S-SNEDDS
FC 9	Tamoxifen	10	0.5% w/w
	Cotton seed oil	100	
	Tween® 80	675	
	PEG 400	225	
	Aerosil® 200	1000	

Characterization of S-SNEDDS of Tamoxifen

Estimation of drug content in nanosuspension

The nano suspension containing 10 mg equivalent amount of Tamoxifen was dispersed in corresponding mobile phase in 100 mL volumetric flask by adding 20ml of mobile phase and sonicated using bath sonicator (Citizen, India) for 10 minutes and made up to the volume with corresponding mobile phase to extract Tamoxifen and glimepiride totally and centrifuged at 3000rpm for 20 minutes separate un dissolved excipients. The supernatant was taken and was passed through a 0.45 micron membrane filter (PALL, USA). The samples were analyzed using RP-HPLC attached with PDA at a max of 228nm.

Reconstitution properties of nanosuspension

The time required for self-emulsification of nanosuspension was determined using USP type II dissolution rate apparatus. 100 mg nanosuspension was taken in to 500 mL of distilled water in a dissolution vessel at 37^0C under gentle agitation at 50rpm. The emulsification time of suspension assessed visually. All the studies performed triplicate to obtain accurate results.

Droplet size determination of reconstituted suspension

The z-average diameter of nanosuspensions droplets formed after reconstitution of 100 mg of nanosuspension into 50ml of deionized water was determined by dynamic light scattering particle size analyzer (Nano ZS, Malvern, UK) at 635nm wavelength of 90^0C scattering angle at 25^0C.

The z-average diameter of droplets obtained from cumulated examination by the auto measured software tool (Malvern Instruments, UK).

Differential scanning calorimetry (DSC)

DSC curves for pure drug and optimized Suspension were obtained in a differential scanning calorimeter (SIIO, 6300, Japan) using platinum pans with 2mg of sample purged with nitrogen gas at a flow rate of 50mL/min with heating rate of 10^0C/min over a temperature range of 25-250^0C.

Scanning electron microscopy (SEM)

The samples of drug (Tamoxifen), colloidal silica and suspension samples were mounted on a double adhesive tape. The samples mounted were sputtered with thin gold palladium layer by VG-microtechsputter coater unit. The surface microphotographs were analyzed with an S- 120 Sterioscan scanning electron microscope (Cambridge, UK).

X-ray powder diffraction (XRPD) study

The diffraction patterns of plain drugs (Tamoxifen), colloidal silica and Suspension were obtained on Philips PW 1729 X-ray diffractogram, with monochromatized Cu Kα radiation (1.542A^0), The samples were analyzed between 2^0 and 50^0 angles of 2θ, voltage of 30 kV and current of 30mA (47).

Stability of nano suspension in simulated gastric fluid (SGF)

10 mg of Suspension of Tamoxifen were introduced into 50 ml of simulated gastric fluid containing pepsin at pH of about 1.2 for 3 hours. The structure and morphological examination of the oil droplets loaded with the drug in nanosuspensions were observed with transmission electron microscopy mentioned in chapter 4.8.1. A drop of sample was placed over the formwar coated grid. The sample droplets were stained negatively for 10 minutes with phosphotungstic acid (1and w/v) solution. Excess liquid is blotted with Whatmann filter paper. The samples were then examined with TEM (HITACHI, H-7500, Japan) operated at 80 kV. The TEM images of droplets observed for the drug precipitation, coalescence and break down of nanosuspension globules.

Stability studies at accelerated conditions

The optimized formulation powder placed into the 0 size hard gelatin capsule shells and sealed manually. Furthermore the capsules were sealed in amber colour glass bottle and placed in stability chambers maintained at 40+ 2^0 C/75 %+5 % (accelerated conditions as per ICH). The studies were conducted for 3 months. The samples were collected for 0, 1, 2, and 3 months and evaluated for Tamoxifen assay using RP-HPLC method. The size of droplets of suspension was determined by method depicted to determine effect of storage conditions on size of droplets of suspension.

RESULTS

Solubility studies of Tamoxifen in oils, surfactants and co surfactants

The SUSPENSION is prepared by one or more surfactants and drug dissolved in oil. At room temperature the mixture should be an opaque, monophasic liquid and supposed to have fine solvent characters to permit solubilisation of drug in solution. During solubility experiments cotton seed oil showed the highest solubility for Tamoxifen compared to other oils like isopropymyristate, soy bean oil, sunflower oil and oleic acid. In the vicinity of triglyceride chains of the cotton seed oil supports absolute solubilization of Tamoxifen. PEG 400 as cosurfactants, cotton seed oil as lipid and Tween® 80as surfactant were selected for the construction of ternary phase diagrams to identify the nanosuspension domains such that at particular concentration of oil and surfactant co -surfactant ratios a stable nanosuspension formulation is formed.

The lipophilic surfactant promote emulsification of oil but it produces crude suspension with large globule size as the lipophilic surfactants have HLB value less than 10. Hydrophilic surfactants HLB >10 are superior at giving fine and uniform suspension droplets which are more likely to empty quickly from the stomach. Large surface area helps in faster and complete absorption. In most cases it is the right blend of low and high HLB surfactants leads to the formation of stable nanosuspension upon exposure to water. Based on the efficiency of self-emulsification, Tween® 80 with HLB value of 15 was selected for the formulation of Tamoxifen SUSPENSION. PEG 400 selected as cosurfactant correspondingly and cottonseed oil was selected as an oil phase (Table 6).

Table 6: Solubility of Tamoxifen in vehicles.

Oil vehicle	Solubility (mg/mL)
Cottonseed oil	6.37±0.075719
Oleic acid	2.42±0.1253
Sunflower oil	2.09±0.179536
Soy bean oil	1.25±0.155349
Isopropyl myristate	0.36±0.045826
Miglyol® 812	1.09±0.020817
Surfactant (HLB)	Solubility(mg/ml)
Tween® 80 (15.0)	22.77±0.452
Cremophor RH40 (13)	17.23±0.351
Span® 20 (8.6)	4.36±0.511
Span® 80 (4.3)	1.30±0.220
PEG400 (13.1)	9.90±0.458
Propylene glycol	0.69±0.03

Construction of the ternary phase diagram based on solubility data of Tamoxifen

Ternary phase diagrams presents the information about the concentration range of the oil (Cotton seed oil), surfactant (Tween® 80) and cosurfactant (PEG 400) concentrations

to form clear nanosuspension after titration with water (spontaneous emulsification method). The shaded area for SUSPENSION region were chosen based on percentage transmission after spontaneous emulsification method. The area shaded by the points in the phase diagram displays the concentration mixture components that resulted in a clear nanosuspension out of all the trial concentrations.

All the combinations under test impulsively formed a nanosuspension in particular concentrations which are located within the shaded area of phase diagram. This is possibly due to spontaneous stabilization of oil droplets by surfactant owing to their high concentration. When cotton seed oil concentration increases more than 40% the percentage transmittance decreased resulted from low accessibility of Smix to emulsify the cotton seed oil to form nanosuspension. The emulsification efficiency was found to be capable, when the Smix amount of concentration was supplementary more than 60% of formulation

Preparation of nanosuspension

Tamoxifen showed maximum solubility in Tween 80 and PEG 400 among surfactants and cosurfactants correspondingly. There are more chances of drug precipitation when the drug concentration is more than its solubility. Only 10mg/gm i.e. 1% w/w drug is loaded into the plain suspension formulations. Upon aqueous dilution, the drug should not precipitate and is established by spontaneous emulsification method. After loading the drug, all the formulation (FC1 to FC12) presented.

Screening of the SUSPENSION for physical and thermodynamic stability

Thermodynamically stable nanosuspension shown in table 7 formed at a particular concentration of oil, Smix and water, with spontaneous emulsification with no phase separation, creaming or cracking. The formulations selected subjected to thermodynamic stability test. All the formulation (FC1 to FC12) presented do not show any drug precipitation after thermodynamic stability test and results. All the formulations emulsified within 1 minute

Table 7: Thermodynamic stability of SUSPENSION formulations.

S. No.	Oil: Smix	Surfactant: Co-surfactant	Cotton seed oil (%)	Tween 80 (%)	PEG 400 (%)	Thermo dynamic Stability
FC1	1:9.00	1:1	10.00	45.00	45.00	Stable
FC2	1:4.00	1:1	20.00	40.00	40.00	Stable
FC3	1:2.33	1:1	30.00	35.00	35.00	Stable
FC4	1:1.50	1:1	40.00	30.00	30.00	Stable
FC5	1:9.00	2:1	10.00	60.00	30.00	Stable
FC6	1:4.0	2:1	20.00	53.44	26.66	Stable
FC7	1:2.33	2:1	30.00	46.66	23.33	Stable
FC8	1:1.50	2:1	40.00	40.00	20.00	Stable
FC9	1:9.00	3:1	10.00	67.50	22.50	Stable
FC10	1:4.00	3:1	20.00	60.00	20.00	Stable
FC11	1:2.33	3:1	30.00	52.50	17.50	Stable
FC12	1:1.50	3:1	40.00	45.00	15.00	Stable

Table 8: Droplet size and PDI of SUSPENSION of Tamoxifen.

S. no.	Oil: Smix	Cotton seed oil (%)	Tween 80(%)	PEG 400 (%)	Z-Avg size (d nm)	PDI
FC1	1:19	10.00	45.00	45.00	98.2	0.725
FC2	1:9	20.00	40.00	40.00	258	0.610
FC3	1:4	30.00	35.00	35.00	670	0.810
FC4	1:2.33	40.00	30.00	30.00	3942	0.422
FC5	1:19	10.00	60.00	30.00	85.50	0.603
FC6	1:9	20.00	53.44	26.66	199.8	0.554
FC7	1:4	30.00	46.66	23.33	805.1	0.493
FC8	1:2.33	40.00	40.00	20.00	2666	0.613
FC9	1:19	10.00	67.50	22.50	143	0.251
FC10	1:9	20.00	60.00	20.00	306.2	0.220
FC11	1:4	30.00	52.50	17.50	722.2	0.432
FC12	1:2.33	40.00	45.00	15.00	918.5	0.744

Optimization of Tamoxifen SUSPENSION using droplet size and Polydispersity Index

Tamoxifen SUSPENSION formulations (FC1-FC12) presented in Table 7 were further tested for their droplet diameter and polydispersity index(PDI) determined by dynamic light scattering particle size analyzer at 635nm wavelength of 90^0C scattering angle at 25^0C. The size and PDI significantly affected with percentage of cottonseed oil and Smix. The z-average diameter and zeta potential confirmed that increased in concentration of cottonseed oil proportionately increased the suspension droplet diameter whereas the increase in surfactant percentage the droplet diameter was decreased. The surfactant Tween 80 adsorbed at interface of oil and water to form thin film and decrease the oil droplet diameter and also helped to stabilize the nanosuspension. SUSPENSION formulation FC 9 containing cotton seed oil yielded a particle diameter of 143±2nm with a PDI of 0.251. Formulation FC 9 was therefore chosen for formulation of S-SUSPENSION of Tamoxifen because of its desirable particle size and low PDI value. The final composition of SUSPENSION preparation FC 9 containing 10 mg of Tamoxifen was therefore as follows: Cotton seed oil (10%), Tween 80 (67.50%) and PEG 400 (22.50%). Zeta potential was found to be -14.9. The results indicate that the drug loaded oil droplets are stable and well distributed without coalescence. The cosurfactant PEG 400 play potential role for forming nanosuspension due to hydrophilic nature (84). The optimized SUSPENSION formulation FC 9 (Table 8).

Characterization of optimized Tamoxifen SUSPENSION

Transmission electron microscopy (TEM)

The microphotograph of the optimized SUSPENSION (FC9) observed as dark globules with bright surrounding. The TEM image demonstrates that nanosuspension come into viewed as spherical oil droplets after dilution with aqueous phase, attributable to nanosize of cotton seed oil droplets loaded with Tamoxifen.

Viscosity determination

Formulation FC9 SUSPENSION of Tamoxifen was yielded a viscosity of 104.7±0.3 cps. The viscosity evaluation confirming that the liquid formulation FC 9 behaves as Newtonian fluid.

Refractive index determination

Formulation FC 9 SUSPENSION of Tamoxifen was yielded a Refractive index of 1.419±0.01819. The refractive index value was nearly closer to water value at 25^0C. Further more the result of RI represents transparent homogenous nature of SUSPENSION.

Characterization of S-SUSPENSION of Tamoxifen

Estimation of drug content in SUSPENSION

The SUSPENSION containing 10mg equivalent amount of Tamoxifen was dispersed in corresponding mobile phase in 100mL volumetric flask by adding 20ml of mobile phase and sonicated using bath sonicator (Citizen, India) for 10 minutes and made up to the volume with corresponding mobile phase to extract Tamoxifen totally and centrifuged at 3000rpm for 20 minutes separate un dissolved excipients. The supernatant was taken and was passed through a 0.45 micron membrane filter (PALL, USA). The samples were analyzed using RP-HPLC attached with PDA at a max of 228nm (73). The experiments were performed in triplicate (n=3) and Tamoxifen content present in S-SUSPENSION.

Invitro drug release studies

For both liquid and dry nanosuspension formulations show greater than 85% drug liberate after 15 min and in case of marketed product it was less than 15 %. Finally from these comparative dissolution studies it was noticed that liquid nanosuspension and dry nanosuspension has shown greater drug release than marketed product. In vitro drug release profile of SUSPENSION showed complete drug release within 30 min but marketed formulation released only 20 percentage of Tamoxifen and the time verses drug release. The SUSPENSION rapidly hydrated during *invitro* dissolution to form oil in water suspension. The nanosized droplets produce in high specific surface are of delivery system results in improved dissolution rate compared to pure Tamoxifen. The in vitro dissolution reports on S-SUSPENSION exposed that Tamoxifen release from porous carriers (Aerosil® 200) was slow when compared to SUSPENSION. This could be because of extra steps involved during dissolution such as disintegration of Solid structure of S-SUSPENSION and desorption of SUSPENSION from the voids of porous carriers. The SUSPENSION when exposed to dissolution medium, leads to desorption of the SUSPENSION from the silica surface due to stronger interaction between silica and dissolution medium than those between silica and SUSPENSION. Drug freed from SUSPENSION was initially slow when compared to SUSPENSION. This could be because of increase in diffusion path length and capillary forces for adsorbed liquid formulation in the matrix of porous Aerosil® 200 carrier. Wicking properties of liquid filled porous carrier upon contact with dissolution medium, could also be responsible for slower drug release. In vitro dissolution, studies confirmed that the self-nanoemulsifying formulations can improve the release profile of Tamoxifen. (Table 9 Table 10).

Physical stability of the medicated nanosuspensions was intensively evaluated over 30 days period. According to the obtained results, the Tamoxifen nanosuspension could retain the particle size distribution in the nanometer range within 14 days. Afterwards, the particle size began to

increase and within 30 days the particle size of the Tamoxifen nanosuspensions had grown rapidly to be very big particles. The particle growth was due to the existence of aggregated particles which follows Ostwald ripening phenomenon.

Table 9: 7 % Drug release of formulations FC1 to FC6.

Time	FC1	FC2	FC3	FC4	FC5	FC6
0	0	0	0	0	0	0
5	29.02	28.04	18.87	27.86	27.73	29.02
10	35.70	35.43	27.19	36.35	35.63	35.70
15	60.92	58.89	57.13	60.24	75.41	60.24
20	66.08	64.53	63.63	66.73	83.84	66.73
30	70.44	69.43	69.71	71.34	102.80	76.34
45	80.90	79.98	79.27	80.17		88.52
60	87.27	83.98	89.02	93.28		98.67

Table 10: 8 % Drug release of formulations FC7 to FC12.

Time	FC7	FC8	FC9	FC10	FC11	FC12
0	0	0	0	0	0	0
5	27.86	30.23	37.47	18.42	19.62	19.89
10	36.35	44.9	59.93	27.73	27.86	28.04
15	41.45	50.87	65.85	35.63	36.35	43.81
20	55.25	66.37	89.55	42.04	41.45	58.89
30	60.24	70.84	96.67	64.33	71.34	79.98
45	79.01	76.56	77.88	75.41	78.52	83.52
60	84.23	90.64	86.6	83.84	80.17	88.65

DISCUSSION

It was possible to obtain Tamoxifen nanosuspensions with fine solubility and dissolution properties, and the nanosuspensions possessed a high drug- loading (10%), which could reduce the dosage administration and gastrointestinal side effects. High pressure homogenization can be employed to produce aqueous drug nanosuspensions that are stable up to one month. The zeta potential values are about -30mv or higher, i.e. in the range for a long-term stable suspension. Aqueous nanosuspension can be converted to dry nanocrystals by lyophilization. The very fine particles of the dried nanocrystals re- disperse completely in water. This characteristic is critical in improving the kinetic solubility and the dissolution behavior of drug.[16,17]

In conclusion, stable Tamoxifen nanosuspension with an increased drug saturation solubility and dissolution velocity can be prepared by high pressure homogenization. The nanosuspensions possessed a high drug-loading, which could reduce the dosage administration and gastrointestinal side effects. Aqueous nanosuspension can be converted to dry nanocrystals by lyophilization. Dried Tamoxifen nanocrystals offer superior physicochemical properties. Further in-vivo studies of Tamoxifen nanosuspension are required for its efficacy, safety and tolerability.

ACKNOWLEDGEMENTS

Authors would like to thank Dr. Senthil, Dr. Mades Srinu, Dr. Narsing Rao, Dr. P.V. Chalam, Dr. Vijayalaxmi for their cooperation.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Müller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in therapy: rationale for development and what we can expect for the future. Advanced drug delivery reviews. 2001 Mar 23;47(1):3-19.
2. Möschwitzer J, Achleitner G, Pomper H, Müller RH. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. European Journal of Pharmaceutics and Biopharmaceutics. 2004 Nov 1;58(3):615-9.
3. Mouridsen H. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. N Engl J Med. 2009;361(8):766-76.
4. De Lima GR, Facina G, Shida JY, Chein MB, Tanaka P, Dardes RC, Jordan VC, Gebrim LH. Effects of low dose tamoxifen on normal breast tissue from premenopausal women. European Journal of Cancer. 2003 May 1;39(7):891-8.
5. Mirzajani F, Rafati H, Atyabi F. Fabrication of biodegradable poly (d, l-lactide-co-glycolide) nanoparticles containing tamoxifen citrate. Iranian Polymer Journal. 2010;19 (6):437-46.
6. Li Y, Dong L, Jia A, Chang X, Xue H. Preparation and characterization of solid lipid nanoparticles loaded traditional Chinese medicine. International journal of biological macromolecules. 2006 May 30;38(3-5):296-9.
7. Jacobs C, Kayser O, Müller RH. Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide. International journal of pharmaceutics. 2000 Mar 10;196(2):161-4.
8. Lindfors L, Skantze P, Skantze U, Rasmusson M, Zackrisson A, Olsson U. Amorphous drug nanosuspensions. 1. Inhibition of Ostwald ripening. Langmuir. 2006 Jan 31;22(3):906-10.
9. Dubernet C. Thermoanalysis of microspheres. Thermochim. Acta. 1995;248:259-69.
10. Corrigan DO, Healy AM, Corrigan OI. The effect of spray drying solutions of bendroflumethiazide/polyethylene glycol on the physicochemical properties of the resultant materials. International Journal of Pharmaceutics. 2003 Aug 27;262(1-2):125-37.

11. Hancock BC, Carlson GT, Ladipo DD, Langdon BA, Mullarney MP. Comparison of the mechanical properties of the crystalline and amorphous forms of a drug substance. International Journal of Pharmaceutics. 2002 Jul 8;241(1):73-85.
12. Böhm BH, Müller RH. Lab-scale production unit design for nanosuspensions of sparingly soluble cytotoxic drugs. Pharmaceutical science & technology today. 1999 Aug 1;2(8):336-9.
13. Müller RH, Jacobs C, Kayser O. Nanosuspensions for the Formulation of Poorly Soluble Drugs In: F. Nielloud and G. Marti-Mestres. Pharmaceutical Emulsions and Suspensions. 2000:383-407.
14. RA Nash. Suspensions, In: J Swarbrick, JC Boylan (ed), Encyclopedia of pharmaceutical technology, 2nd Edition; New York: Marcel Dekker; 2002:3:2045-3032.
15. Müller RH, Peters K. Nanosuspensions for the formulation of poorly soluble drug I: Preparation by size reduction technique. Int. J. Pharm. 1998;160:229-37.
16. Dianrui Z, Tianwei T, Lei G, Wenfa Z, Peng W. Preparation of Azithromycin Nanosuspensions by High Pressure Homogenization and its Physicochemical Characteristics Studies, Drug Development and Industrial Pharmacy. 2007;3(5):569-75.
17. Soo PL, Cho J, Grant J, Ho E, Miller MP, Allen C. Drug release mechanism of paclitaxel from a chitosan-lipid implant system: Effect of swelling, degradation and morphology, European Journal of Pharmaceutics and Biopharmaceutics. 2008;69:149-57.

Cite this article as: Reddy SM, Sriganth NN, Kumar CS, Gursale SC, Ragavan VV. In-vitro study of formulation and evaluation of nanosuspension of tamoxifen. Int J Basic Clin Pharmacol 2018;7:926-34.