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Parenteral Docetaxel Emulsion System and Its Stability

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ABSTRACT – Docetaxel is an anticancer agent with low aqueous solubility. More extensive clinical use of this drug is somewhat delayed due to lack of appropriate delivery vehicles. An attempt was made to adopt an o/w emulsion as the drug carrier which incorporated docetaxel in the propyleneglycerol stabilized by a mixed-emulsifier system. A suitable formulation was found in this study: 10 mg/mL docetaxel, 10% (w/v) oil blend, 4% (w/v) PG, 3% (w/v) Solutol HS 15 in 2.25% (w/v) glycerol solution. The formulated emulsion has very good stability when stored at 40°C, and the docetaxel containment efficiency can be maintained above 95% and the mean emulsion diameter around 10 μm for at least 3 months. The formulated emulsion is a promising carrier for docetaxel and other lipophilic drugs.

Key words – Emulsion, Docetaxel, Anticancer therapy, Drug delivery system, Stability

Docetaxel is an effective treatment approved in five key cancers, breast cancer,[1] non-small -cell lung cancer (NSCLC),[2] gastric adenocarcinoma,[3] hormone-refractory prostat cancer,[4] head and neck cancer[5] but its effectiveness in clinical practice can be compromised by sub-optimal side effect management. The published specific docetaxel-related side effects are pertained to six side effects identified as being common to the majority of docetaxel regimens and indications of particular relevance to the oncology for examples, febrile neutropenia, hypersensitivity reactions, fluid retention, nail changes, asthenia, neuropathy.[6]

Furthermore, there is a need for a systematic approach to manage cutaneous reactions associated with weekly docetaxel administration.[7]

On the other hand, a comparative analysis on pharmaceutical quality of docetaxel generic versus originator drug product was reported,[8] where 31 commercially available generic formulations of docetaxel was evaluated in terms of docetaxel content, impurity levels and pH versus those of the proprietary product Taxotere® but 90% of the generic docetaxel formulations evaluated contained insufficient active drug, high levels of impurities or both from an analytical point of view, which has the potential to affect both efficacy and safety of the drug.

At the pharmaceutical point of view, highly efficient system to deliver docetaxel into tumor cells should be taken into consideration and drug carriers such as hydrophobically modified glycol chitosan nanoparticles,[9,10] thermosensitive micelles,[11] solid dispersion,[12] pegylated conjugation,[13-15] liposome,[16-18] nanoassembly,[19] albumin-conjugate,[20] submicron lipid emulsion[21] loaded with docetaxel have been reported.

In this study, docetaxel was formulated by introducing the combination of 30% PEG and 70% PEG esters, which manipulate hydrophilic lipophilic balance (HLB) in order to stablize the emulsion system O/W for practically insoluble docetaxel anhydrous. The stability of formulated emulsion system was investigated at 40°C or room temperature and accelerated condition for 3 months. In comparison study with Taxotere® docetaxel content, impurity levels and pH versus those of the proprietary product were also investigated.

Experimantal Part

Materials

Docetaxel reference standard was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Docetaxel anhydrous was obtained from Sicor Chemical Co. branch plant of Teva Pharmaceutical Co. Ltd. (Tel aviv, Israel). HPLC grade methanol and acetonitrile were from Aldrich Chemical Co. (St. Louis, MO, USA).

Method

Preparation of O/W emulsion system - The o/w microemulsion systems containing 1% (w/w) of docetaxel were prepared using the selected surfactant and cosurfactant for the stability study and the content of the mixture of S/CoS were varied from 4 to 8% as described in Table I.

Each formulated microemulsion system was filling in transparent ample by using filling machine (Bausch Sprobel, Germany). After sealing leaky test was performed and then autoclaved at

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Table I–*Pharmaceutical Composition of Docetaxel-loaded Microemulsion Systems for Parenteral Delivery*

Pharmaceutical composition		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Active ingradient	Docetaxel	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg
Surfactant	Solutol HS 15	260 mg	260 mg	260 mg			
	Cremophore EL				260 mg	260 mg	260 mg
Dissolving agent	Propylene glycol	200 mg	150 mg	100 mg	200 mg	150 mg	100 mg
Tonicity	Mannitol	50 mg	50 mg	50 mg			
	Sorbitol				50 mg	50 mg	50 mg
pH adjust	citric acid	q.s	q.s	q.s	q.s	q.s	q.s
solvent	WFI	q.s					

121°C, 110 psi for 15 minutes. The prepared samples were cooling down and foreign materials were visually observed.

Quantity test - Preparation of standards : Docetaxel standard 40.0 mg was weighed accurately and dissolved in 100 mL volumetric flask filled with mobile phase upto meniscus. Taken 10 mL standard soln from the properly mixed solution was diluted with 20 mL mobile phase to make 0.2 mg/mL.

Sample processing : Docetaxel sample 0.5 g was weighed precisely and dissolved with EtOH 5 mL in 100 mL volumetric flask and then filled with mobile phase up to meniscus. Each standard and sample was withdrawn in the quantity of 20 μL and tested according to HPLC method in KP.

HPLC conditions : The prepared standard and docetaxel samples were separated on a Lichrospher® STAR 100RP-18 column (4.0 × 250 mm, particle size 5 μm; Phenomenex, USA) protected by a 0.5 μm precolumn filter and a 4 mm guard cartridge. The mobile phase consisted of acetonitrile : MeOH : water (260 : 320 : 420 v/v) delivered at a flow rate of 1.5 mL/min. The column temperature was maintained at 28°C and the UV detector was operated at 232 nm.

$$\text{Docetaxel (\%)} = \frac{AT \times MR \times d \times C}{AR \times MT \times 2 \times 42.68} \quad (1)$$

where each abreviations represent for MR : docetaxel standard amount (mg); MT : sample amount (g); AR : docetaxel peak area obtained from standard chromatogram; AT : docetaxel peak area obtained from standard chromatogram; d : density of docetaxel(1.1) (g/mL);C : standard content (%).

Validation - The analytical methods were validated regarding linearity, recovery, accuracy, precision, sensitivity and sample volume based on the criteria.

Linearity, accuracy and precision

For docetaxel, the linearity was determined by analysing calibration standards in duplicate at five levels using single samples. The validation for this compound included five calibrator curves analysed over a period of 8~10 days. In the analysis of docetaxel, QC samples in triplicate at three levels were included in each run and used for determination of accuracy and precision. Additional samples (5 at each level) containing docetaxel were included to verify the limit of quantitation. All calibrator curves were calculated using weighted least squares regression (WLS) using a weighing factor of 1/x, where x is the analyte concentration.

Stability test - Three quality control (QC) samples at each level were prepared separately and used for determining docetaxel content, impurity levels and pH versus those of the proprietary product for the docetaxel analysis method. Amples containing 2 mL of the microemulsion were stored in a stability chamber (Fine Scientific Instrument, FLT-600D, Korea) at 40°C for 12 weeks. At 0, 2, 4, 6, 8 and 12 weeks, samples were withdrawn and the physicochemical changes in the micro-emulsion system were observed and docetaxel content was measured in Table II matrix format.

Visual microscopic observations - Visual microscopic observations (Nickon microscope) calculated the number of droplets with diameter >2.5 μm. The dilution of the emulsions was 1:10 in order to obtain a final lipid concentration of 2 g/100 mL. The observations were made in a cell counting chamber (Thoma's grads) at × 500 magnification. A significant increase in particles with diameter >50 μm indicated destabilisation and the presence of particles with diameter >60 μm revealed a breakdown of the emulsion. Polaroid microphotography (×1000 magnification) confirmed the observations.

Microemulsion particle size distribution - Stored samples for 3 months were mixed with n-hexane and their particle size was analyzed by using laser diffraction, Mastersizer (Malvern

Table II–*Fundamental Analytical Validation Data for Docetaxel*

Sample	Accuracy			Precision		Repeatability		Specificity	
	Mean ± SD	ABS* %	Recovery %	Mean ± SD	%CV	Mean ± SD	RSD	Mean ± SD Rt(min)	Difference %
1	98 ± 0.49	0.30	97%	98 ± 0.49	1.00	98 ± 0.45	0.45	25.36 ± 0.0026	− 0.01
2	105 ± 2.50	0.77	99%	105 ± 2.50	0.83	105 ± 0.85	0.85	25.16 ± 0.0015	− 0.02
3	103 ± 1.03	0.34	101%	103 ± 1.03	0.97	103 ± 0.67	0.67	25.13 ± 0.0015	− 0.01

*ABS(Absolute Percent Error)

Instruments Ltd., Malvern, UK). Measured beam obscuration was ranged from 10% to 30% in polydisperse system and the residual was less than 3%.

Results and Discussion

The limits of quantitation were 1 nM for docetaxel and linearity was confirmed from the limit of quantitation up to 1000 nM in mobile phase. The recoveries ranged between 92% and 118% for docetaxel. Accuracy and precision were within international acceptance criteria, that is within ±15%, except at the limit of quantitation where values within ±20% are acceptable (in Table II). As shown in Figure 1-a, representative chromatogram for standard docetaxel with the retention time 7 min designated individual substance less than 2.5% and total related substance less than 5.5% respectively. For the docetaxel assay, we used a sample size of two milliliters of undiluted stock soln. The method was linear over the range tested, from 5 mg/mL to 80 mg/mL docetaxel. WLS regression of five calibrator curves with calibration standards at five levels gave the linear equation: Amount = 277(± 6) × Ratio − 0.6(± 0.3), R^2 ranged between 0.999 and 1.000 (Figure 1-b).

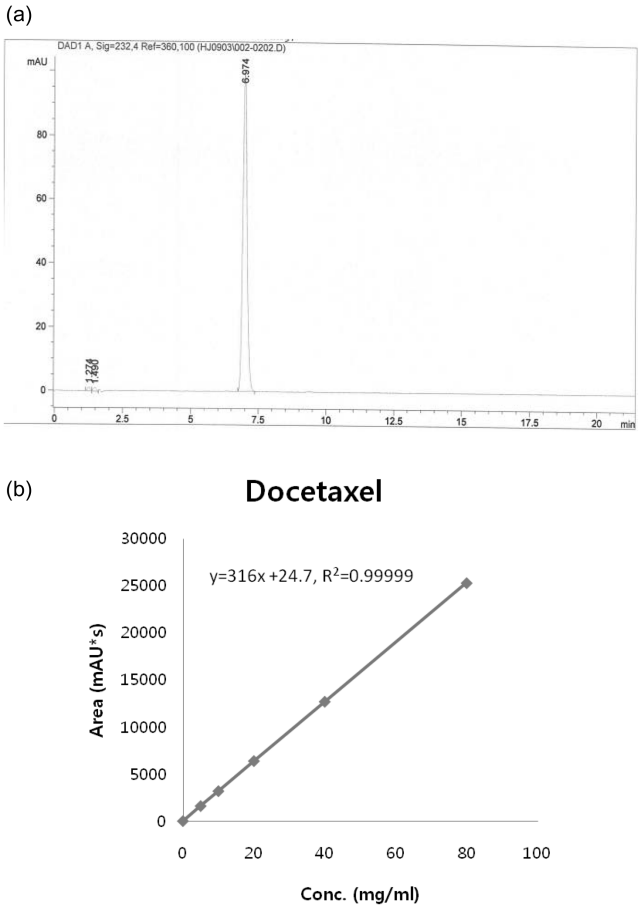

Figure 1–(a) Representative chromatogram for standard docetaxel with the retention time 7 min designated individual substance less than 2.5% and total related substance less than 5.5% respectively. (b) Standard calibration curve for docetaxel anhydrous in duplicate at five levels using single samples.

Chromatograms

Commercial available product, Taxotere® has been applied as following delivery using premixing in infusion parenteral system. Representative chromatogram for this control was shown in Figure 2.

Taxotere®	13%(w/w) EtOH/water	Total volume
20 mg/0.5 mL	1.88 - 2.08 mL	1.8 mL
80 mg/2 mL	6.96 - 7.70 mL	7.1 mL

Figure 2 showed that total impurity level was less than 5.5% and major peak was presented at retention time 25 min. In addition to it, representative chromatogram (Figure 3) for docetaxel loaded microemulsion samples was correspond to that of Taxotere® at same retention time.

In Figure 3, the early peaks represented for polyglycol monoesters of 12-hydroxystearic acid, polyglycol diesters of 12-hydroxystearic acid, free polyethylene glycols, and propylene glycols at retention time 1.5 min, 2.9 min, 4.7 min and 6.0 min respectively. Three quality control (QC) samples at each level were prepared separately and stored at room temperature and accelerated conditions for 3 months, which used for determining docetaxel content, impurity levels and pH versus those of the proprietary product by the docetaxel analysis method. The results (Table III) showed the conformity of doc-

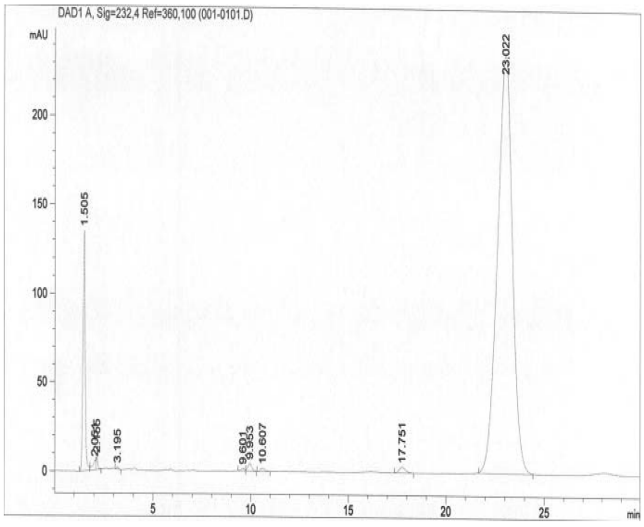

Figure 2–Representative chromatogram for commercial docetaxel product (Taxotere®).

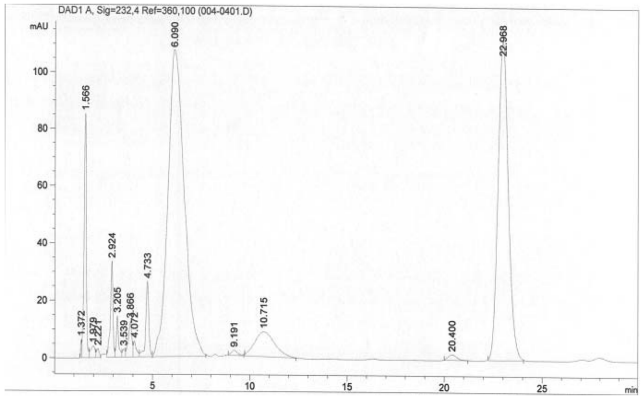

Figure 3–Representative chromatogram for docetaxel loaded microemulsion system for parenteral drug delivery.

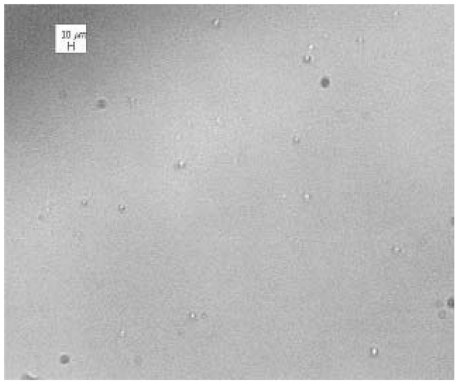

Figure 4–Photograph for docetaxel loaded microemulsion taken by Nickon microscope and particle size is around 10 μm.

etaxel content as well as impurity levels without pH changes.

Docetaxel loaded microemulsion particle characterization

The prepared microemulsion was taken into photos shown in Figure 4 and imply that the transparent small particles around 10 μm is thermodynamically stablized microemulsion system. Analysis of particulate samples was done to find the particle size and distribution from a representative sample. A particle size distribution can be displayed either tabular form in Table IV or graphically (Figure 5), and either as a cumulative distribution or as a differential distribution. To a sphere, the size is the diameter (or radius) of the sphere. To a real particle of arbitrary shape, the commonly acceptable size for 3-dimentional measurement techniques such as laser diffraction, Mastersizer (Malvern Instruments Ltd., Malvern, UK).

The importance of particle size in emulsions has been discussed in many papers. It is a determinant of emulsion stability, coating, break rate and cure rate. This is, of course, not

Table III–*Docetaxel Content Changes for 3 Months Stability Test*

Conditions	Time (weeks)	Control			Sample content			Sample average	
		content	impurity	pH	1	2	3	Impurity*	pH
25°C, 60%	0	97	<5%	conform	98	105	103	<5%	4.2
	2	95	<5%	conform	91	99	93	<5%	3.7
	4	99	<5%	conform	98	99	101	<5%	4.0
	8	103	<5%	conform	98	105	103	<5%	3.8
	12	95	<5%	conform	91	99	93	<5%	3.7
40°C, 75%	0	97	<5%	conform	98	105	103	<5%	4.2
	2	96	<5%	conform	97	93	94	<5%	4.2
	4	98	<5%	conform	104	100	105	<5%	3.8
	8	101	<5%	conform	100	103	98	<5%	3.7
	12	98	<5%	conform	97	93	94	<5%	3.8

*under development for generic product.

Table IV–*Docetaxel Loaded Microemulsion Particle Size Analysis Report*

Size Low (um)	In %	Size High (um)	Under %
3.09	0.00	3.60	0.00
3.60	0.02	4.19	0.02
4.19	0.10	4.88	0.13
4.88	0.40	5.69	0.52
5.69	1.19	6.63	1.72
6.63	2.83	7.72	4.54
7.72	5.17	9.00	9.71
9.00	7.80	10.48	17.51
10.48	9.65	12.21	27.16
12.21	10.55	14.22	37.72
14.22	10.45	16.57	48.16
16.57	9.62	19.31	57.79
19.31	8.38	22.49	66.17
22.49	7.12	26.20	73.29
30.53	5.35	35.56	84.75
35.56	4.73	41.43	89.48
41.43	4.04	48.27	93.52
46.27	3.25	56.23	96.77
56.23	2.16	65.51	98.93
65.51	1.07	76.32	100.00
76.32	0.00	88.91	100.00

the whole story, as formulation, raw materials and aggregates are also critical. However, particle size and particle size distribution are important variables and are controllable with formulation, raw materials and the equipment used to manufacture the emulsion. Many of the processes of breaking and curing are directly dependant on it. Therefore, normal distribution ranged from 6 μm to 70 μm and Gausnian distribution with mean diameter 21.6 μm.

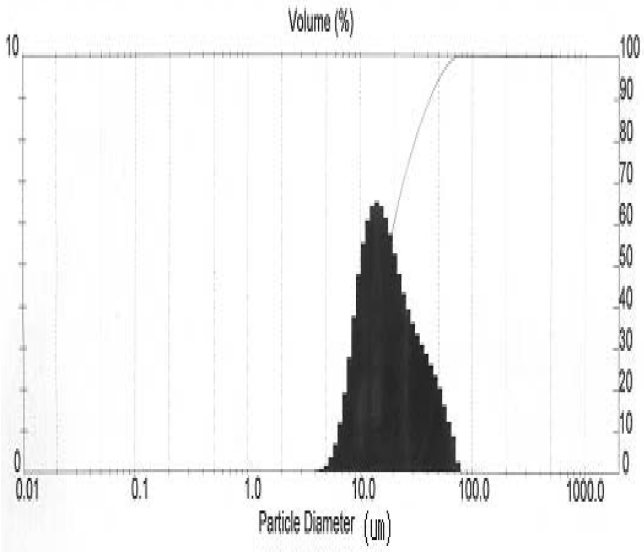

Figure 5–Docetaxel loaded microemulsion particle size distribution.

References

1) R.M. Strother and C. Sweeney, Lessons learned from development of docetaxel. *Expert Opin Drug Metab Toxicol.* **4**(7), 1007-19 (2008 Jul).
2) E. Saloustros and V. Georgoulias, Docetaxel in the treatment of advanced non-small-cell lung cancer. *Expert Rev Anticancer Ther.* **8**(8), 1207-22 (2008).
3) V. Gebbia, C. Gridelli, C. Verusio, L. Frontini, E. Aitini, B. Daniele, T. Gamucci, G. Mancuso, M. Di Maio, C. Gallo, F. Perrone and A. Morabito, Weekly docetaxel vs. docetaxel-based combination chemotherapy as second-line treatment of advanced non-small-cell lung cancer patients The DISTAL-2 randomized trial. *Lung Cancer.* 2008 Jul 14. [Epub ahead of print]
4) J.Y. Joung, I.G. Jeong, K.S. Han, T.S. Kim, S.O. Yang, H.K. Seo, J. Chung, K.S. Cho and K.H. Lee, Docetaxel Chemotherapy of Korean Patients with Hormone- refractory Prostate Cancer:Comparative Analysis between 1st-line and 2nd-line

Docetaxel.*Yonsei Med J.*, **49**(5), 775-82 (2008).
5) G.H. Yoo, G. Subramanian, M.P. Piechocki, J.F. Ensley, O. Kucuk, O.E. Tulunay, F. Lonardo, H. Kim, J. Won, T. Stevens and H.S. Lin, Effect of docetaxel on the surgical tumor microenvironment of head and neck cancer in murine models.*Arch Otolaryngol Head Neck Surg.*, **134**(7), 735-42 (2008).
6) J. Baker, J. Ajani, F. Scotté, D. Winther, M. Martin, M.S. Aapro and G. von Minckwitz, Docetaxel-related side effects and their management.*Eur J Oncol Nurs.*, **12**(3), 253-68 (2008).
7) L. Chew and V.S. Lee Chuen, Cutaneous reaction associated with weekly docetaxel administration. *J Oncol Pharm Pract.* 2008 Aug 27. [Epub ahead of print]
8) J. Vial, M. Cohen, P. Sassiat and D. Thiébaut, Pharmaceutical quality of docetaxel generics versus originator drug product: a comparative analysis. *Curr Med Res Opin.*, **24**(7), 2019-33 (2008). PMID: 18544188 [PubMed - indexed for MEDLINE]
9) H.Y. Hwang, I.S. Kim, I.C. Kwon and Y.H. Kim, Tumor targetability and antitumor effect of docetaxel-loaded hydrophobically modified glycol chitosan nanoparticles.*J Control Release.*, **128**(1), 23-31 (2008).
10) M.V. Lozano, D. Torrecilla, D. Torres, A. Vidal, F. Domínguez, and M.J. Alonso, Highly efficient system to deliver taxanes into tumor cells: docetaxel-loaded chitosan oligomer colloidal carriers. *Biomacromolecules.*, **9**(8), 2186-93 (2008).
11) B. Liu, M. Yang, R. Li, Y. Ding, X. Qian, L. Yu and X. Jiang, The antitumor effect of novel docetaxel-loaded thermosensitive micelles. *Eur J Pharm Biopharm.*, **69**(2), 527-34 (2008).
12) J. Chen, L. Qiu, M. Hu, Y. Jin and J. Han, Preparation, characterization and in vitro evaluation of solid dispersions containing docetaxel. *Drug Dev Ind Pharm.*, **34**(6), 588-94 (2008).
13) F. Esmaeili, M.H. Ghahremani, S.N. Ostad, F. Atyabi, M. Seyedabadi, M.R. Malekshahi, M. Amini and R. Dinarvand, Folate-receptor-targeted delivery of docetaxel nanoparticles prepared by PLGA-PEG-folate conjugate. *J Drug Target.*, **16**(5), 415-23 (2008).
14) M. Senthilkumar, P. Mishra and N.K. Jain, Long circulating PEGylated poly(D,L-lactide-co-glycolide) nanoparticulate delivery of Docetaxel to solid tumors. *J Drug Target*. Jun; **16**(5), 424-35 (2008).
15) S.S. Chandran, S.R. Banerjee, R.C. Mease, M.G. Pomper and S.R. Denmeade, Characterization of a targeted nanoparticle functionalized with a urea-based inhibitor of prostate-specific membrane antigen (PSMA). *Cancer Biol Ther.* Jun; **7**(6), 974-82 (2008).
16) Y. Qiu, Y. Gao, K. Hu and F. Li, Enhancement of skin permeation of docetaxel: a novel approach combining microneedle and elastic liposomes. *J Control Release.*, **129**(2), 144-50 (2008).
17) A. Fernaìndez-Botello, F. Comelles, M.A. Alsina, P. Cea and F. Reig, A Monolayer Study on Interactions of Docetaxel with Model Lipid Membranes. *J. Phys Chem B.*, **112**(44), 13834-13841 (2008).
18) Z. Xu, L. Chen, W. Gu, Y. Gao, L. Lin, Z. Zhang, Y. Xi and Y. Li, The performance of docetaxel-loaded solid lipid nanoparticles targeted to hepatocellular carcinoma. *Biomaterials.*, **30**(2), 226-32 (2009).
19) Y. Gao, L. Chen, W. Gu, Y. Xi, L. Lin and Y. Li, Targeted Nanoassembly Loaded with Docetaxel Improves Intracellular Drug Delivery and Efficacy in Murine Breast Cancer Model. Mol Pharm. 2008 Oct 9. [Epub ahead of print]
20) F. Esmaeili, R. Dinarvand, M.H. Ghahremani, M. Amini, H. Rouhani, N. Sepehri, S.N. Ostad and F. Atyabi, Docetaxel-albumin conjugates: Preparation, in vitro evaluation and biodistribution studies. *J Pharm Sci.* 2008 Oct 28. [Epub ahead of print]
21) K. Gao, J. Sun, K. Liu, X. Liu and Z. He, Preparation and characterization of a submicron lipid emulsion of docetaxel: submicron lipid emulsion of docetaxel. *Drug Dev Ind Pharm.*, **34**(11), 1227-37 (2008).