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Accelerated Stability Testing of Microcapsulated Sorafenib-loaded Carbon nanotubes Prepared by emulsification/internal gelation Method

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ABSTRACT:

This work aims to evaluate the effect of environmental factors, including temperature, humidity, and light, on the quality of a drug substance or a formulated product which is utilized for prediction of its shelf life, to determine proper storage conditions and to suggest labeling instructions. Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any new drug or formulation. In this work, stability studies were performed on Microcapsulated Sorafenib-loaded Carbon nanotubes (Microcapsulated SFN-loaded CNTs) which were developed for studying the effect of different storage conditions such as morphological shape, drug content and release profile of the drug from Microcapsulated SFN-loaded CNTs. Microcapsules were stored for three months at 30 ˚C and 40 ˚C in 75±5% Relative Humidity (RH) in thermostatically-controlled cabinet. The stored formulae showed accepted properties of morphological shape and drug content. Regarding the release profile of drug, the obtained results indicated that the storage of Microcapsulated SFN-loaded CNTs for three months at stress conditions has a minimum effect on the rate of drug release.

KEYWORDS:

Carbon nanotubes, Sorafenib, Accelerated stability study, Microcapsules**.**

INTRODUCTION:

The word "capsule" implies a core and shell structure, and the term "Microcapsules" are small particles that contain an active agent or core material surrounded by a coating or shell. Microcapsules according to the French Pharmacopoeia are solid material consisting of a solid envelope containing a liquid or solid or a pasty substance. Microcapsules occur in the form of powder with particles less than 1250 µm in diameter. Microcapsulation of pharmaceuticals was first investigated in the year 1931 by preparing spheres of gelatin using coacervation technique **[1].** Microcapsulation is a process by which solids, liquids or even gases may be enclosed in microscopic particles formation of thin coatings of wall material around the substances **[2].** Microcapsulation, in other definition, is a process or technique by which thin coating can be applied reproducibly to small particles of solids, droplets of liquids, or dispersions, thus forming microcapsules. It can be differentiated readily from other coating methods in size of the particles involved, these ranges from several tenths of a micron to 5000 in size **[3].**

Carbon Nanotubes (CNTs) have been investigated as an excellent candidate for drug delivery purpose due to their unique properties including high aspect ratio, high surface area, nanosized stability and facile functionalization by different ways **[4].** Recently various types of functionalized CNTs have been prepared for a diversity of applications ranging from delivery of drugs, proteins, peptides, nucleic acids (for gene transfer or gene silencing), to in vivo tumor imaging **[5].**

Sorafenib (SFN) is a multikinase inhibitor that has shown efficacy against a wide variety of tumors in preclinical models. It has been shown to block tumor cell proliferation and angiogenesis by inhibiting serine/threonine kinases (c-RAF, and mutant and wild-type BRAF) as well as the receptor tyrosine kinases vascular endothelial growth factor receptor 2 (VEGFR2), VEGFR3, platelet-derived growth factor receptor (PDGFR), FLT3, Ret, and c-KIT. It has also been reported that SFN induces apoptosis in human leukemia cells and other human tumor cell lines through the inhibition of the translation and down-regulation of myeloid cell leukemia-1 (Mcl-1), a Bcl-2 family member **[6].** It has been approved by the U.S. Food and Drug Administration for treatment of patients with advanced renal cell carcinoma, unresectable hepatocellular carcinoma, and differentiated thyroid carcinoma **[7].**

The quality of a drug product changes with time under the influence of environmental factors such as temperature, humidity and light **[8].** Stability of a pharmaceutical product may be defined as the ability of a particular formulation in a specific container/closure system to remain

within its physical, chemical, microbiological, toxicological, protective and informational specifications **[9].** Stability studies aimed to evaluate the effect of environmental factors (temperature, humidity, and light) on the quality of a drug substance or a formulated product which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labeling instructions. Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any drug or formulation **[10].**

In the current work, stability studies were performed on the SFN loaded CNTs prepared in microcapsules dosage form.

Microcapsulated SFN-loaded CNTs was stored for three months at room temperature (~25 ^{o}C) , 30 ^{o}C and 40 ^{o}C and RH 75% ± 5% in thermostatically-controlled cabinet **[11].**

The degradation reactions of Microcapsulated SFN-loaded CNTs at the investigated temperatures and conditions were studied. By applying Arrhenius equation and substituting the experimentally established specific rate constants at the three temperatures employed, the energy of activation and the decomposition reaction rate constant at room temperature were determined. The Microcapsulated SFN-loaded CNTs was stored in tightly closed light protected bottles wrapped using aluminum foil and tested for stability under the accelerated stability conditions. The order of the degradation reaction and the decomposition rate constants and the shelf-life (t_{90}) of the drug were determined **[12].**

MATERIALS AND METHODS:

Materials:

CNTs (number of walls: 3-15, outer diameter: 30-50 nm, length: 10-30 μm, purity of ≥95%) were purchased from Sisco Research Laboratories Pvt. Ltd., India. Sorafenib and polyethylene glycol 6000 g/mol (PEG-6000), sodium alginate (NaA) were purchased from Sigma Chemical Co., St. Louis, USA. High-quality water employed to prepare solutions was obtained by using a Milli-Q All other chemicals, reagents, and solvents were of analytical grade were obtained from standard suppliers, and were used without further purification.

Methods:

Preparation of Microcapsulated SFN-loaded CNTs:

Microcapsules were prepared by emulsification/internal gelation technique. A basal encapsulation protocol was used to prepare Microcapsules, as following **[13].**

Three grams of CNTs-SFN were dispersed in 100 ml NaA aqueous solution (2.5%) using a magnetic stirrer for 10 minutes. Using a 10 ml syringe, this drug alginate (1:2) dispersion was transferred drop-wise to a 50 ml $CaCl_2$ solution (0.4 M) with mild agitation within a period of 7 minutes at ambient temperature. The mixture was then stirred slowly for 6 minutes to cure the formulated Microcapsulated SFN-loaded CNTs which were subsequently dried under vacuum at 65 ˚C for 24 hours.

Characterization of the prepared Microcapsulated SFN-loaded CNTs:

Determination of drug content:

The drug content of the prepared Microcapsulated SFN-loaded CNTs determined by the following method **[14].**

One hundred mg of Microcapsulated SFN-loaded CNTs were crushed carefully in a glass mortar and transferred to a 100 ml volumetric flask using phosphate buffer pH 7.4. The volumetric flask was completed to the volume with phosphate buffer pH 7.4 then agitated for 5 minutes each hour for 5 hours. The sample was filtered and the drug concentration was determined spectrophotometerically at 265 nm. The same procedure was applied for the plain formula, which was used as a blank. The concentration was calculated using the standard calibration curve of SFN in phosphate buffer pH 7.4.

***In vitro* drug release study:**

In vitro release of SFN from the formulated Microcapsules, equivalent to 200 mg SFN, was performed at 37 ˚C according to a dissolution medium pH shift method with a paddle type dissolution test apparatus, SR II, 6 flasks (Hanson Research Co., USA) adjusted at 50 rpm as described in literatures. In brief, 500 ml of simulated gastric fluid (pH 1.2) was used as a release medium for two hours, followed by the addition of 5 ml of 7 M KH_2PO_4 containing 16.75% (w/v) NaOH in order to shift the medium pH to 7.4 and the experiment was continued for another six hours **[14].** Throughout the whole experimental time, a three ml aliquot was aspirated and

filtered every 15 minutes interval to measure the absorbance at the predetermined λ_{max} of each media against a corresponding blank **[15].**

Scanning electron microscope (SEM) imaging:

It has the advantage of providing a direct visual representation of the particles being measured. SEM can provide details about morphological shape and homogeneity of the tested sample. According to the microscopic method, diluted suspension of Microcapsulated SFN-loaded CNTs in liquid paraffin was mounted on a slide. Then a photograph for each Microcapsules was taken from the prepared slide at different magnification powers.

Stability study of the Microcapsulated formula:

Evaluations of the stability of the prepared Microcapsules were carried out after storage at room temperature (25 ˚C), 30 ˚C, and 40 ˚C for three months in a RH of 75±5% using a thermostatically-controlled cabinet.

The Microcapsulated SFN-loaded CNTs were tested for changes in the morphological shape, the drug content, and the amount of drug released within 6 hour in comparison to the corresponding properties of a freshly prepared microcapsules

The kinetic parameters were calculated in order to obtain the suitable kinetic order for Microcapsulated SFN-loaded CNTs stability. The expiry date (t_{90}) was estimated for the Microcapsulated SFN-loaded CNTs. In accelerated stability testing, a product is stressed at several high (warmer than ambient) temperatures and the amount of heat input required to cause product failure is determined. This is done to subject the product to a condition that accelerates degradation. This information is then projected to predict shelf life **[16].**

The kinetics of drug decomposition at elevated temperatures were studied by plotting some functions of remaining amounts of drug against time according to different models (zero and first-order). Using of Arrhenius equation and by substituting the experimentally established specific rate constants at the two elevated temperatures, the energy of activation can be determined as follow **[17].**

$$\log\left(\frac{K2}{k1}\right) = \left(\frac{Ea}{2.303R}\right)\left(\frac{1}{T2} - \frac{1}{T1}\right)$$

Where, K1 is the specific decomposition reaction rate constant at temperature T_1. K_2 is the specific reaction rate constant at temperature T_2. Ea is the energy of activation (Cal. /mole). R is the gas constant (1.987 cal. / mole. degree). T_1 is the absolute temperature in Kelvin (T_1 ^{o}C +273). T_2 is the absolute temperature in Kelvin (T_2 ˚C +273).

Activation energy (Ea) was determined from the slope and the Arrhenius factor (A) was determined from the intercept of the straight lines. By substituting the values of (Ea) and (A) in Arrhenius equation, the specific rate constant at room temperature (K_{25}) was calculated. The expiration date (t_{90}) was calculated by applying the kinetics equations using K_{25} value **[18].**

RESULTS AND DISCUSSION:

Preparation Microcapsulated SFN-loaded CNTs:

Microcapsulated SFN-loaded CNTs were prepared by the emulsification/internal gelation technique. This method is generally known to be simple, reproducible and economic **[13].** In this work, the evaporation process in oil phase (external phase) using liquid paraffin was employed. Since, solvents with dielectric constants between 10 and 40 showed poor compatibility with liquid paraffin and the systems of this solvent/liquid paraffin were reported to be applicable to micropelletization process. NaA was used as polymer in the concentration of 5%, which produce the best Microcapsulated SFN-loaded CNTs.

Characterization of Microcapsulated SFN-loaded CNTs:

Drug content determination:

In order to estimate the actual weight of SFN itself in the finally formulated drug-loaded Microcapsules, the drug content was calculated by measuring the deviation from the theoretical weight. The SFN contents in the prepared Microcapsulated SFN-loaded CNTs were found to be 191.2/200 g (95.6%).

***In vitro* drug release study:**

The in vitro release profile of SFN from the Microencapsulated formula was assessed according to a dissolution medium pH shift method. Under acidic conditions mimicking those in the stomach (pH 1.2); only small amount of the drug was released during the first 2 hours. This could

correspond to the drug molecules deposited on the surface of the Microcapsules. Once these drug molecules were consumed, the insoluble nature of the pH-dependent polymer, NaA prevented the drug release from Microcapsulated SFN-loaded CNTs. On the contrary, the pH shift towards the alkaline medium (pH 7.4) that resemble that of the intestine, apparently enhanced the drug release to be efficiently completed within 5 hours (Figure 1).

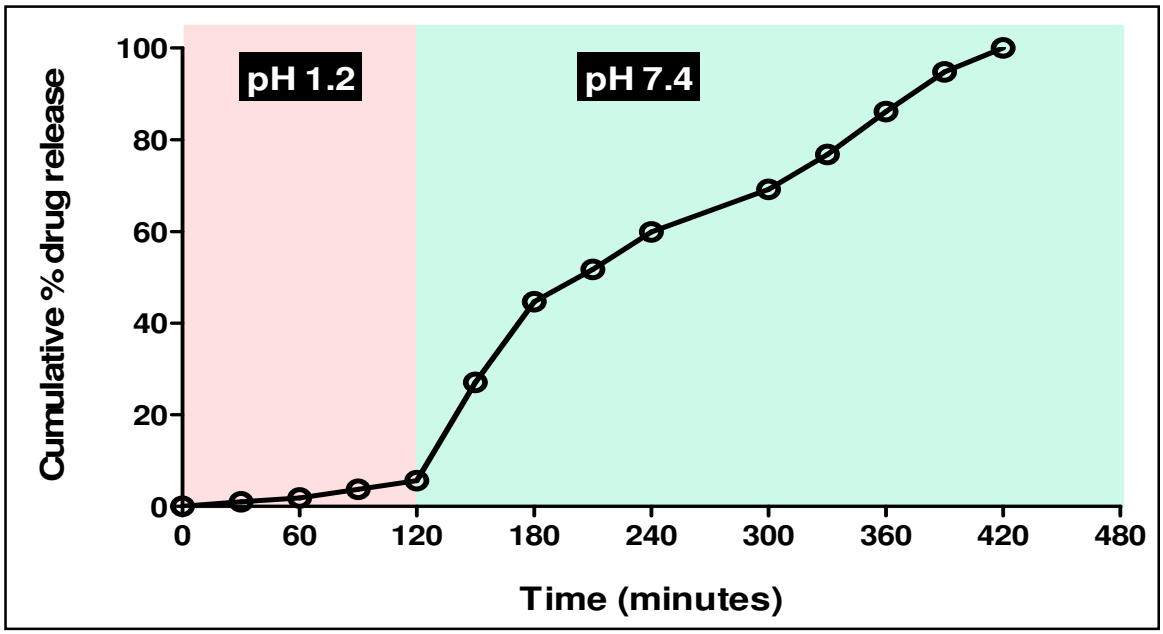

Figure 1: pH-dependant *in vitro* release profiles of SFN from the Microcapsules formula, pH 1.2 and in pH 7.4.

SEM imaging:

SEM technique was used to give a clear picture about the shape and the surface of the prepared Microcapsulated SFN-loaded CNTs. The obtained SEM images showed also that the finally prepared Microcapsulated SFN-loaded CNTs. Its appear spherical in shape and exhibit smooth surfaces with an obvious indication for the efficient drug loading in the case of the Microcapsulated SFN-loaded CNTs formulation (Figure 2).

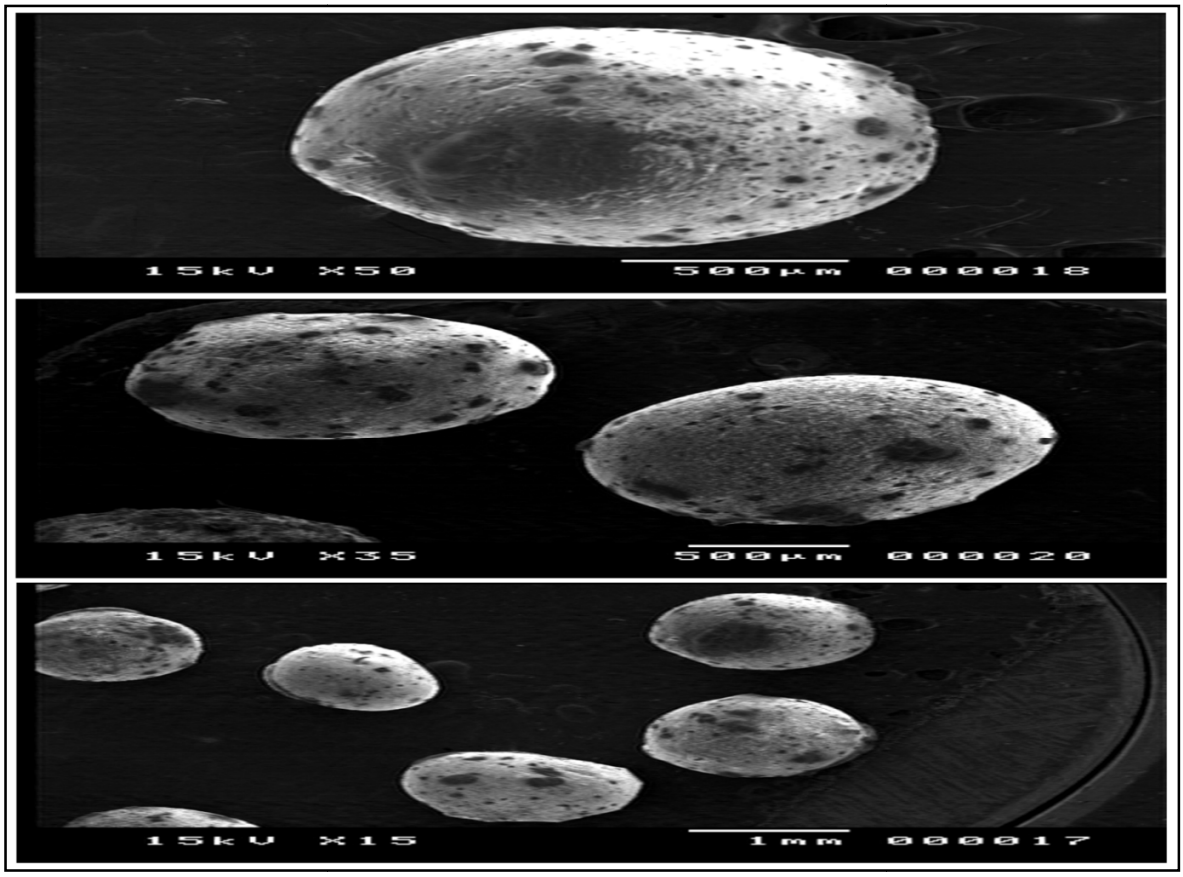

Figure 2: SEM image of Microcapsulated SFN-loaded CNTs at different magnification powers**.**

Assessment of the stability of the Microcapsulated SFN-loaded CNTs:

Estimating the shelf life of Microcapsulated SFN-loaded CNTs is a critical step in evaluating new formulations. The shelf life of a product may be defined as the time through which essential performance characteristics are maintained under specific handling conditions **[19].** After a three months storage period at 25 ˚C, 30 ˚C and 40 ˚C in a RH of 75±5%, no significant changes were observed in the morphological shape of the Microcapsulated SFN-loaded CNTs as detected by SEM analysis (Figure 3). As well, these storage conditions were unable to significantly affected the drug content of the Microcapsulated SFN-loaded CNTs as illustrated in Table 1.

Regarding the drug release profile, the obtained results indicated that storage of Microcapsulated SFN-loaded CNTs for three months at stress conditions induced a negligible decrease in the rate of the drug release from the stored Microencapsules as illustrated Table 1 and Figure 4.

Table 1: Effects of storage conditions on the drug content and rate of drug release from the stored Microcapsulated SFN-loaded CNTs.

	Fresh Microcapsules	Stored Microcapsules		
		25 ˚C	30 ˚C	40 ˚C
Drug content (%)	95.60 ± 1.74	94.85 ± 2.93	94.15 ± 1.15	93.42 ± 2.03
SFN released within 6 hour (%)	98.41 ± 1.58	97.80 ± 2.04	96.19 ± 3.01	95.99 ± 3.11

Data are presented as the mean ± SD (n = 3). SFN, Sorafenib

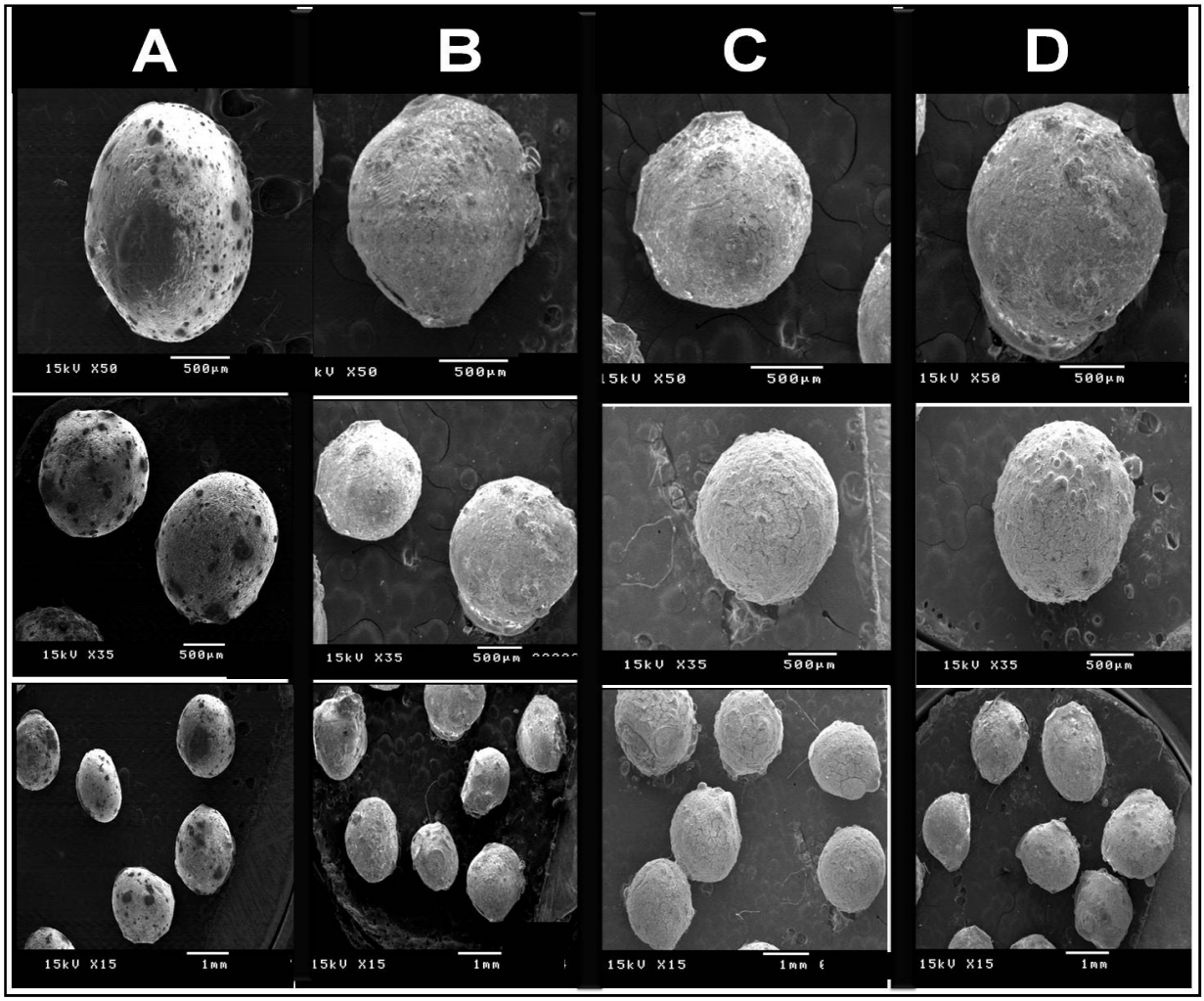

Figure 3: SEM images of the drug-loaded Microcapsules after a three months storage period at 25 ˚C (B), 30 ˚C (C), and 40 ˚C (D) in a RH of 75±5%, in comparison to freshly prepared Microcapsulated SFN-loaded CNTs (A) at different magnification powers.

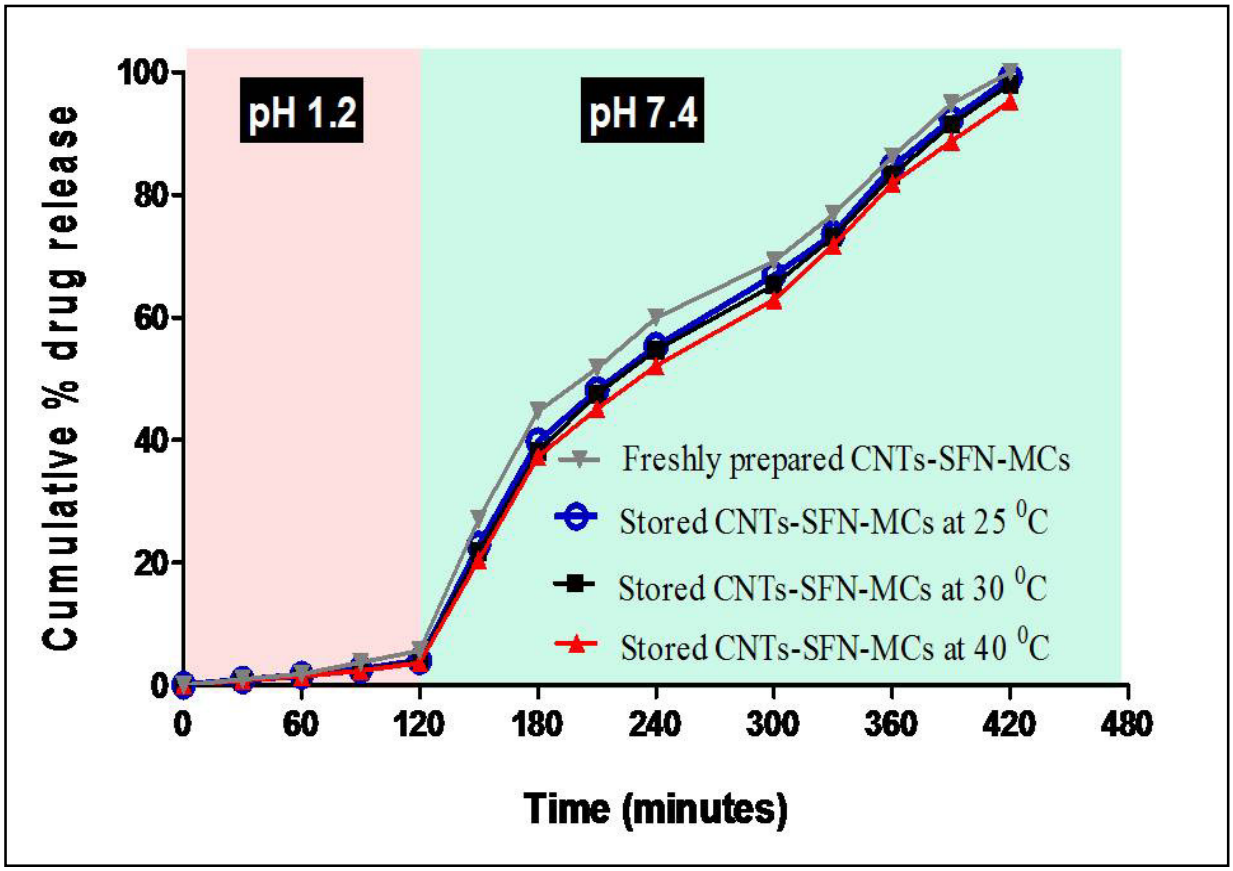

Figure 4: Release profile of Microcapsulated SFN-loaded CNTs after shelf-storage at 25 ˚C, 30 ˚C and 40 ^{o}C and RH 75 % ±5% for three months compared with for three months compared with corresponding freshly prepared Microcapsulated SFN-loaded CNTs**.**

Kinetic treatment of the accelerated stability tasting of Microcapsulated SFN-loaded CNTs at different storage conditions was represented in Tables 2-3 and graphically illustrated in Figure 5.

Table 2: Accelerated stability testing of Microcapsulated SFN-loaded CNTs stored at 30 ^{o}C and 40 ^{o}C and RH 75 % ±5%.

Time	**Percentage of SFN remained**	
	30 $^{\circ}$C+ 75%RH	**40 $^{\circ}$C+ 75%RH**
0	100	100
7	99.45	99.25
15	98.85	98.45
30	98.02	97.22
45	97.01	95.37
60	96.89	92.07
75	95.95	90.78
90	94.69	90.04

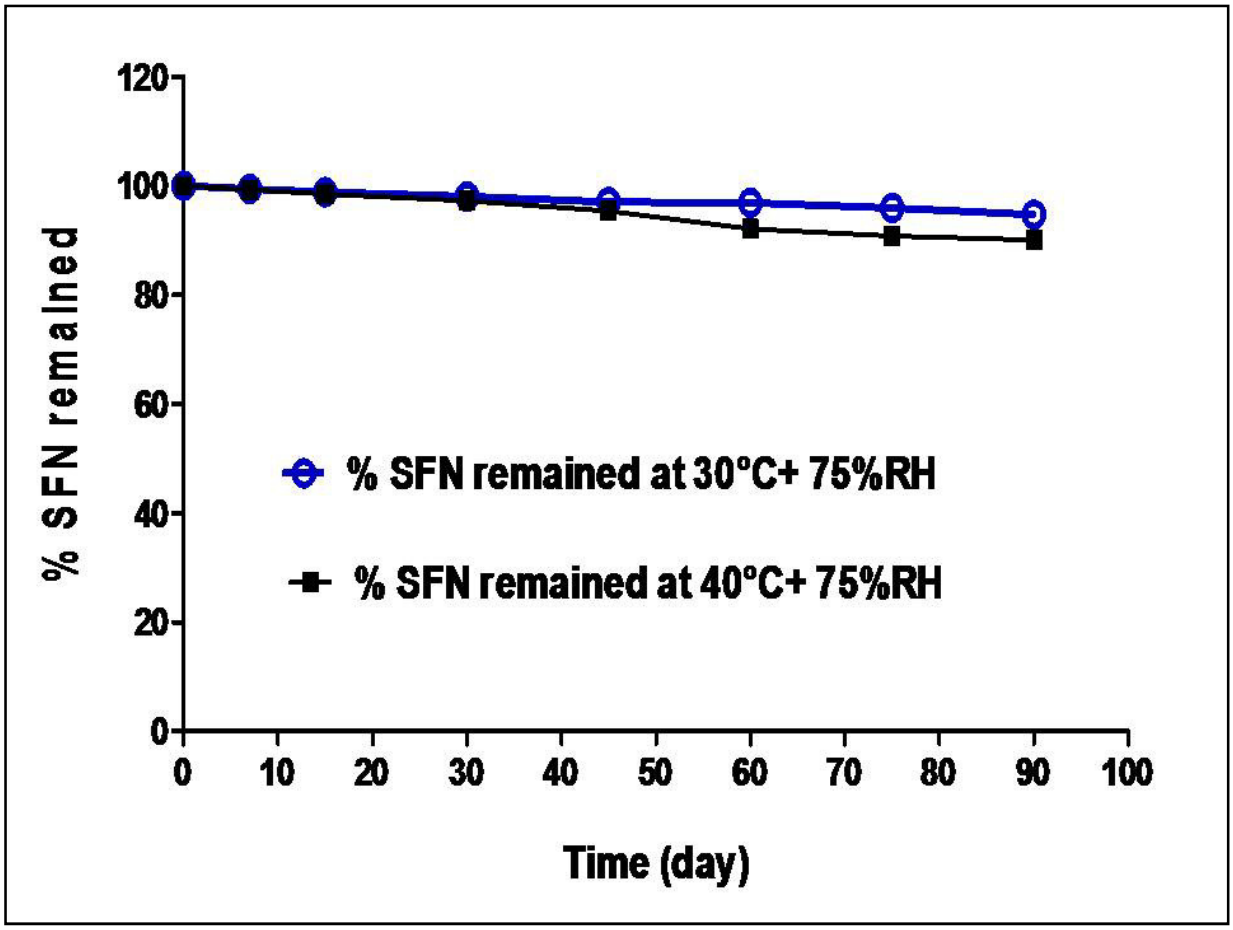

Figure 5: Accelerated stability testing of Microcapsulated SFN-loaded CNTs at 30 ˚C and 40 ˚C and RH 75 % ±5%.

Table 3: Accelerated stability testing of Microcapsulated SFN-loaded CNTs stored at 30 ^{o}C and RH 75% ± 5% and 40 ^{o}C and RH 75 % ±5% for three months according to zero order kinetics and first-order Kinetic**.**

	Zero order kinetics		**First-order Kinetic**	
	Percentage of SFN decomposed		**Log percentage of SFN remained**	
Time	**30 °C+75%RH**	**40 °C+75%RH**	**30 °C+75%RH**	**40 °C+75%RH**
7	0.55	0.75	1.9976	1.9967
15	1.15	1.55	1.9949	1.9932
30	1.98	2.78	1.9913	1.9877
45	2.99	4.63	1.9868	1.9794
60	3.11	7.93	1.9862	1.9641
75	4.05	9.32	1.9820	1.9575
90	5.31	9.96	1.9763	1.9544

Kinetic parameters (intercept, slope, r, k and $t_{1/2}$) for the accelerated stability testing of Microcapsulated SFN-loaded CNTs was represented in Table 4.

Table 4 Accelerated stability testing of Microcapsulated SFN-loaded CNTs stored at 30 ^{o}C and RH 75% ± 5% and 40 ^{o}C and RH 75 % ±5% for three months according to zero order kinetics and first-order Kinetic**.**

Storage conditions	**Intercept**		**Slope**		**r**		**K(hr^{-1})**		**$T_{1/2}$ (Days)**	
	Zero-order	First-order	Zero-order	First-order	Zero-order	First-order	Zero-order	First-order	Zero-order	First-order
30 ^{o}C	0.29528	1.99881	0.05302	0.00024	0.98886	0.98859	0.05302	0.00055	943.01	1269.31
40 ^{o}C	0.30764	2.00181	0.12134	0.00056	0.98692	0.98680	0.12134	0.00128	412.04	539.65

Determination of the shelf-life of the Microcapsulated SFN-loaded CNTs:

The shelf life is determined from the data obtained from the accelerated stability studies. The calculated t_{90} for accelerated stability testing was found to be 1.2865 years as represented in Table 5.

Table 5: Kinetic data for the accelerated stability testing of Microcapsulated SFN-loaded CNTs

(k_{30}) Day^{-1}	**(k_{40}) Day^{-1}**	**E_a Cal/mole**	**Calculated (k_{20}) Day^{-1}**	**$t_{1/2}$**	**(t_{90})**	**(t_{90}) (Year)**
0.0530	0.1216	15604.7	0.021894	2283.695	456.7391	1.2865

CONCLUSION:

The Microcapsulated SFN-loaded CNTs formulae were physically and chemically stable after storage for three months at room temperature with non-significant changes in comparison with the freshly prepared Microcapsulated SFN-loaded CNTs. Accelerated stability study showed that the differences in physical and chemical properties between the stored and freshly prepared Microcapsulated SFN-loaded CNTs were non-significant. The accelerated stability testing of Microcapsulated SFN-loaded CNTs at 30 ^{o}C and 40 ^{o}C and RH 75% obey USP specification. Shelf-lives (t_{90}) of the Microcapsulated SFN-loaded CNTs formulae were 1.2865 years.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interests.

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