ORIGINAL ARTICLE

DESIGN AND OPTIMIZATION OF NSAID LOADED NANOPARTICLES

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ABSTRACT

The objective of the study was to design and evaluate NSAID loaded Nanoparticles drug delivery system, where Flurbiprofen (model drug) Nanoparticles with suitable size range are envisaged to concentrate at inflammation sites due to increase fragility of blood vessels at those sites and increased aggregation and prostaglandin synthesis. Materials used were surfactant (pluronic F 68) and polymer (poly lactic co glycolic acid; PLGA). The flurbiprofen loaded nanoparticles were prepared by solvent diffusion nano-precipitation method. Experiment was carried out following 3^2 factorial designs, where drug-polymer ratio was varied to optimize the formulation. From I.R studies no drug-polymer interaction was found. Particles size analysis was done using Malvern Mastersizer. Two parameters, namely, drug-polymer ratio and solvent-nonsolvent ratio were chosen for optimization following the factorial design. Amount of drug loading and surfactant were kept constant, and only polymer load was varied. The in-vitro drug release profile from nanoparticles was found to follow Higuchi square root kinetics implying a diffusion dependent release as is expected of an insoluble, non-swellable nature of PLGA. It indicated that nanoparticles formed were matrix in nature, in which flurbiprofen dispersed uniformly. Suitable polynomial models were generated and statistically validated using ANOVA for the different responses, namely drug release (maximization) and particle size (minimization). Those models were solved numerically and simultaneously to optimize the required formulation. Optimized formulation were found to have a polymer-drug ratio of 18.89:1 and manufactured at a nonsolvent-solvent ratio of 4:1 to maximized release after 8 hrs and minimized particle size. The methodology avoids the use of organic solvent and thus provides a safe, reproducible and fast method of production of nanoparticles. The study collaborates on the feasibility and suitability of aqueous polymeric drug delivery system, employing statistical design to develop a clinically useful Nanoparticle system with targeting potential.

Keywords: Nanoparticles, NSAID, PLGA, pluronic F 68, optimization, factorial design.

INTRODUCTION

Nanoparticles with suitable size range have been found to concentrate at inflammation site due to increased fragility of blood vessels at those sites and increased bio-adhesiveness (Tripathi, 2003). The extravasations of drug bearing nanoparticles through endothelial fenestrations near inflammation sites has the potential to target drug delivery to the desired site and thus minimize unwanted side-effects of NSAIDs. However, a narrow size distribution is essential since capillary wall permeability is a function of particle size (Hite *et al*., 2001). A sustained rate of delivery would be an added advantage since it is known to improve biopharmaceutical efficacy of the delivery system. In the present investigation an attempt has been made to develop nanoparticles of flurbiprofen, a model NSAID of propionic acid series, using FDA approved bio-compatible poly lactic-co-glycolic acid (PLGA) polymer and characterize the release profile as well as particle size distribution, so that a predictable flurbiprofen loaded nanoparticulate system could be developed. The Pluronic F-68, the dispersing agent is also FDA approved surfactant, used in this preparation. The drug has been found to inhibit platelet aggregation (Illum *et al.*,1989). Orally it is more potent than indomethacin in inhibiting prostaglandin synthesis. It is a peripherally acting analgesic. NSAIDs are commonly responsible for the side effects like ulceration & gastric irritation. Flurbiprofen Nanoparticles, being colloidal drug delivery systems (Jorg, 1994), are expected to overcome these limitations. Further, the factorial design methodology is used in persuit of optimization of nanoparticles. Experimental designs are known to yield robust products at minimum time with conservations of valuable resources. Hence, a 3^2 factorial design was adopted in this study (Bolton, 1997). Therefore, the developed flurbiprofen loaded nanoparticulate system is expected to be clinically effective in better management of systemic and ocular inflammation with greater degree of safety and accuracy.

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MATERIALS AND METHODS

Materials

Flurbiprofen (Model drug) was obtained as gift sample from FDC, Mumbai and surfactant (Pluronic-F 68) and polymer (PLGA) were received as gift samples from Sun Pharmaceuticals Ltd., Baroda. Acetone and all other reagents were procured commercially and used as obtained.

Method

The nanoparticles were manufactured by solvent-diffusion nanoprecipitation methodology (Fessi *et al*., 1992). Briefly, the drug and the polymer were dissolved in acetone and this solution was poured in the water containing Pluronic F68, under mild agitation conditions. Stirring (2500rpm) was continued for about 1.5 hrs until acetone gets slowly but completely evaporated and rigidized nanoparticle suspension was formed. Literature gives indication for increasing the polymer concentration to get sustained release of the drug. But considering the very low water solubility of flurbiprofen and preformulation trial data, the polymer quantity was varied upto above 76.92% of the formulation. It was found that increase in polymer beyond this limit caused increase in particle size above nano-range (Rajaonarivony, *et al*. 1993). Different formulations were developed by varying drug polymer ratio from 1:15 to 1:20 and by changing nonsolvent-solvent (water-acetone) ratio from 2:1 to 4:1 (Table 1). The design choosen for the study was a 3^2 factorial design, taking nonsolvent-solvent ratio (NSSR) and polymer-drug ratio (PDR) as two factors at three levels. Process parameters such as time of evaporation and drying time were optimized during preformulation. The prepared nanoparticles were subjected to various physico chemical studies, which included study of particle size distribution, drug content analysis, drug-polymer compatibility study (IR spectroscopy) (Montgomery, 1996), surface topography (SEM), and in-vitro release (dialysis method) study. The experiments were carried out following 3^2 factorial designs. It was expected that properties of nanoparticle could be dependent on the polymer load as well as the volume of the acetone and water. Therefore an experimental design was employed to investigate the effect of these parameters on the quality and performance of the desired nanoparticle (Devissaguet, 1990).

Drug content analysis

Flurbiprofen in solution can be determined by spectrophotometric method directly in the U.V region at 243nm (λmax). Amount (mg) of drug present per ml of nanoparticle suspension was calculated by determining the amount of drug remaining in the residue plus amount of non-entrapped drug. The residue remained in the beaker was first mixed with 20 ml 0.2 M NaOH and shaken with a glass rod for 15 minutes and then filtered through cotton plug. The volume was then made upto 50 ml and amount of drug was determined spectro-photomerically at 243 nm wavelength. The amount of non-entrapped drug determined by dialysis membrane and this value was added to the residual value. The result obtained was taken as loss and the amount of drug present in the nanoparticle was calculated by deducting this value from initial amount of drug added.

Compatibility study (IR spectroscopy)

The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to infrared spectrophotometric study using a Buck Scientific Model 500 IR Spectrophotometer.

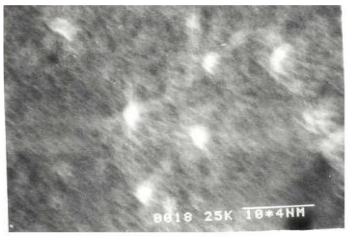
Surface topographic study

The surface topographical features of the nanoparticles

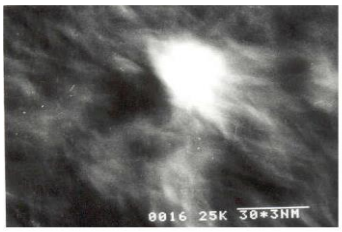
Table 1: Formulation design

Batch code	COMPONENTS						
	Polymer (%w/w)	Drug (%w/w)	Surfactant (%w/w)	Non solvent: Solvent Ratio (NSSR)	Drug loading (mg/ml)	Drug loading (mg/mg)	Drug entrapment efficiency (%)
PF 1	76.92	3.84	19.23	2.0	0.9607	0.04803	96.06
PF 2	70.17	3.63	27.27	3.0	0.9646	0.04823	96.46
PF 3	66.51	3.22	32.25	4.0	0.9626	0.04813	96.26
PF 4	76.46	6.25	21.27	2.0	0.9596	0.0548	95.96
PF 5	67.30	3.84	28.84	3.0	0.9618	0.05496	96.18
PF 6	61.40	3.50	35.08	4.0	0.9639	0.05508	96.39
PF 7	71.42	6.76	23.80	2.0	0.9297	0.06198	92.97
PF 8	63.82	6.25	31.91	3.0	0.9632	0.06421	96.32
PF 9	57.69	3.84	38.46	4.0	0.9712	0.06475	97.12

a. PDR 15:1(magnification: 750x)

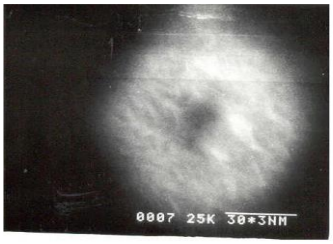


b. PDR 15:1 (magnification: 300x)

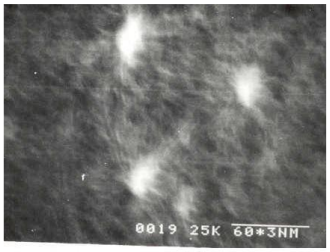


c. PDR 17.5:1 (magnification: 1000x)

d. PDR17.5:1 (magnification: 1000x)



e. PDR 20:1 (magnification: 1000x)



f. PDR 20:1(magnification: 500x)

Fig. 1: SEM photomicrographs of nanoparticles.

were studied using a Scanning Electron Microscope (SEM) (JEOL JSM 5200, Jeol, Japan) at 15 KV accelerating voltage, tilt angle of 0^0 and at various magnifications after coating the samples with a thin conducting gold film in a sputter coater.

Particle size determination

Particle size distribution study was carried out using a Malvern Mastersizer (Malvern Instruments, UK) by diluting the sample (5ml suspension) in purified water and then uniformly dispersing the nanoparticles by agitation and then size was determined by quasi-electric light scattering technique.

Free drug separation and release rate studies

In the dialysis method for separation of non-entrapped from entrapped drug, the nanoparticle was taken in a dialysis membrane (made of by cellulose acetate; Himedia) and suspended in a bulk of 100 ml phosphate buffer medium of pH 7.4. The dialysis membrane was permeable to the drug but not to the nanoparticles. The cut off size of dialysis membrane was 12kD. The non-entrapped drug was separated from the nanoparticles and diffuses through the membrane, which was assayed in the receptor buffer medium (Gasco, 2001).

Dialysis method is also useful for determining the release profile of the particles over prolong period of time. For determining release profile, 2ml sample was taken in the dialysis membrane and kept suspended in 500 ml phosphate buffer (pH 7.4) under mild agitating condition. The release drug diffused out in the buffer was assayed by spectrophotometric method at 243 nm, against appropriate blank.

Stability testing

Stability testing of the nanoparticles was carried out by keeping them at 40ºC and at 4ºC for six months. Then the samples were analyzed for particle size and drug content by Malvern Mastersizer (Cavalli *et al*., 2002).

RESULTS AND DISCUSSION

After the pre-formulation trials for suitable nanoparticle preparation, it was found that the desired delivery system could be successfully manufactured employing solvent displacement or nanoparticle precipitation method. The FDA approved biodegradable polymer PLGA was used as the matrix polymer in which the drug was dispersed during manufacturing (Nagai, 1993).

Drug content

The drug content analysis indicated high flurbiprofen loading 100% presumably due to low aqueous solubility of the drug (table 1).

Compatibility and surface topographic study

From the I.R study no potential drug-polymer interaction was found (data not shown). SEM study indicated nanoparticles to be spherical and non-porous in nature. No obvious aggregations of the nanoparticles were found.

Particle size determination

Aliquot samples of the nanoparticle suspension in which nanoparticle were uniformly dispersed was subjected to particle size analysis using Malvern Mastersizer. It was found that the freshly prepared formulations have a size range distribution of 0.31µ to 10µ with 50% particles lying between 0.38µ to 0.91µ and 62.39 (PF1) to 100%

Table 2: Particle size distribution at normal condition

Particle size (µ)	Cumulative % undersize								
Batch Code →	PF 1	PF 2	PF 3	PF 4	PF 5	PF 6	PF 7	PF 8	PF 9
0.31	3.66	6.21	8.44	5.05	21.69	9.32	7.23	9.66	6.77
0.36	10.33	9.55	13.11	14.56	34.22	29.61	15.44	18.76	23.31
0.42	18.69	16.34	17.69	26.68	45.11	54.35	28.66	24.55	46.08
0.49	24.56	19.63	31.22	39.08	50.23	76.01	30.11	29.33	68.72
0.58	31.05	29.88	43.26	49.62	56.28	90.29	36.22	35.62	85.78
0.67	38.95	45.38	50.36	56.97	70.23	97.23	45.69	46.33	95.33
0.78	46.32	52.55	56.66	60.95	78.65	99.53	48.5	65.33	99.04
0.91	50.88	60.33	61.39	72.42	86.33	99.97	56.33	72.11	99.91
1.06	62.39	64.96	67.33	75.62	92.33	100	68.77	79.33	100.00

Table 3: Particle size distribution after six months storage (4°C/40°C)

Particle size (µ)	Cumulative % undersize (4°C/40°C)				
	PF 2	PF 4	PF 5	PF 6	PF 8
0.31	9.89 / 5.69	12.33 / 6.88	12.47 / 9.01	10.23/ 10.29	13.36 / 15.46
0.36	15.46 / 12.36	16.97 / 7.49	18.7 / 12.36	19.89/ 15.94	14.56 / 17.41
0.42	18.69 / 17.85	18.46 / 10.47	20.13 /14.72	25.45/ 18.4	22.31 / 19.37
0.49	23.11 / 24.12	25.99 / 12.43	26.88 / 19.48	31.24/ 20.13	25.36 / 22.13
0.58	24.53 / 27.33	28.63 / 24.13	39.56 / 23.44	40.02/ 27.39	31.26 / 24.93
0.67	31.27 / 32.46	36.87 / 25.45	43.56 / 31.26	46.59/ 35.49	36.78 / 32.11
0.78	39.26 / 39.87	42.37 / 36.45	54.36 / 39.25	50.23/ 36.16	41.66 / 38.9
0.91	48.76 / 40.23	54.39 / 39.55	61.47 / 44.33	58.39/ 47.69	47.23 / 40.12
1.06	51.33 / 41.79	58.99 / 52.14	65.89 / 50.19	67.56/ 52.36	53.23 / 44.39

Table 4: Design layout

Std	Factor 1	Factor 2	Response 1	Response 2	Response 3
	A:PDR	B:NSSR	CR8%	d micron	DL mg/ml
PF 1	15.00	2.00	34.56	0.82	0.06198
PF 2	17.50	2.00	33.18	0.59	0.0548
PF 3	20.00	2.00	34.69	0.95	0.048035
PF 4	15.00	3.00	49.5	0.7	0.06421
PF 5	17.50	3.00	39.97	0.49	0.05496
PF 6	20.00	3.00	39.92	0.76	0.04823
PF 7	15.00	4.00	80.76	0.45	0.06475
PF 8	17.50	4.00	45.55	0.42	0.05508
PF 9	20.00	4.00	36.13	0.62	0.04813

Table 5: Response Parameters Models and their ANOVA

Response	Polynomial Model	n	F	P>F	R^2 adj
CR8	$1.0/ (CR8) = +0.094904 - 3.28550\times10^{-3}$ *PDR – 0.031271*NSSR + 1.54039×10^{-3} *PDR*NSSR.	9	26.92	0.0016	0.9067
d	d = +11.13167 – 1.18933*PDR – 0.14500*NSSR + 0.034667* PDR^2	9	27.62	0.0015	0.9089
DL	$DL = +0.10988 - 3.10300\times10^{-3}$ *PDR	9	411.14	< 0.0001	0.9809

(PF6 and PF9) particles lying within 1µ size range (table 2).

Release rate studies

The *in vitro* dissolution study of the nanoparticle was found to follow predominantly Higuchi squre root kinetics implying diffusion dependent release as is expected due to the insoluble non-swellable nature of PLGA (Nagai *et al.*, 1993; Yang *et al.*, 1999). Hence these observations also indicate indirectly that the nanoparticle formed were matrix in nature in which flurbiprofen, the model drug was dispersed uniformly. The release profiles are shown in fig. 2. The release of the formulations ranged from around 25 – 40% after 8 hours

except for the sample PF9, which shows a marked increase in drug release profile. This may be explained based on its composition (Polymer: drug : surfactant = 57.69 : 3.84 : 38.46% w/w) that shows relatively less polymer and highest surfactant composition in the formula. The reduced release retarding effect of lower polymer concentration and higher leaching/solubilizing effect due to high surfactant concentration may be responsible for faster and higher release of drug from PF9.

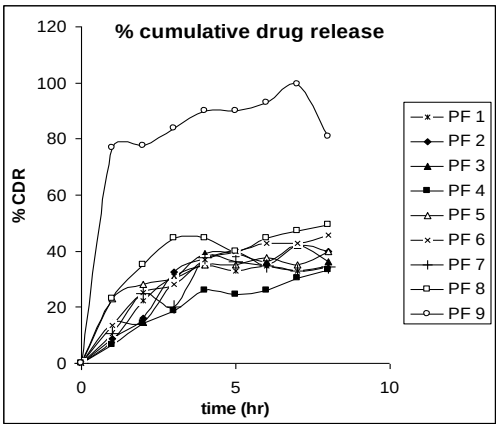


Fig. 2: Release Profile of the drug from nanoparticles.

Stability testing

Particle size distribution after stability study at 4^0C was changed and was shifted to 0.31µ to 100µ with 50% particles lying between 0.68µ to1.89µ and 51.33% (PF2) to 67.56% (PF6) particles lying within 1µ size range for freezing. For elevated temperature (40^0C), 50% particles were lying between 0.95µ to 1.06µ and 41.79 (PF2) % to 52% (PF4, PF7) particles lying between 1µ range (Table 3). However judging the total period of storage and temperature of storage, it may be concluded that with the existing system of nanoparticle in suspension form, the variation of particle size was within the tolerable limit. No appreciable change in drug content was observed after storage (data not shown).

Optimization of the nanoparticles

Having prepared the nanoparticle and standardized the system, three response parameters were chosen to optimize the properties of nanoparticle. Two parameters, namely, polymer-drug ratio PDR (15:1 to 20:1) and nonsolvent-solvent ratio NSSR (2:1 to 4:1) were selected for optimization. The amount of drug and surfactant were kept constant. Only the polymer load was varied. Nonsolvent used was water, whose volume was changed keeping the acetone volume constant. So it was expected that the volume available for acetone solution and polymer to disperse, would affect the drug loading and particle size of nanoparticles (Muller *et al*., 2002). The investigated response parameters were cumulative release (CR8), particle size (d), and drug loading (DL) (Radtke *et al.*, 2000). The individual responses are shown in table 4. The experimental design followed was a 3^2 factorial design.

Suitable polynomial models were developed for each response parameters and validated through ANOVA (table 5 and figs. 3 & 4).

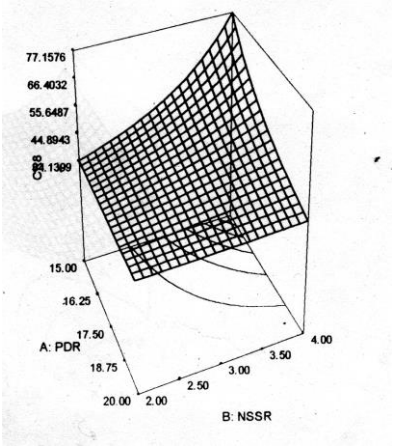


Fig. 3: Response surface for CR8

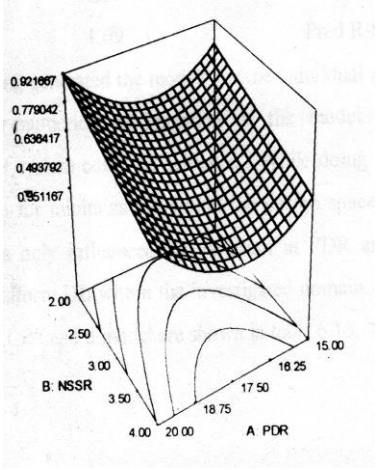


Fig. 4: Response surface for d

Since DL response was, relatively uniform and near maximum, it was left out the optimization process. It may be easily visualized from table 5 and figs. 3 & 4, that with increase in nonsolvent-solvent ratio and decrease in polymer-drug ratio, the drug release increased and hence, minimum release was obtained at high polymer loading and at low nonsolvent-solvent ratio. This may be explained considering the fact that higher polymer level decreases the drug diffusivity in the nanoparticle matrix, while low nonsolvent-solvent ratio yield particles of bigger size having smaller dissolution surface area. However, the minimum particle size was obtained with medium level of PDR and highest level of NSSR – the former being at least 10 times more influential than NSSR in determining mean particle size (d equation; table 4). This may be due to the higher solvent diffusion rate achieved at those levels of the parameters that quickens the loss of solvent from particles leading to finer precipitation, and hence smaller size.

Table 6: Average error in prediction

Replicates	Cumulative % release at 8 hr (CR8)	Average % error for CR8	Particle size of 50% particles (d)	Average % error for d
F 1	42.56	9.96	0.48	6.67
F 2	41.33		0.50	
F 3	42.27		0.46	
Predicted	41.3924	-	0.455787	-

Hence, to prolong release characteristics and minimize size, CR8 and d response models were numerically optimized (Jenning et al., 2000). Optimum formulation was found to have a polymer-drug ratio of 18.89:1 and manufactured at a nonsolvent-solvent ratio of 4:1 for maximum drug release after 8 hrs and minimum particle size attainable with the followed method.

The optimum formulation was prepared in triplicate and was found to agree reasonably well to the predicted values of the response parameters (table 6).

Therefore, through experimental design methodology, a complex nanoparticulate system could be optimized employing a minimum of formulations.

CONCLUSIONS

This methodology provides a safe, reproducible and fast method of production of nanoparticles, whose characteristics could be predictably modified with the help of experimental design to control process and formulation parameters. The study elaborates on the feasibility and suitability of aqueous polymeric colloidal drug delivery system, employing statistical design to develop a clinically useful nanoparticle system with targeting potential.

An added advantage of this methodology is its cost effectiveness since very few batches are required to arrive at the optimized formulation, keeping in view the cost of the drugs and polymers used in nanoparticle formulation.

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REFERENCES

Bolton S (1997). Factorial designs, in 'Pharmaceutical statistics: practical and clinical application. 3rd Ed, Marcel Dekker, New York, pp.326-351.

Cavalli R, Gasco MR, Chetoni P, Buralassi S and Saettone MF (2002). Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. *Int. J. Pharm*., **238**: 241-245.

Devissaguet JP (1990). Nanocapsules as carrier for oral peptide delivery. *J. Control Rel.,* **13**: 133-136.

Fessi H and Devissaguet F (1992). Nanocapsules preparation by interfacial polymer deposition following solvent displacement. *Int. J. Pharm.,* **55**: R1.

Gasco MR (2001). Solid lipid nanoparticles for drug delivery. *Pharm. Tech. Eur*., **32**: 137-141.

Hite GH and Foye WO (2001). Analgesic. *In*: principles of Medicinal Chemistry. 5th Ed. Lippincott Wiliams & Wilkins, Boston, p.771.

Illum L and Davis SS (1989). Targeting of Microsphere to sites of inflammation. *Int. J. Pharm*., **52**: 221-225.

Jenning V, Thunemann AF and Gohla SH (2000). Characterization of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. *Int. J. Pharm.,* **199**(2): 167-177.

Jorg Kreuter (1994). Nanoparticles: Colloidal drug delivery system. Marcel Dekker, New York, p.25.

Montgomery DC (1996). Design and analysis of experiments, 4th ed., John Wiley and Sons, New York, p.145.

Muller RH, Radtke M and Wissing SA (2002). Nanostructured lipid matrices for improved micro encapsulation of drugs. *Int J Pharm.*; **242**: 121.

Nagai T (1993). Encapsulation of hydrophilic and lipophilic drug in PLGA nanoparticles by the nanoprecipitation method. *Drug Develop. Ind. Pharm.,* **25**(4): 471.

Radtke M and Muller RH (2000). Comparison of structural properties of solid lipid nanoparticles (SLN) versus others lipid particles, *Proc. Int. Symp. Control Rel. Bioact. Mater*., **27**: 309.

Rajaonarivony M, Vauthier C, Couarraze G, Puisieux F and Couvreur P (1993). Development of a new drug carrier made from alginate. *J. Pharm. Sci.,* **82**(9): 912-917.

Tripathi KD (2003). Essentials of Medical Pharmacology. 5th Ed, Jaypee Brothers, New Delhi, p.177.

Yang S, Zhu J, Lu Y, Liang B and Yang C (1999). Body distribution of camptothecin solid lipid nanoparticles after oral administration. *Pharm. Res*., **16**(5): 751.

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