INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

DEVELOPMENT AND EVALUATION OF NANOCARRIERS OF AN ANTICANCER DRUG FOR THE TREATMENT OF BREAST CANCER

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Article Received on: 13/02/19 Approved for publication: 04/04/19

DOI: 10.7897/2230-8407.1005173

ABSTRACT

The current goal of the research work was to develop and evaluate Docetaxel nanoparticles as potential carrier system to facilitate targeted delivery to tumour cells. Central composite design was used to optimize Docetaxel loaded Pluronic F68 nanoparticles. Drug loaded Pluronic F68 nanoparticles were prepared by anti solvent precipitation method and was characterized for FTIR, DSC, Entrapment efficiency, Drug Content, % Yield, SEM and *in vitro* release studies. Docetaxel with Pluronic F68 showed no interaction based on the results from FTIR and DSC. Polymer concentration affected the entrapment efficiency, drug content and percentage yield. The more the polymer concentration the more was the yield, the more was the entrapment efficiency. The *in-vitro* release study was performed and at 12th hour the release ranged between 81.28-91.71 %. The average particle size was found to be 62.51 nm.

Keywords: Docetaxel, Nanoparticles, Anti solvent precipitation, Pluronic F68.

INTRODUCTION

Nanotechnology involves use and manipulation of materials with a size in the range of 10-1000 nm. Studies of nanotechnology and nanoscience have emerged rapidly and providing opportunities for material development, including medical applications, where conventional technologies can reach their limits[1]. By Nanotechnology, we can obtain better therapeutic work, better bioavailability, and better patient compliance. Several nanomaterials like polymeric nanoparticle, solid lipid nanoparticles, liposomes, dendrimers, Nano emulsions, Nano suspension and ligand mediated Nano-systems are successfully used to deliver drug to the active site[2]. Docetaxel is a BCS class - IV drug with poor solubility as well as poor permeability. Docetaxel is effective against breast, ovarian, prostate and non-small cell lung cancer[3]. Nearly 40 % of new drugs have low solubility in water, resulting in reduced oral bioavailability; hence, the urge to develop new treatments for cancer which are effective and affordable. The novel formulations showed more advantages over conventional anti-cancer drugs, including increased solubility, bioavailability, protection against toxicity, enhanced pharmacological activity, improved stability, improved cell distribution, sustained delivery and protection from physical and chemical degradation[4]. Breast cancer is the second major mortality cause in women, as it is a very diverse ailment with several indicators associated to typical features of tissues, therapeutic evaluation and reaction to therapy[5], it is commonly presents as a lump in the breast, the use of screening has also allowed early cancers to be diagnosed before they can be detected clinically[6].

MATERIALS AND METHODS

Docetaxel trihydrate pure drug was a gift sample from INM Technologies bangalore-560059. Pluronic F68 was purchased from Yarrow Chem Product Mumbai-400037 (India). Acetone and acetonitrile were purchased from Loba Chemie Pvt. Ltd. and Merck respectively. Ethanol was purchased from Changshu Hongsheng Fine Chemical Co, Ltd.

Drug-Excipient Compatibility Studies

FTIR Spectroscopy

To identify any possible interactions, the physical mixtures of the drug , the polymers and the drug with polymer was analysed using the Fourier transformed infrared (FTIR) spectroscopy. The samples were scanned in the range of 400 to 4000 cm^{-1}. The shifts in the spectra of the drug in the presence of polymers were investigated to determine physical interactions between the drug and the polymers. (FTIR Module : αE (ATR Module) BRUKER[7].

Differential scanning calorimetry (DSC)

Differential scanning calorimetry is a thermo-analytical strategy whereby the distinction in the amount of heat required increasing the temperature of a sample and reference is estimated as an element of temperature. Five milligrams of samples were fixed in the aluminium pan and explored by differential scanning calorimeter at a temperature heating rate of 10°C/min that from 35 to 350 ºC with nitrogen at a flow rate of 100 mL/min and pressure of 40 bar. The DSC was calibrated with indium, with an unfilled pan utilized as reference.[8]

Estimation of Docetaxel

Standard Graph of Docetaxel

Determination of λmax

λmax was determined by scanning 10 µg/ml solution of docetaxel against acetonitrile as a reagent blank in spectrum mode between 200-400 nm.

Preparation of stock solution

Primary stock solution of docetaxel in acetonitrile (1000 µg/ml) was prepared by dissolving 25 mg docetaxel in 25 ml acetonitrile. Then 1 ml of the above mixture was diluted to 10 ml using acetonitrile to get 100 µg/ml which is referred to as the stock solution.

Preparation of calibration curve in acetonitrile

From the stock solution containing 100 µg/ml docetaxel is transferred to 10 ml volumetric flask and then diluted to 10 ml using acetonitrile. The absorbance of all the prepared solutions of 10, 15, 20, 25, 30, 35 and 40 µg/ml was then measured at 229 nm using UV Spectrophotometer (Model UV 1700 Shimadzu). The reading was recorded in triplicate and a graph of concentration verses absorbance was plotted.[9]

Design expert

By using Stat-Ease software, by implying the central composite design we took two variables that is the Polymer concentration and the Speed while taking 5 responses which included 1 h release, 4 h release, 8 h release, 12 h release, Entrapment Efficiency and % Yield. The levels are given in Table 1.

Preparation of nanoparticles by Anti solvent Precipitation

A 30 mg/mL solution of Docetaxel with 15-30 mg/mL Pluronic F-68 in acetone (organic solution) was prepared at room temperature. An aqueous solution of Pluronic F-68 from 15 to 30 mg/mL was pre cooled in a temperature controlled water bath at 3°C. To precipitate the drug, the organic solution at room temperature was introduced into the cooled aqueous solution to achieve the desired suspension. Homogenize for 20 minutes then using a Magnetic stirring at approximately 500 rpm was utilized to enhance heat and mass transfer during mixing. As given in Table 2.[10]

Encapsulation efficiency

The percentage encapsulation of DTX in Pluronic F68 nanoparticles was determined by separating the un-entrapped drug from the nanoparticles by taking about 50 mg of Docetaxel tri-hydrate equivalent suspension nanoparticles was dissolved in 50 mL of acetonitrile then centrifugation at 14,000 for 20 minutes. The clear supernatant was analysed for the contents of DTX by measuring absorbance in a UV-Visible spectrophotometer 229 nm (Model UV 1700 Shimadzu).[11] The percentage encapsulation efficiency was calculated as follows:

$$\%\ \text{Entrapment Efficiency} = \frac{\text{amount of drug entrapped in nanoparticles}}{\text{total amount of drug in nanoparticle}} \text{X100}$$

Drug content

Docetaxel trihydrate content was estimated by taking a known volume of the nanosuspension formulation and then ultra-centrifuged at 25000; the amount of drug in the supernatant was estimated by U.V at 229 nm (Model UV 1700 Shimadzu).[12] The formula used to calculate drug content is given below:

$$\%\text{Drug content} = \frac{\text{weight of the drug in nanoparticles}}{\text{total weight of drug in nanoparticle}} \text{X100}$$

Percentage yield

Percentage practical yield of docetaxel trihydrate was determined to find about percentage yield. Practical yield was determined as the weight of docetaxel trihydrate recouped from each batch in relation to the total of beginning material.[13] The percentage yield of docetaxel trihydrate prepared was determined by utilizing the formula.

$$\%\ \text{Yield} = \frac{\text{Actual weight of product}}{\text{total weight of drug and polymer}} \text{X100}$$

***In vitro* drug release**

In vitro release profiles of Docetaxel loaded pluronic F68 was studied using USP Apparatus I at 37 ± 0.5°C with a rotating speed of 100 rpm in phosphate buffer 6.8 as the dissolution media. During the study, 10 ml of aliquots were removed at predetermined time intervals (1-12 h) from the dissolution medium and replaced with fresh media. The amount of Docetaxel released in the dissolution medium was determined by UV-visible spectrophotometer at 229 nm (Model UV 1700 Shimadzu).[14]

Particle size analysis

The particle size analysis was performed using Malvern Master sizer. The average particle size and size distribution of optimized nanosuspension was recorded.[15]

Scanning Electron Microscopy (SEM)

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of the prepared nanoparticles. Docetaxel nanoparticles were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of Docetaxel nanoparticles were taken by random scanning of the stub.[13]

RESULTS AND DISCUSSION

FTIR

The FTIR of Pure Docetaxel and the physical mixture (1:1) of Drug and Pluronic F68 given in Figures 1 and 2 respectively. The IR spectra of the Pure Docetaxel shows peaks at 3368.20 cm^{-1} (N-H stretch), 1737.29 cm^{-1} (C=O stretch), 1496.71 cm^{-1} (C-C stretch in Ring), 1455.34 cm^{-1} (C-H bend) and 1349.29 cm^{-1} (C-H rock). On the other hand it shows peaks at 3373.44 cm^{-1}, 1737.19 cm^{-1}, 1496.35 cm^{-1}, 1453.99 cm^{-1} and 1341.43 cm^{-1}. Hence this concludes that the physical mixture of Docetaxel does not show any major interaction with PluronicF68. As shown in Table 3.

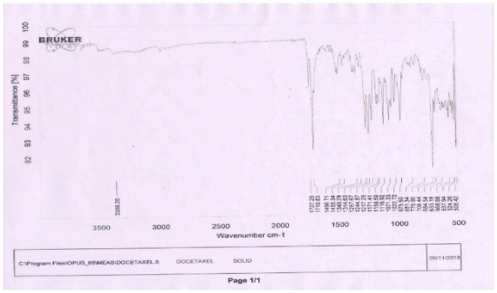

Figure 1: FTIR of pure Docetaxel drug

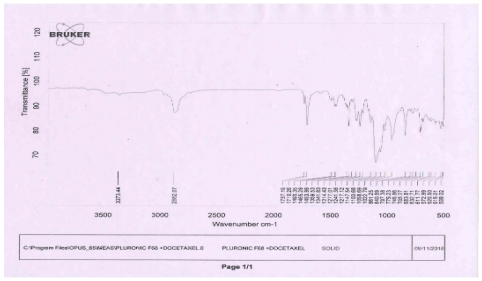

Figure 2: FTIR of pure Docetaxel drug and Pluronic F68

DSC

Dsc thermogram of Docetaxel exhibits a sharp endothermic peak at 199.68ºC corresponding to its melting temperature. Thermogram of physical mixture of Drug and Polymer showed endothermic peaks at 211.04ºC and 55.20 ºC respectively. The DSC analysis clearly indicates that there was No interaction between the Docetaxel and Pluronic F68. That is the fact why Pluronic F68 was selected in the preparation of the nanoparticles. The thermograph of Docetaxel and mixture of Docetaxel and Pluronic F68 is given in Figure 3 and 4 respectively.

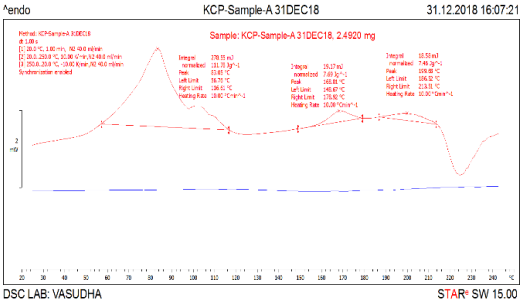

Figure 3: DSC of pure Docetaxel drug

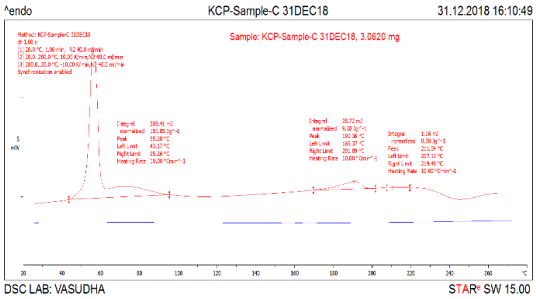

Figure 4: DSC of pure Docetaxel drug and Pluronic F68

Table 1: Variables taken along with the Levels

Variable factors	Level				
	-1.14	-1	0	1	1.14
Polymer concentration (mg/ml)	11.89	15.0	22.5	30.0	33.1
Speed (rpm)	198	250	375	500	551

Table 2: Formulation design of Docetaxel Nanoparticle

Ingredient	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (mg/ml)	30	30	30	30	30	30	30	30	30
Pluronic F68 (mg/ml)	15	30	15	30	11.89	33.1	22.5	22.5	22.5
Acetone (ml)	20	20	20	20	20	20	20	20	20
Water (ml)	10	10	10	10	10	10	10	10	10

Table 3: FTIR Interpretation of Docetaxel and Docetaxel With Pluronic F68

S. No.	Group	Range	Docetaxel	Docetaxel and Pluronic F68
1	N-H stretch	3500-3300	3368.20	3373.44
2	C=O stretch	1760-1665	1737.29	1737.19
3	C-C stretch (in ring)	1600-1585	1496.71	1496.35
4	C-H bend	1470-1450	1455.34	1453.99
5	C-H rock	1370-1350	1349.29	1341.43

Table-4: Spectrophotometric data for construction of standard graph Docetaxel

Concentration (µg/mL)	Absorbance (nm)
0	0
10	0.167 ± 0.001
15	0.255 ± 0.001
20	0.342 ± 0.002
25	0.424 ± 0.002
30	0.508 ±0.001
35	0.596 ± 0.001
40	0.68 ± 0.002

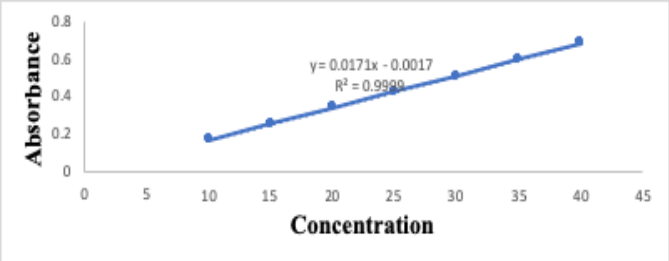

Figure 5: Standard Curve of Docetaxel

Table 5: Drug content, % Entrapment Efficiency and % Yield

Evaluation	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
% EE	70.76	87.13	72.51	85.38	69.59	90.64	76.60	80.70	78.94
% Drug Content	85.38	75.43	83.62	72.51	88.88	70.76	78.94	76.60	77.77
% Yield	69.59	80.70	68.42	83.62	65.49	87.13	78.94	76.60	75.43

Table 6: Release studies

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
	% CDR	% CDR	% CDR	% CDR	% CDR	% CDR	% CDR	% CDR	% CDR
0.5	19.88	15.98	19.88	16.37	21.83	14.91	17.05	16.96	17.25
1	24.65	22.12	24.26	22.51	27.48	21.15	24.65	24.27	23.68
2	30.89	29.04	29.92	29.24	31.87	26.12	32.65	29.92	32.65
3	37.13	32.45	35.86	33.33	38.4	30.6	37.13	35.87	37.13
4	42.2	37.52	40.25	38.69	44.15	35.67	42.59	40.25	41.42
5	48.53	43.08	47.56	44.54	49.9	41.22	49.9	47.56	49.9
6	55.45	49.31	52.34	49.7	55.84	48.05	55.84	52.34	55.85
7	61.5	55.65	60.52	55.26	63.45	55.65	62.57	60.14	62.57
8	66.76	60.33	67.83	60.72	70.95	63.74	67.74	68.52	68.42
9	76.9	66.96	73.29	67.74	77.77	69.98	73.88	72.61	73.88
10	81.77	70.46	79.82	72.02	82.75	75.83	78.46	76.12	78.46
11	85.28	77	85.96	77.78	89.57	78.75	82.36	80.7	82.36
12	88.01	82.75	88.98	83.23	91.71	81.28	87.03	84.51	85.38

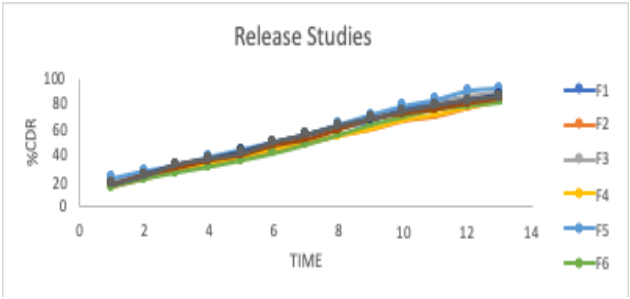

Figure 6: Release Studies

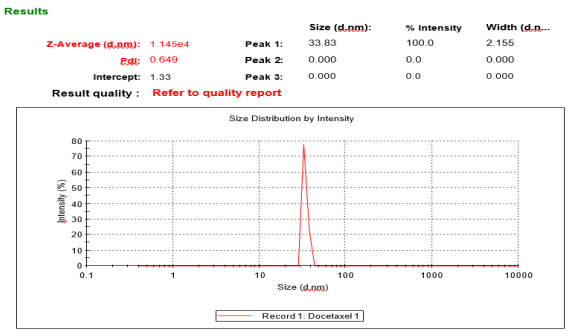

Figure 7: Average particle size of Docetaxel-Pluronic F68 nanoparticles

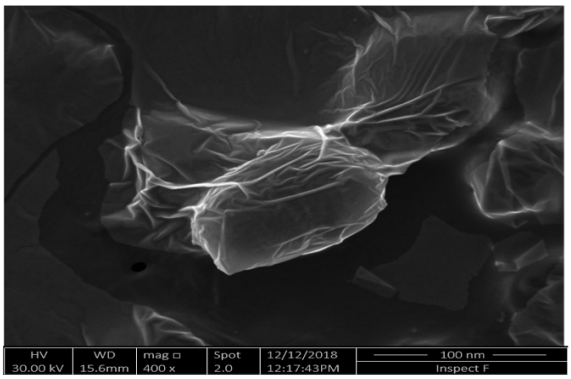

Figure 8: SEM for the prepared formulation

Estimation of Docetaxel

Determination of λ max

Based on the spectrophotometric scanning of Docetaxel (10 µg/ml), the maxima was obtained at 229 nm in acetonitrile, hence chosen as the analytical wavelength.

Calibration Curve of Docetaxel

Table 3 shows the mean absorbance values along with the standard deviation of Docetaxel in acetonitrile. The high correlation coefficient in the acetonitrile indicated that absorbance and concentration of the drug was linearly related. Beer's law was found to be obeyed in the range of 10 to 40 µg/ml in acetonitrile. As shown in Table 4

Entrapment Efficiency (EE), Drug Content (DC) and % Yield

The EE, DC and Yield for all the 9 prepared formulation ranged from 69.59-90.64 %, 75.43-88.88 % and 65.49-87.13 % respectively. The results are given in Table 5.

***In-vitro* Drug release**

The *in vitro* release studies of the 9 prepared formulations ranged between 81.28 %-91.71 % after 12 h of release. As shown in Table 6.

Scan Electron Microscope

SEM analysis of the prepared nanoparticles showed that the particles are partially spherical with a slightly rough surface.

CONCLUSION

Docetaxel-Pluronic F68 nanoparticles were formulated and it proved that the nanoparticles are a suitable option compared to the conventional dosage forms, as it improves the drug permeation, drug solubility, drug bioavailability as well it can be used to reach the targeted site of action in a sufficient dose. The Pluronic F68 was selected as a carrier due to its ability to retard the release rate of the drug hence controlling the release rate of the drug. Most of the BCS class-IV drugs can be formulated as nanoparticles. The nanoparticles can be used to target a specific site of interest. Among all the prepared formulations, F6 showed a better result concluding that the nanoparticles can be used to control the rate at which the drug is released from the polymer matrix. From the *in vitro* results, F6 showed the minimum drug release of 81.28 % at 12th hour.

ACKNOWLEDGEMENT

Our sincere thanks to Karnataka College of pharmacy for the support and would like to thank INM Technologies bangalore-560059 for giving the Docetaxel pure drug as a gift sample.

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Cite this article as:

Ahmed Mohammed Abdalla *et al*. Development and Evaluation of Nanocarriers of An Anticancer Drug for the treatment of Breast Cancer. Int. Res. J. Pharm. 2019; 10(5):106-111 http://dx.doi.org/10.7897/2230-8407.1005173

Source of support: Nil, Conflict of interest: None Declared