Comparative *in-vivo* Evaluation of Anti-Cancer Drugs Loaded Nanospheres

Prakash Katakam[1], Phalguna Yadagiri[2]*

[1]Professorand Prinicipal SSS Indira college of Pharmacy, Sahayog Educational Campus, Vishnupuri, Nanded, Maharashtra, INDIA.
[2]Department of pharmaceutics Jyothismathi college of Pharmacy, Turkapally(V), Shameerpet(M), Medchal-Dist. Telangana-500078, INDIA.

ABSTRACT

Objectives: objective of present research was to formulate and evaluate nanospheres of selected anticancer drugs, viz., Capecitabine (CPN), Tamoxifen (TAM) and Doxorubicin (DXO). The adverse effects associated with anticancer drugs which include are bone-marrow depression, cardio toxicity, diarrhoea, nausea and vomiting, stomatitis and dermatitis. **Materials and Methods:** Drug loaded nanospheres of polycaprolactone-chitosan in various drug: polymer ratios, cross linked with Tripolyphosphate were prepared by double emulsion solvent evaporation and solvent diffusion methods. Male white New Zeeland Rabbits(weighing about 2500 gm) were selected as the animalmodel. The rabbits selected for the study had no medicationfor two weeks prior to the study. **Results and Discussion:** The parameters like AUC(0-24) of DXO nanospheres 2362.0 ng.h/mL, whereas DXO pure drug was 1956.5 ng.h/mL. AUC (0-24) of TAM nanospheres 5646.00 ng.h/mL. Whereas TAM unadulterated medication was 4786.30ng.h/mL. AUC (0-24) of CPN nanospheres 4927.40 ng.h/mL. Whereas CPN pure drug was 4027.5ng.h/mL. **Conclusion:** *In vivo* results showed a significant increase in the bioavailability of drugs from DXO6, CPN6 and TAM6 nanospheres when compared to those of the standard drugs. This enhanced bioavailability could be helpful in reducing the dose of DXO, CPN and TAM and also reduce their toxicities. This enhanced bioavailability could be helpful in reducing the dose and also reduce the toxicities of the selected drugs.

Key words: Doxorubicin, Tamoxifen, Capecitabine, Nanospheres, *in vivo* studies.

INTRODUCTION

Tamoxifen (TAM) is a nonsteroidal specialist that ties to estrogen receptors (ER), propose a conformational change in the receptor. The outcome is a blockage or change in the indication of estrogen qualities.[1] TAM is utilized to treat a breast tumor that has reach out to different parts of the body, to treat breast growth in specific patients after surgery and radiation treatment. Doxorubicin (DXO) has antimitotic and cytotoxic activity through a numeral of future components of activity. DXO shapes edifices with DNA by intercalation between base sets, and it restrains topoisomerase II movement by settling the DNA-topoisomerase II complex. DXO is an anthracycline sort of chemotherapy that is utilized alone or with different medications to treat a few distinct sorts of breast tumor.[2] Capecitabine (CPN) is a prodrug that is specifically tumor-initiated to its cytotoxic moiety[3] fluorouracil, by thymidine phosphorylase.[4] CPN is utilized alone or with different medicines/drugs to take care of positive sorts of malignancy like colon, rectum.[5] The adverse effects associated with anticancer drugs which include are bone-marrow depression, cardio toxicity, diarrhoea, nausea and vomiting, stomatitis and dermatitis.[6] Therefore the objective of the present study was to evaluate the bioavailability studies of these drugs in order to eliminate its adverse effects.

Nanoparticles take an interest to a great degree basic obligation in tumor study. Because of a massively minimal size of nanoparticles they are basically and included

deliberately taken up by the human body.[7] Nanoparticles are steady strong colloidal particles comprise of biodegradable polymer or lipids and size range 10-1000 nm. Formulation, characterization and *in vitro* evaluation of DXO,[8] TAM,[9] and CPN,[10] loaded nanospheres have already been discussed in our previous articles. Moreover this *In vivo* evaluation has not been reported in the literature. Therefore in this article we evaluate the bioavaliability studies for different anticancer drugs and compared with pure drugs.

MATERIALS AND METHODS

Doxorubicin was a gift sample from Sun Pharmaceutical Ltd., Mumbai, India. Tamoxifen was a gift sample from the Cipla Limited, Mumbai, India. Capecitabine was a gift sample from the Dr. Reddy lab's., Hyderabad, India. Polycaprolactone (Mw 14, 000), chitosan (Mw 60, 000-120, 000), sodium tripolyphosphate (Mw 367.86) and polyvinyl alcohol (Mw 7, 200) were purchased from Sigma Aldrich Pvt. Ltd., Bangalore, India. Dialysis membrane (average diameter 21.5 mm and capacity 3.63 ml/cm) was from Himedia Pvt. Ltd., Mumbai, India. All other reagents used were of analytical grade.

Preparation of nanospheres

The DXO loaded nanospheres were formulated using two methods. Polycaprolactone (PCL), chitosan, sodium tripolyphosphate (TPP), polyvinyl alcohol (PVA) combinations at various concentrations. Formulations DXO1–DXO3, TAM1-TAM3, CPN1-CPN3 were prepared by double-emulsion solvent evaporation method.[11] using polycaprolactone and chitosan each at concentrations of 0.5, 1.5 and 2.5% w/w respectively. Similarly formulations DXO4–DXO6, TAM4-TAM6, CPN4-CPN6 were prepared by emulsion solvent diffusion method.[12,13] using the same formula. In all, the six formulations, polyvinyl alcohol (4% w/w) and tripolyphosphate (1% w/w) were used as external aqueous phase (EAP) and cross-linking agent respectively.

In vivo bioavailability studies of nanospheres

All the animal investigations were performed as per the requisite protocol approved by the Animal Ethics Committee, Albino Research and Training Institute, Hyderabad, India. Approval letter No: ARTI/CPC-SEA/2014/05. Male white New Zeeland Rabbits (weighing about 2500 gm) were selected as the animal model. The rabbits selected for the study had no medication for two weeks prior to the study. Animals were divided into seven groups each containing 3 rabbits. Group-I (control group), Group-II (CPN pure drug), Group – III (DXO pure drug), Group – IV (TAM pure drug), Group – V (CPN6 -nanospheres), Group – VI (DXO6-nanospheres) and Group – VII (TAM6- nanospheres). The rabbits were placed on their side on the surgery table and 0.5 mL of blood sample was collected from a retro – orbital vein at the time interval of 0, 1, 4, 8, 12, 16, 20, and 24 h for all the seven groups. The blood samples were allowed to coagulate and whole blood samples were centrifuged and plasma was separated and stored at -20°C until analyzed.

Analysis of Plasma samples by LC/MS method

The chromatographic system consisted of an Agilent HPLC with a UV-visible detector (UVD170U). The separation was achieved by using Orochem PD, RP18, (4.6 mm i.d. ×15 mm) analytical column (Waters, Milford, MA, USA) which is operated at 40°C. The mobile phase was an isocratic elution with a flow rate of 1200 **µ**L/min and consisted mixture of 5 MM Ammonium Format: Methanol (20:80, v/v.[14] Under these conditions, RTs were typically 1.10 min for Capecitabine approximately. The Column effluent was introduced into the mass. The temperature of the auto sampler was kept 4°C and the run time was 2.0 min.

The chromatographic system consisted of an Agilent HPLC with a UV-visible detector (UVD170U). The separation was achieved by using YMC Triat C18 column (5**µ**, 100×4.6 mm i.d.) analytical column (Waters, Milford, MA, USA) which is operated at 40°C. The mobile phase was an isocratic elution with a flow rate of 600 **µ**L/min and consisted mixture of 10 MM Ammonium Acetate/Acetonitrile (40/60, v/v).[15] Under these conditions, RTs were typically 1.10 min for Doxorubicin approximately. The Column effluent was introduced into the mass. The temperature of the auto sampler was kept 4°C and the run time was 2.0 min.

The chromatographic system consisted of an Agilent HPLC with a UV-visible detector (UVD170U). The separation was achieved by using Symmetry Shield, C18, (4.6 mm i.d. ×50 mm) analytical column (Waters, Milford, MA, USA) which is operated at 40°C. The mobile phase was an isocratic elution with a flow rate of 600 **µ**L/min and consisted of 2 mL of Ethyl Acetate.[16] Under these conditions, RTs were typically 1.10 min for Tamoxifen approximately. The Column effluent was introduced into the mass. The temperature of the auto sampler was kept 4°C and the run time was 2.0 min.

Mass spectrometry conditions for CPN, DXO, and TAM

An API-4000 triple-quadrupole mass spectrometer (Applied Bios stems/MDS SCIEX, Foster City, CA/ Concord, Ontario, Canada) was equipped with an electro

spray source (ESI), operating in the positive ion mode. Data were collected and processed using Scitex Analyst 1.4.2 Data collection and integration software on a DELL compatible computer using multiple reaction monitoring (MRM). Data were acquired on a Dell Precision 370 workstation and were processed using the Analyst 1.4.2 software package (MDS Scitex). Calibration curve Range: 5 ng/mL to 5000 ng/Ml.

Statistical analysis

The measurable investigation of exploratory information used the student's t-test, and the outcomes are introduced as mean ±sd. Statistical significance was accepted at a level of *p*<0.05.[17]

RESULTS AND DISCUSSION

DXO1-DXO3, TAM1-TAM3, CPN1-CPN3 were successfully prepared by double emulsion solvent evaporation and DXO4-DXO6, TAM4-TAM6,CPN4-CPN6 prepared by solvent diffusion method. Nanospheres were characterized in terms of particle size, zetepotential, polydispersity index, refractive index, SEM and *in vitro* release profile.

Drug entrapment, PSA, ZP, SEM, PDI, RI and *in vitro* release of DXO

Drug entrapment for DXO6 was found to be 93.62±0.17%, particle size of the nanospheres was found in the range of 700±105 to 770±115 nm, Zeta potential was showed negative with 34.05±1.5 mV, Polydispersity index (0.316), Refractive index (1.33), *In vitro* drug release for DXO6 was 94.16±0.85% for a period of 24 h.

Drug entrapment, PSA, ZP, SEM, PDI, RI and *in vitro* release of TAM

The drug entrapment for TAM6 was showed 90.56±0.45, Particle size of nanospheres was found to be 620±50 to 770±100 nm, zeta potential was found to be -72.59 mV, Polydispersity index(-5.83), Refractive index(1.33), The formulation TAM 6 showed 92.57±0.32% release of the drug for a period of 24 h.

Drug entrapment, PSA, ZP, SEM, PDI, RI and *in vitro* release of CPN

Drug entrapment for CPN6 was found to be 96.53±0.86, maximum diameter of the particles is found to be 616±110 to713±115 nm, zeta potential was found to be -17.30 mV, Poly dispersity index(0.316), Refractive index(1.33), SEM photographs showed nanospheres of discrete nature, and distinct particle size and shape

with a smooth surface, *in vitro* release profile showed maximum in CPN6 drug release of 94.28±0.20 % for a period of 24 hrs.

Kinetic studies

The drug release profiles are fitted to release kinetics the slope for Kosmeyer-peppas was in the range of 0.45 to 1 indicating both diffusion and erosion of both biodegradable polymers. The release mechanisms for all the formulations followed by non-fickian diffusion mechanism.

Bioavailability studies

Based on the best release profiles of DXO6, TAM6 and CPN6 formulations were selected for *In vivo* studies. Bioavailability studies of optimized formulations were evaluated to prove their therapeutic efficacy. Bioavailability of developed nanospheres were also compared with pure drugs. The results of these studies are given in Table 1-3 and Figure 1-3 Nanospheres of DXO experienced in beginning an appearance with in 1st h. nanospheres demonstrated higher blood concentration when contrasted with pure drug through the study. T_{max} of DXO nanospheres and standard drug was found to be 12 h and 8 h. Yet, C_{max} and $AUC_{(0-24)}$ were higher for DXO nanospheres. The C_{max} of nanospheres was found to be 156.30 ng/mL, whereas for pure DXO it was 126.80 ng/mL. The other parameters like $AUC_{(0-24)}$ of DXO nanospheres 2362.0 ng.h/mL, whereas DXO pure drug was 1956.5 ng.h/mL.

Nanospheres of TAM experienced in introductory appearance with in 1st h. The nanospheres demonstrated higher blood focus when contrasted with pure drug through the study. T_{max} of TAM nanospheres and standard medication was found to be 4 h. Be that as it may, C_{max} and $AUC_{(0-24)}$ was higher for TAM nanospheres. C_{max} of nanospheres was discovered to be 459.20 ng/mL, whereas pure drug discovered to be 442.20 ng/mL. The other parameter like $AUC_{(0-24)}$ of TAM nanospheres 5646.00 ng.h/mL. Whereas TAM unadulterated medication was 4786.30ng.h/mL.

Nanospheres of CPN experienced in beginning an appearance with in 1st h. Nanospheres demonstrated higher blood focus when contrasted with pure drug through the study. T_{max} of CPN nanospheres and standard medication was found to be 4 h. Yet, C_{max} and AUC (0-24) were higher for CPN nanospheres. C_{max} of nanospheres was discovered to be 563.20 ng/mL. Whereas pure drug discovered to be 456.20 ng/mL. The other parameter like AUC (0-24) of CPN nanospheres 4927.40 ng.h/mL. Whereas CPN pure drug was 4027.5ng.h/mL.

Table 1: *In vivo* studies of standard DXO and optimized DXO6 Nanospheres.		
Time in h	Concentration (ng/mL) for standard DXO	Concentration (ng/mL) for optimized DXO6 nanospheres
1	23.4±1.28	42.3±1.11
4	86.2±0.78	96.3±1.21
8	126.8±0.71	145.3±1.17
12	106.2±1.15	156.3±0.95
16	89.2±1.83	99.2±1.02
20	65.2±1.34	66.2±0.88
24 $AUC_{(0-24)}$(ng.h/L) C_{max}(ng/L) T_{max}	29.20±0.45 1956.50±23.86 126.80±0.711 8	36.2±1.05 2362±21.31 156.3±0.95 8

Table 2: *In vivo* studies of standard TAM and optimized TAM6 nanospheres.		
Time in h	Concentration (ng/mL) for standard TAM	Concentration (ng/mL) for optimized TAM6 nanospheres
1	66.2±1.41	86.2±1.25
4	442.2±1.47	459.2±1.48
8	326.0±1.07	339.2±1.32
12	223.3±1.34	269.8±0.95
16	106.4±1.16	208.2±1.22
20	89.7±1.07	106.3±1.34
24 $AUC_{(0-24)}$(ng.h/L) C_{max}(ng/L) T_{max}	62.1±1.38 4785.09±25.4 442.20±1.47 4	86.2±1.09 5646.02±28.86 459.2±1.4 4

Table 3: *In vivo* studies of standardCPN and optimized CPN6 nanospheres.		
Time in h	Concentration (ng/mL) for standard CPN	Concentration (ng/mL) for optimized CPN6 nanospheres
1	38.60±0.95	45.9±1.08
4	456.20±1.08	563.2±1.32
8	312.0±1.03	382.6±1.11
12	156.0±1.11	186.2±1.47
16	78.2±1.13	89.2±1.39
20	36.1±1.17	46.3±1.46
24 $AUC_{(0-24)}$(ng.h/L) C_{max}(ng/L) T_{max}	12.2±1.21 4027.50±19.59 456.20±1.08 4	23.6±1.13 4927±29.84 562.7±1.32 4

Statistical analysis

It was observable that the distinction in bioavailability was P=0.027 for CPN, P=0.018 for DXO and P=0.016 for TAM beteen the standard and tested nanospheres. This shows that the arranged nanospheres demonstrated fundamentally better bioavailability with that of the standard.
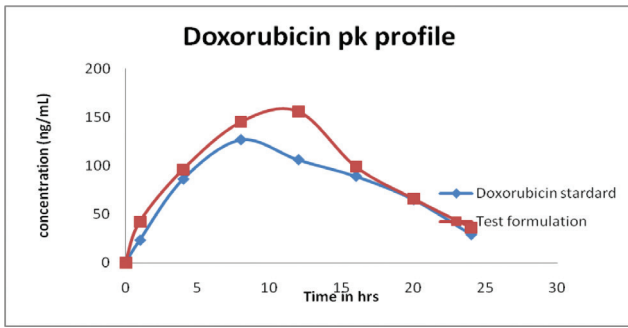
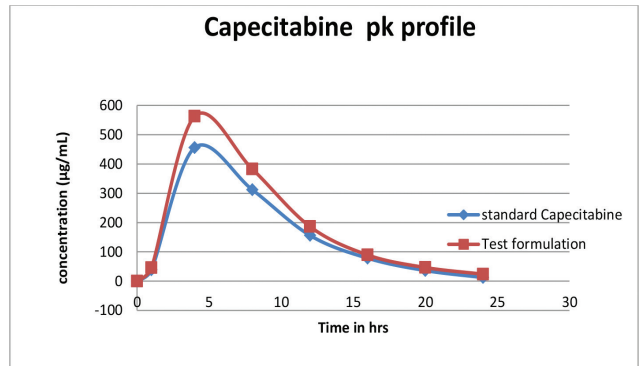**Figure 1: DXO comparative Pharmacokinetic (s) profile.**

Figure 2: Mass spectra for DXO.

Figure 3: TAM comparative Pharmacokinetic (s) profile.
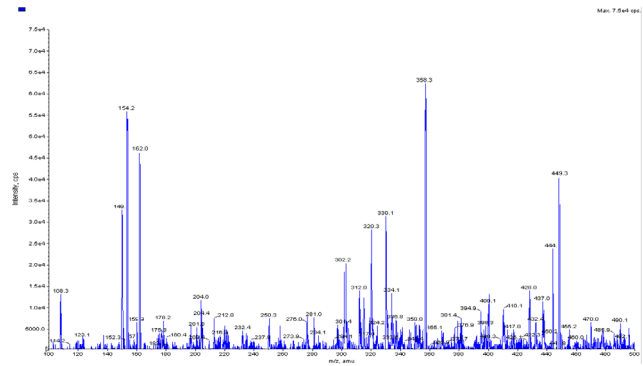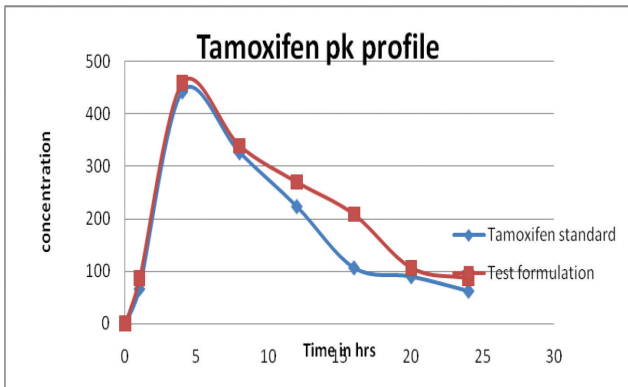

Figure 4: Mass spectra for TAM.

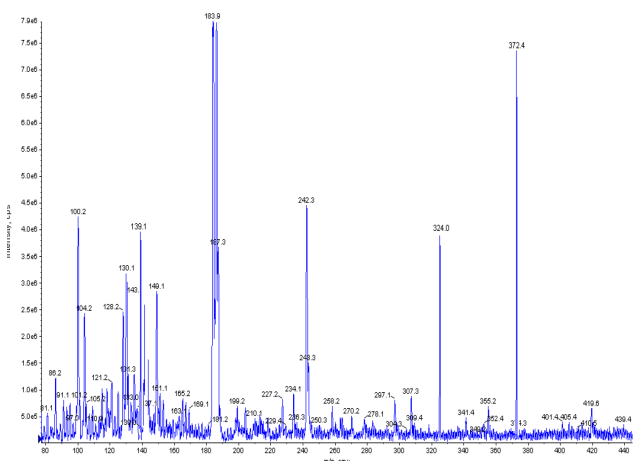**Figure 5: CPN comparative Pharmacokinetic (s) profile.**

Figure 6: Mass spectra for CPN.

CONCLUSION

In vivo results showed a significant increase in the bioavailability of drugs from DXO6, CPN6 and TAM6 nanospheres when compared to those of the standard drugs. This enhanced bioavailability could be helpful in reducing the dose of DXO, CPN and TAM and also reduce their toxicities. The present investigation shows a promising future on the nanospheres formulations of the selected anticancer drugs, viz., DXO (p=0.018), CPN (p=0.027) and TAM (p=0.016). This research throws light on the novel drug delivery systems for the selected drugs. From the results it can be concluded that the developed nanospheres have great potential for parenteral application of DXO, TAM and CPN.

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CONFLICT OF INTEREST

There are no conflicts of interest.

ABBREVIATION USED

TAM: Tamoxifen **DXO:** Doxorubicin; **CPN:** Capecitabine; **ER:** Estrogen receptors; **EAP:** External aqueous phase; **ESI:** Electro spray source.

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PICTORIAL ABSTRACT

Capecitabine Nanospheres
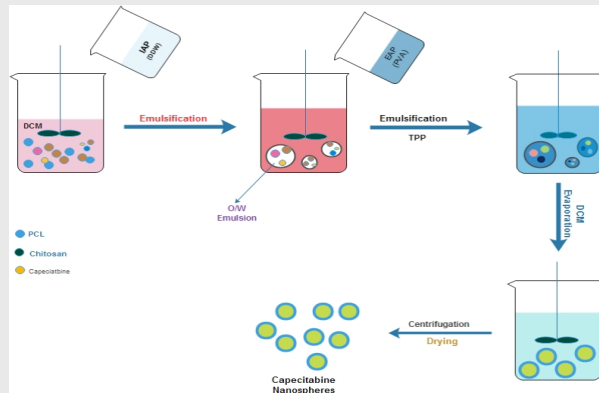
SUMMARY

An attempt was made to formulate and evaluate nanospheres of selected anticancer drugs, viz., Capecitabine (CPN), Tamoxifen (TAM) and Doxorubicin (DXO).DXO1-DXO3, TAM1-TAM3, CPN1-CPN3 were prepared by double emulsion solvent evaporation and DXO4-DXO6, TAM4-TAM6, CPN4- CPN6 prepared by solvent diffusion method. All the evaluation parameters were within the standard limits. The bioavailability of developed nanospheres were compared with standard and found to be much effective with better bioavailability.

About Authors

Dr.Prakash Katakam: Principal and professor, SSJ Indira college of pharmacy, Nanded, Maharastra,India. Professional Experience of over 20 years of teaching, research and administrative experience in pharmaceutical education. Five years of International Exposure worked in Libyan Govt. University and Visiting Professor of Belgrade University, Serbia.
Contributed 117 publications in national and international journals, Published 06 Indian patents. Presented research works in 15 conferences. Guided 08 PhD students and guiding 03 students.

Dr.Y.Phalguna: HOD in Dept.of Pharmaceutics, Jyothismathi college of pharmacy, Turkapally. He has 13 years of experience in teaching and research. He published various papers in national and international journals.