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Research Article

Fabrication, characterization and *in-vitro* anticancer activity of sulforaphane magnetic nanoparticles using MDA-MB-231 cells

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One of the main causes of death for women is breast cancer. Surgery, radiation therapy, and chemotherapy are common forms of treatment, and they have a number of adverse effects. Sulforaphane (SFP) is a naturally occurring isothiocyanate that can be found in cauliflower, broccoli, and other vegetables. It exhibits potential anticancer activity against breast, pancreatic, bladder, hepatic, prostate, osteosarcoma, melanoma. In order to demonstrate its targeted medication delivery utilizing a magnetic field, magnetic nanoparticles are essential. This study's primary objective was to investigate how magnetic nanoparticles can improve bioavailability, stability, and dissolution. By encapsulating a herbal medication called SFP in iron salts, the magnetic nanoparticles were developed. They were then characterized using FTIR, XRD, SEM, TGA, drug loading efficiency, zeta potential, VSM, and stability studies. *In-vitro* dissolution and *in-vitro* anticancer activity were conducted to determine which formulation was the best among them using MDA-MB-231 cells. According to HRSEM data, the average particle size of MNPs 100–250 nm following loading with sulforaphane had a consistent spherical shape. At pH 6, the zeta potential value was determined to be 15 mv and -9 mv, respectively. According to *in-vitro* dissolution studies, pH has an impact on the amount of drug release. that all four varieties of magnetic nanoparticles exhibit adequate magnetic response for drug targeting in the presence of an external magnetic field, have a good size range, a high surface area, and a sufficient percentage of elements on their particle surface. SFP/MCM-41MNP (F2) shown more cytotoxicity on MDA-MB-231 cells than other positive control groups and pure SFP, according to the MTT-assay. The IC_{50} of F_2 was close to that of standard Doxorubicin. Hence it was considered as best formulation, useful for the effective management of breast cancer.

Keywords: *Sulforaphane, FTIR, Loading Efficiency, dissolution study, MDA-MB-231*

Introduction

Cancer is predicted to be the second leading cause of mortality worldwide in 2020, accounting for 9.9 million deaths, or one in six deaths. In males, the most often affected cancers were those of the liver, lungs, stomach, colon, and prostate. In case of women, the most commonly observed cancer types were breast, cervical, thyroid, colon and lung cancer. Breast

cancer is the most common type of cancer in women, affecting 85% of the mucosal epithelium of the milk ducts and 15% of the glandular tissue of the breast lobules. According to 2020 Global Statistics data, 34,65,951 women in India received a cancer diagnosis, and 11,21,413 of them lost their lives to the disease (Sathishkumar et al., 2022). Ailments can be identified, prevented and treated with natural herbal remedies in a variety of ways. When used, natural medicines are more compatible with all polymers used in formulations and preparations, have less side effects, and are less toxic. Even though having flexibility in all issue, as the same way it has limitation of poor bioavailability and stability issues when compared with modern system of medicine. The Sulforaphane has been chosen for this work. Brussels sprouts, cabbage, broccoli, cauliflower, and other cruciferous vegetables are natural sources of sulforaphane, a naturally occurring isothiocyanate. It has potential antineoplastic properties and used for the effective management of Hepatic Cancer, Bladder, Prostate, Pancreatic Cancers. It is also shows powerful anticancer activity in case of osteosarcoma & Melanoma. It shows instability in presence of oxygen, Heat, alkaline condition (Vanduchova et al., 2019).

Oral therapy considered as Primary choice for both Physicians and patients alike. Due its convenience and It improves patient's quality of life. The another reason for preferring this route by its economy; but sometimes it is restricted by various constraints. The constraints such as Absorption, Bioavailability. Due to protein binding, Metabolism (First pass effect) sometimes unable to obtain good clinical outcome. This lead to Patient Non-compliance. The magnetic nanoparticles are promising carriers for the efficient delivery of antineoplastic drugs because there is no burst impact and scaling up is easy. High encapsulation of both hydrophilic and lipophilic drug molecules resulted in significantly lower drug ejection during storage. By changing the lipid matrix, it is possible to modify the drug release profile and noticeably increase stability in the GIT at various pH levels (Prijic & Sersa, 2011; Huang et al., 2016). The Delivery systems containing particles in Nano size range (<200 nm) exhibits wide range of benefits for better dissolution, absorption, bioavailability and good clinical outcome. This proven in many of chemotherapeutic agents as they produced excellent therapeutic efficacy in animal studies (Yusuf et al., 2023). Using a magnetic field, magnetic nanoparticles are essential for exposing their centered drug delivery. Due to their attractive properties and potential wide range of biomedical uses, magnetic nanoparticles have recently been the focus of numerous investigations. Those includes environmental cleanup, MTCPC, data storage, MRI, material imaging, nanofluids, optical filters, disease detection, magnetic cooling, cation sensors, tissue-specific targeting, and catalysis (Liu et al., 2019; Stueber et al., 2021; Mittal et al., 2022).

Materials and methods

Sulforaphane (SFP) powder in its pure form was a complimentary gift from Vanco Herbals in Solan, Himachal Pradesh, India. Aman Scientifics provided the additional chemicals, which included Pluronic P123, Tetra Ethyl Ortho Silicate (TEOS), Conc. HCl, Ethyl Alcohol, Hexane, and Butanol. Cros Chemicals Pvt Ltd provided the iron, acetyl acetone, cetyltrimethyl ammonium bromide (CTAB), ammonium hydroxide, and nitric acid (HNO3). We purchased the MDA-MB-231 cell lines from the Ambala Cancer Institute in Maharashtra, India. In this work, we created four magnetic nanoparticles loaded with sulforaphane using the calcified co-precipitation approach. Table 1 lists the ingredients and quantities needed to prepare the four varieties of MNP. All of the ingredients from step 1 were weighed out, and the mixture was stirred overnight at 32°C. Then the contents were transferred to container bottle made of Teflon and heat the mixture to 100°C for 2 days. After this step the contents were subjected to straining and the obtained marc or precipitate was then washed (DD water only) and then dried. After drying the contents were subjected to calcination for 8 hr at 500°C. The specified quantities of ingredients such as Iron, acetyl acetone, nitric acid as per the formulations were mixed gently and heated at 80°C (The quantities of above mentioned ingredients were shown in Table 1).

This process is continued for 4 hr. The Nano particles were suspended overnight in the above mixture with occasional stirring to remove excess solvent. Then the resultant mixture was subjected to heating to 500°C using furnace for 2hr with incremental effect i.e increasing the temperature of 2°C per minute (Zhou et al., 2018; Madhulatha et al., 2022).

Preparation of Sulphoraphane MNP by encapsulation technique

Iron silica (Fe-SiO2), Fe-MCM-41, Fe-KIT-6, and Fe-SBA-15 samples weighing a certain amount as per table 1 were taken separately (coded as F1, F2, F3, and F4 accordingly) and add to 20 milliliters of Sulforaphane-hexane solution (containing 1.4 gram SFP) to the above mixture, and it was macerated for three days while being stirred occasionally. Resulting mixture was subjected to centrifugation, filtration followed by washing with hexane to obtain Sulforaphane MNP. Obtained MNPs were subjected to Vacuum drying for 10 hr at 60°C.

Table 1. Preparation of Four Magnetic Nanoparticles

Part	Name of the Ingredient	Quantity for Formulation			
		F_1	F_2	F_3	F_4
1	Pluronic-123 (gm)	1.4	-	1.23	0.17
	Concentrated Hydrochloric Acid (mL)	2.7	-	2.25	2.9
	Ethanol (mL)	6	-	-	-
	Tetra Ethyl Ortho Silicate (TEOS, mL)	5.2	10	10	1.09
	Cetyl Trimethyl Ammonium Bromide (CTAB, gm)	-	2.4	-	-
	Ammonium Hydroxide (mL)	-	8	-	-
	Deionized Water (mL)	-	120	44	202.6
2	Iron (gm)	0.3153	0.3153	0.3153	0.3153
	Acetyl Acetone (mL)	3	3	3	3
	Nitric Acid (mL)	0.3	0.3	0.3	0.3
	Silicon Di Oxide (gm)	0.25	-	-	-
	MCM-41 (gm)	-	0.25	-	-
	KIT-6 (gm)	-	-	0.25	-
	SBA-15 (gm)	-	-	-	0.25
3	Sulforaphane (gm) for Encapsulation	1.4	1.4	1.4	1.4

Evaluation or characterization of sulforaphane MNPs

Drug response study

UV-Vis spectroscopy (UV-Vis) is a simple, low-cost characterization technique that is widely used to study substances at the nanoscale. To identify, indicate, and examine those compounds as well as assess the stability of NP colloidal solutions, UV-Vis spectroscopy is a crucial instrument. Near the NP floor, the optical characteristics of NPs can be affected by size, shape, concentration, agglomeration state, and refractive index. An important instrument for assessing development of MNP in aqueous suspension is frequently a UV-visible spectrophotometer (Soni et al., 2018). The samples were examined using a UV-Visible spectrophotometer for absorption at 202 nm.

FTIR

The surface adsorption of useful structures on nanoparticles can be studied by using FTIR, which is specifically employed for the identification of unknown compounds. One advantage is that users can examine a layer of nanoparticles covered at the ATR element while also modifying the section above it. It is commissioned to study the chemistry of magnetic nanoparticles (Kumari et al., 2021). These pellets have undergone FTIR Spectrophotometric analysis. Then, data was obtained within the 400–4000 cm^{-1} wave range.

Loading Efficiency

Drug loading performance is defined as the ratio of the amount of drug contained within the nanoparticle to the total amount of drug used in the nanoparticle formula. The encapsulation performance, which is a measure of the drug's loading performance, is one of the most important factors in developing nanoparticle transport architectures. Utilizing a UV-Visible spectrophotometer set to 202 nm, the absorbance of the samples was measured. Loading efficiency was determined using following formula

Drug loading efficiency = $\{(W-W_D)/W_{MNP}\}X100$

Where, W, W_D,W_{MNP} indicate Sulforaphane's initial weight, the weight of the Sulforaphane that was found in the solution, and the MNP's weight, respectively (Zhu et al., 2019).

Zeta potential (ζ)

Zeta potential, also known as electrokinetic potential, measures the "effective" electric powered charge at the surface of colloidal nanoparticles to evaluate their charge balance. The charge is "filtered" by ions with heightened attention at the nanoparticle surface when it has a net floor charge. The layer of oppositely charged ions that accompany the nanoparticle

and the layer of surface charge make up the electric double layer. The Zeta Potential, which is related to the surface of nanoparticles, is a measurement of the difference in ability between the layer of fluid that contains the oppositely charged ions and the majority fluid in which a particle is dispersed.

Negatively charged surfaces will form bonds with positively charged particles, and vice versa. Greater electrostatic repulsion and, hence, greater balance are demonstrated by better significance potentials. Information about particle balancing is provided by the relevance of the Zeta Potential. Zetameter was used to measure the Zeta potential.

in-vitro dissolution study

The *In-vitro* dissolution study was performed as per the standard test procedures using dissolution media pH 5.5 buffer. USP Type-II apparatus was used for conducting the drug relase study. The standard set of conditions such as Temperature 37±0.5 °C, agitation rate 100 rpm.[31] This test is performed for Sulphoraphane pure drug as well MNP also. Samples were collected as per the predetermined time intervals (0,2,4,8,12,16,20,24,30,36 hr). Filter the obtained samples to avoid fluctuations in calculating absorbance's. Dilute the samples with fresh buffer if necessary. Measure the absorbances for collected sample at 202 nm using UV-Visible spectrophotometer for calculating amount of Drug Release.

in-vitro cytotoxicity study for best formulation

The Cytotoxicity Study of SFP loaded MNPs (best formulation of all) was conducted on particular breast cancer cells utilizing the Cell Proliferation Assay with MTT Reagent, including MDA-MB-231. Using the 3-(4, 5-dimethylthialzol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay, the cytotoxic effects of free MNP MCM-41, SFP, and SFP MNP were evaluated on MDA-MB-231 (breast cancer cell). Over the course of a day, the MDA-MB-231 cells were exposed to different doses (0–40 µg/mL) of free MNP MCM-41, SFP, and SFP/MCM-41 (F_2).

MDA-MB-231 cells were plated and cultured in serum-free medium for a full night in a 96-well plate that contained 5000 cells per well. After receiving 0, 2, 5, 10, 20, 40, 80, and 100 µg/ml of free MNP MCM-41, SFP, SFP/MCM-41 (F_2), and doxorubicin (standard medication) for 24 hours at 37°C, the medium was disposed of, and the resulting cells were treated with 0.1% MTT and incubated at 37°C for around 4 hours. Dimethyl sulfoxide (DMSO) was used to dissolve the resultant formazan crystals, and the absorbance was immediately measured at 202 nm using a microplate reader. At this experiment, dimethyl sulfoxide (DMSO) was employed as a control at amounts and dosages that were comparable.Plates were shaken or rattled for two minutes before to reading. The main idea behind the assay is to quantify the amount of tetrazolium molecule that is converted into the formazan product by metabolically active (viable/live) cells (El-Boubbou et al., 2021).

$$\% Vc = (1\{N_B/N_T\}100)$$

Where Vc- Viable Cells; N_B-No.of Blue cells and N_T- Total number of Cells
Using the aforementioned equation, the percentage of cell proliferation inhibition was determined. The IC50 values were determined and curves were constructed.

$$\% \text{ Inhibition of Growth} = [(A_0-A_S)/(A_0)]*100$$

A_0- Absorbance Zero; A_S- Samples Absorbance

Results and Discussion

The Fe_2O_3 and SFP absorption bands overlapped at 202 nm, indicating optimal absorption. The findings of the drug response investigation verified the existence of surface interaction between iron nanoparticles and silica materials. In Figure 1, the spectrum was displayed. In FTIR study, the results reveals that there is compatibility between drug with formulation additives. It was confirmed by observing characteristic peak in spectra. The characteristic peaks such as 3410, 2922,1646 $cm^{-1}$for O-H, C-H, C=C, respectively. The results were shown in Figure 2. Figure 3 displays the results of the loading efficiency calculation of four MNPs loaded with sulforaphane (SFP), shows that SFP/MCM-41 (F_2) has a higher percentage (98%), compared to other. The inclusion of iron molecules gives the Fe-MCM-41 MNP (F_2) a negative charge, according to the estimated zeta potential values for electrostatic stabilization at pH ranges 3 to 11. On the other hand, the final SFP/MCM-41 (F_2) nanoparticle product, shown in Figure 4, has a positive charge because it contains the stabilized medicine sulforaphane.

Figure 5 shows the data obtained from for the *in-vitro* dissolution rate study. The findings show that SFP loaded MNPs have a higher drug release than pure SFP with a larger surface area, which increases bioavailability. Estimating the cytotoxicity producedby the SFP/MCM-41MNP for its potential anticancer benefit is required. By using the MTT assay method, cytotoxic activity was assessed. Following a 24-hour incubation period, the cytotoxic effects of doxorubicin, SFP, MCM-41 MNP, and SFP/MCM-41 MNP were evaluated on MDA-MB-231 cells (breast cancer cells). Over a 24-hour period, the MDA-MB-231 cells were exposed to several dosages ranging from 0 to 100 µg/ml. The vitality of the cancer cells was dramatically reduced after SFP treatment in a concentration-dependent manner (0–100 µg/ml). At each of the measured doses, the SFP/MCM-41 MNP (F_2) pattern was the same. As seen in the analysis of percent viability, the MTS assay data showed that SFP and SFP/MCM-41 MNP formulations had a concentration-dependent anti proliferative impact (0-100 µg/ml). The concentration dependent percentage viability was shown in Figure 6. On MDA-MB-231 cells, the SFP/MCM-41 MNP (F_2) demonstrated notable inhibitory effects. Figure 7 illustrates the enhanced rate of inhibitory effects on cell viability as a result of focused medication administration (Targeted drug delivery). The data for IC_{50} was presented in Figure 8.

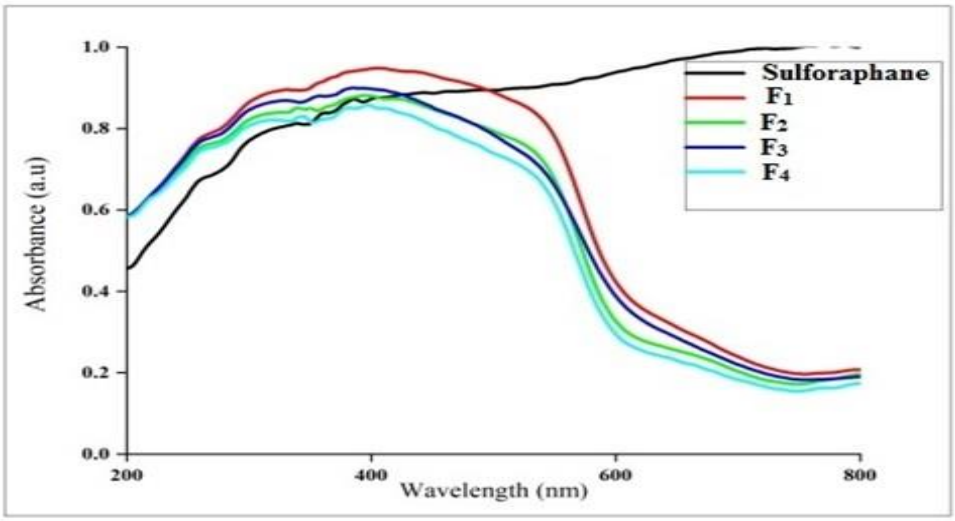

Figure 1. DRS UV-VIS Spectra SFP MNPs

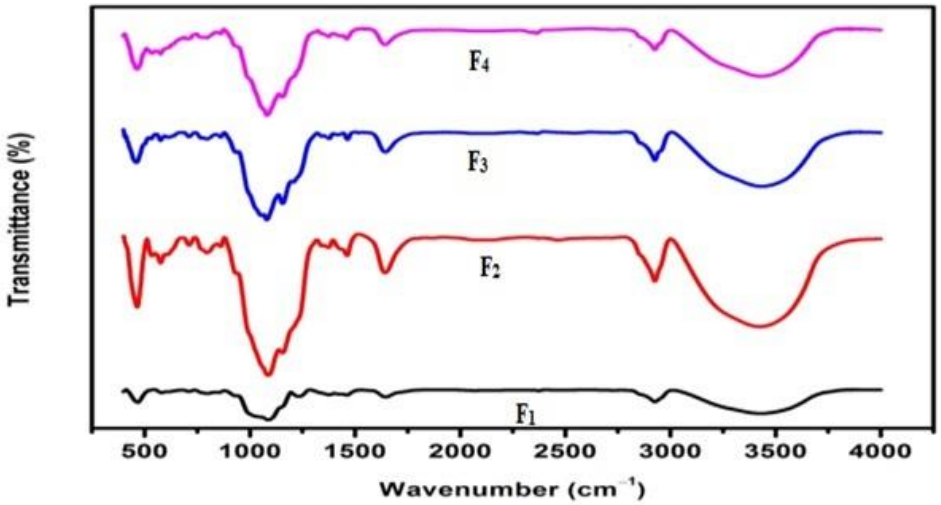

Figure 2. FTIR curves of SFP MNPs

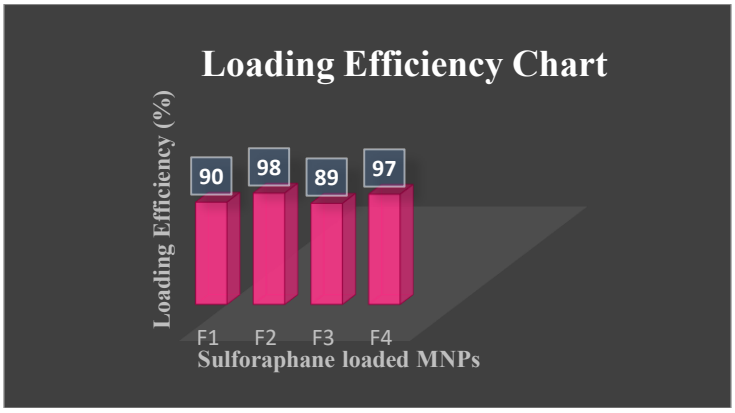

Figure 3. Loading efficiency SFP loaded MNPs

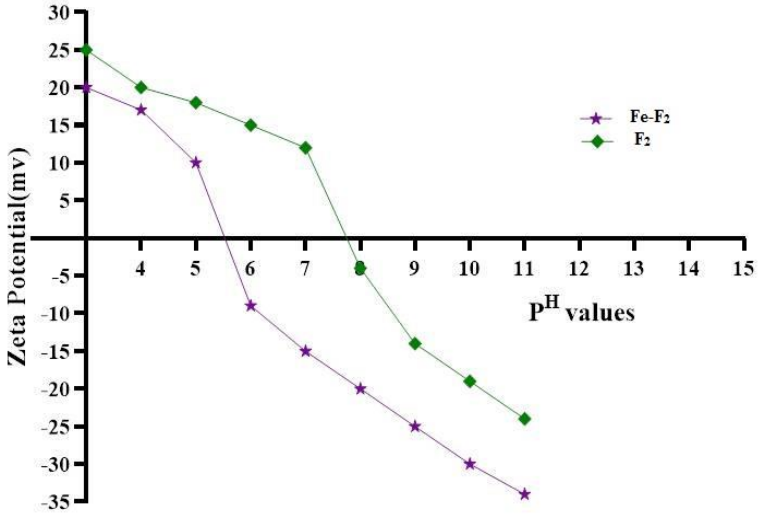

Figure 4. Zeta potential of Fe MCM-41 and SFP/Fe MCM-41

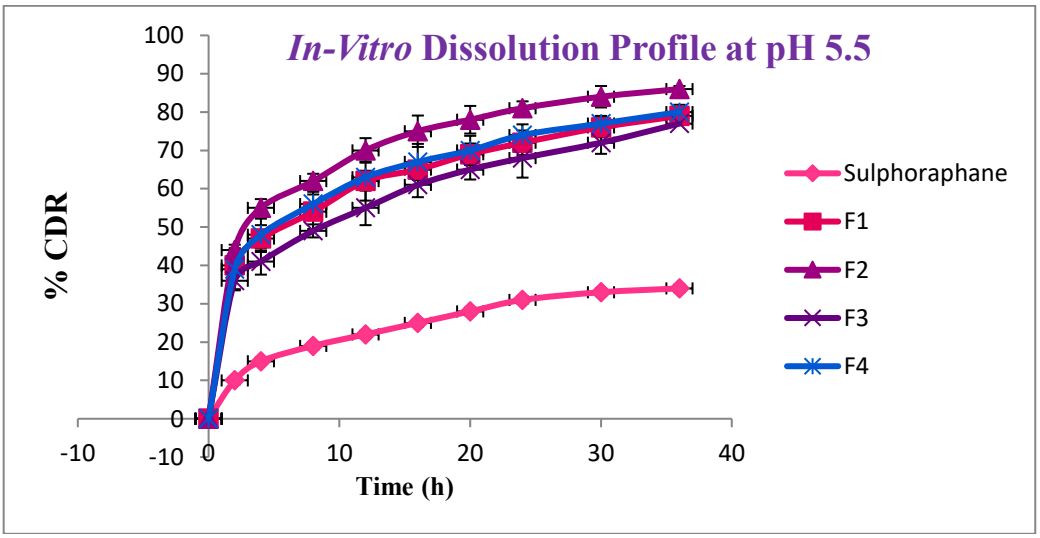

Figure 5. *In-vitro* Dissolution Profile for Sulforaphane MNPs at pH 5.5

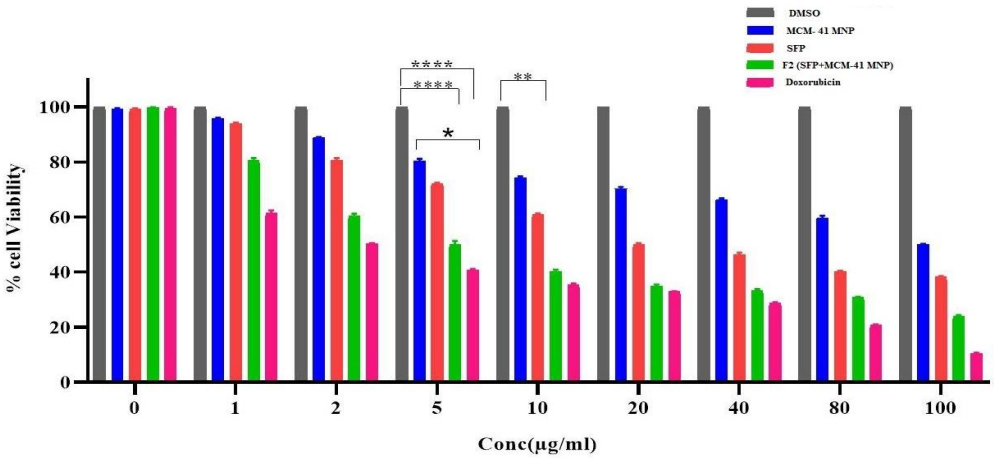

Figure 6. % Cell Viability plot

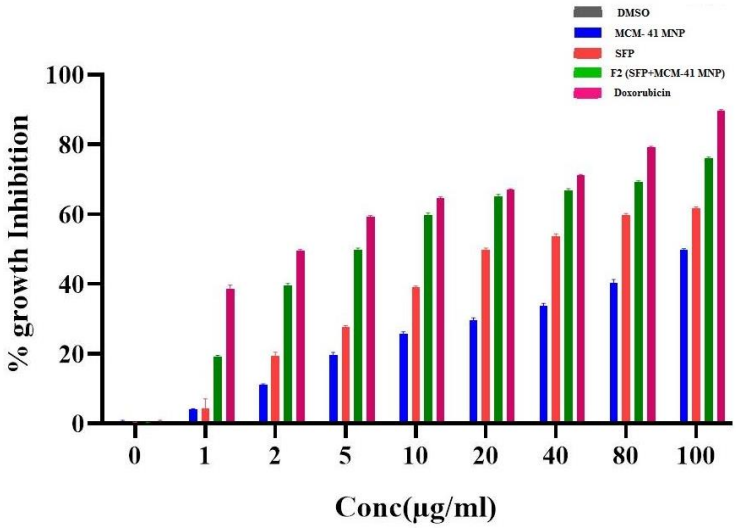

Figure 7. % Growth Inhibition plot

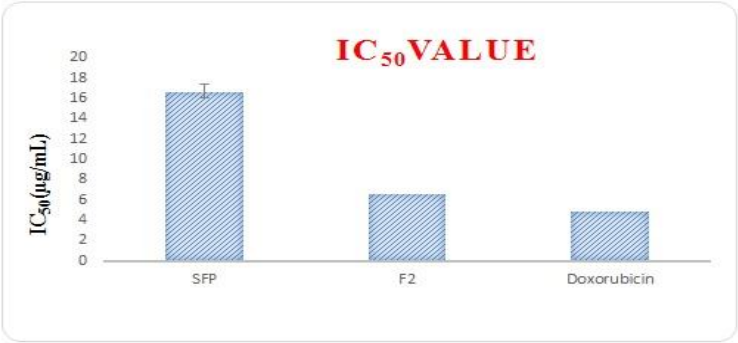

Figure 8. IC_{50} Data

Conclusion

Using the Pluronic block co-polymer P123 as a surfactant template, four distinct mesoporous magnetic nanoparticles for sulforaphane were created. Iron oxides were subsequently added by co-precipitation and calcination processes. The study then proceeded to final characterization tests, including FTIR, loading efficiency, in-vitro drug dissolving rate study, drug response study, and in-vitro cytotoxic potential. According to the findings of the Drug Response Study, Sulforaphane's greatest absorbance was seen at 202 nm. Based on the findings we came to the conclusion that all four varieties of magnetic nanoparticles have a large surface area and pore volume, are structurally well-arranged, have a good size range, and a sufficient percentage of elements on their particle surface. They also demonstrate magnetic responsiveness for drug

targeting when an external magnetic field is present. The greatest drug release was seen at pH 5.5 in the dissolution analysis of Sulforaphane loaded MNP of Fe-MCM-41 (F2). The magnetic nanoparticles showed possible anticancer activity against breast cancer cell lines (MDA-MB 231) using the MTT assay. SFP, SFP/MCM-41 MNP (F2) IC50 values and percentage cell viability were computed and contrasted with Doxorubicin (Standard medication). Therefore, the nano formulation containing sulforaphane and MCM-41 (F_2) proved helpful for the efficient treatment of cancer.

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Author contributions

Raghavendra Kumar Gunda: Conceptualization and methodology; Prasadarao Manchineni and Madhavi Latha Chennuru: Supervision; Venkata Ramana Golla and Ameer Pasha Shaik: Procurement of Raw materials and other sources; Ravi Shankar Kunderu: Drafting and proof reading.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

Not applicable.

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