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In silico modeling, formulation, optimization, and *in-vitro* evaluation of candidone microsponges for the management of colorectal cancer

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ABSTRACT

Cancer is one of the most lethal diseases of the twenty-first century. The present research aimed to screen out natural bioactive with the help of bioinformatics tools. The screened molecule (Candidone) was formulated as a microsponge and evaluated for the effective and safe treatment of colorectal cancer (CRC). The Microsponges were developed using Eudragit RS 100, a pH-sensitive polymer using the quasi-emulsion solvent diffusion method. A total of 9 batch formulations were prepared using 3^2 full factorial designs. The prepared microsponges were evaluated by various parameters such as particle size (PS), entrapment efficiency (EE), and drug release study, optimization was done by the design of an expert, which was further subjected to statistical analysis that was kinetic release mechanism, compatibility studies were performed such as fourier transform infrared spectroscopy. Using factorial design 3^2 for the maximum possible formulation, the optimized formulation was found to be F7 in terms of better release for a longer time and with better by reducing the frequency of administration of the medication, reducing the dose, improving the patient compliances (ease of administration), by reducing the drug fluctuation level in the blood, and by enhancing the efficacy and safety of the treatment. The F7 formulation has a PS of 44.26 ± 0.84 µm, (%) EE of 76.57 ± 0.26, drug loading of 49.67% ± 0.94%, and good % CDR 94.26% ± 2.64% for an extended period of 12 hours. In addition, the candidone shows a decrease in the viability of HT 29 colon cancer cells in a dose-dependent manner. The result indicated that the prepared microsponges could release the Candidone in a sustained manner and help in the management of CRC.

INTRODUCTION

Cancer is the second most significant cause of mortality in underdeveloped countries and is among the most challenging diseases to treat. It has become a global health issue in recent decades, and progress in curing the condition has been made. The second deadliest cancer and the third most prevalent diagnosis for both sexes is colorectal cancer (CRC). Strong links between the environment and genetic risk factors exist for CRC. Life-threatening cancers originate from CRC (colon and rectal cancer) [1–3]. The likelihood of having CRC is between 4% and 5%, and certain characteristics or behaviors including age, a history of chronic illness, and lifestyle choices are linked to an increased risk of the disease. The gut microbiota is important in this setting, and conditions of dysbiosis can cause chronic inflammation, which in turn can lead to colonic carcinogenesis [4,5]. Mutations in specific genes can cause colon cancer to grow, just as other malignancies. These mutations can affect genes involved in DNA repair pathways, tumor suppressor genes, and oncogenes. Depending on where the mutation originated, colorectal tumors can be categorized as sporadic, inherited, or familial. Point mutations only affect specific cells and their offspring during life; they do not result in hereditary disorders. Sporadic malignancies, or tumors brought on by point mutations, account for 70% of all colon cancer cases. Because mutations can affect different genes, sporadic cancer has a complex molecular etiology [6–8].

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On the other hand, approximately 70% of CRC cases follow a certain set of mutations that result in a definite morphological sequence, starting with the development of an adenoma and ending in the carcinoma stage. The initial mutation in the tumor suppressor gene Adenomatous polyposis coli causes benign adenomas, or polyps, to appear [9,10]. Colon cancer affects 4%–5% of persons globally. Many personality traits or behaviors are also considered risk factors since they increase the likelihood of developing CRC or polyps. The main risk factor for CRC is age. The chance of acquiring CRC increases dramatically beyond the age of fifty, and the disease is uncommon in younger adults. (Except for hereditary malignancies). Aside from age, other inherent risk factors are unchangeable. Individual history of inflammatory bowel disease or CRC: Ulcerative colitis patients are 3.7% more likely to have these disorders [11,12].

Candidone is a powerful flavanone found in *Derris indica*. Candidone has demonstrated substantial cytotoxic effects against human cancer cells and antibacterial potential for treating numerous drug-resistant bacterial infections. In addition, candidone has claimed several powerful applications, including ulcers, rheumatoid arthritis, wounds, and diabetes [13,14]. The candidone can be absorbed into the body in several ways: orally, parenterally, transdermal, inhalational, transmucosal, and subcutaneously. The simplest, safest, and most effective way to provide the medication to patients is by oral route [15]. This oral route is ideal for long-term treatments that require frequent dosing. It is necessary to increase the rate at which an active component is released from the system and the GIT's gastric transit time to develop a drug delivery system for the oral route. Oral administration of complete drugs in the conventional dosage type has short-range restrictions due to this failure to recognize and suppress the device in the gastrointestinal system [16–18].

Studies on candidone cytotoxic effects have demonstrated that it can be used as a therapeutic agent to treat HepG2 cancer cells by blocking the production of p65 phosphorylation, Bcl-xl, Mcl-1, Bcl2, and survivin, which activates caspase-3 and caspase-9 [13]. In addition, it has been demonstrated that candidone can sensitize tumors with multidrug resistance to anticancer drugs such as daunorubicin and mitoxantrone; as such, it may be considered a chemosensitizer. Considering the importance of biological characteristics, it is crucial to assess its anticancer properties against colon cancer [19]. Based on the literature there was no delivery system present for the candidone. Therefore, a microsponge-based novel drug delivery system has been proposed in the present work. Microsponges have been critical to this delivery system's development because of their smaller size and efficient carrier capacity. Microsponges are a porous polymer micro-specific drug delivery system made up of a minor, spherical item that resembles a sponge with a porous surface. The colon's macrophages pick up microsponges that are less than 200 mm. Thus, successful localized drug activity at the necessary spot can be demonstrated [20,21].

This study aimed to create microsponges loaded with candidone for colonic delivery. Using Eudragit RS100 and a water-soluble porogen, a quasi-emulsion solvent diffusion approach was used to create candidone-loaded microsponges. To optimize microsponges, 3^2 full factorial designs were used. The Eudragit RS100 concentration and stirring speed were taken as an independent variable. The dependent variables studied were percentage cumulative drug release (CDR), entrapment efficiency (EE), and particle size (PS). Finally, an *in vitro* test utilizing a colon cancer cell line was conducted to check the anticancer properties of the formulation.

MATERIALS AND METHODS

Materials

Candidone (assay 98%) was purchased from Sigma, St. Louis, MO. Eudragit RS 100, Triethyl Citrate, and Polyvinyl alcohol (PVA) were taken from CDH, New Delhi, India. Ethyl alcohol was obtained from Rankem, New Delhi, India. Distilled water was used in all experiments.

Ligand preparation

26 natural flavonoids of plant origin were screened from the searched literature; their molecular profiles and chemical properties were recovered from the NCBI PubChem database. The standard drug, butein, was used as a control in the study.

Protein preparation

The protein, NF-kappa B p50 homodimer bound to DNA, was prepared by sorting out the crystal 3D structure of human PDB ID:1SVC, as managed by the RCSB PDB website. Finally, the protein crystals cleared off all unwanted matter initially present in it.

Molecular docking using AutoDock and PatchDock

The computational molecular docking method was employed to screen anticancer plant-derived compounds against the structure of NF-kappa B p50 homodimer bound to DNA. PatchDock and AutoDock 4.2 software executed the molecular docking. The drug-complex platform of PatchdDock was opted for performing the computational docking. The anticancer compound was sorted at the end of the docking process based on the PatchDock docking score produced via PatchDock software. The highest score value was the basis for selecting the most stable conformation anticancer compound of plant origin. The study of target–ligand interactions was used by Autodock 4.2 following the genetic algorithm of Lamarckian to assess the binding energy and inhibitory constant. For the target protein, NF-kappa B p50 homodimer bound to DNA, the binding points of the prepared grid with 60 (x) × 60 (y) × 60 (z) were analyzed by CASTp, setting other parameters for molecular docking (by default). The docking study was run in Intel®Core™i5-8250U CPU at 3.40 GHz using Dell info system (RAM: 8 GB DDR; Windows 8 professional OS). The complex docked in the cluster of lowest energy levels was only considered for further studies on chemical interaction, as described by Morris *et al.* [22] and Baig *et al.* [23].

Cell culture and cell maintenance

The cell lines (HT-29) were procured from NCCS, Pune, and maintained in the animal tissue culture facility of

the department. RPMI-1640 medium enriched with penicillin-streptomycin-amphotericin B (100 µg/ml) and heat-inactivated FBS (10%) procured from Himedia, India, Ltd., Mumbai, India, was used to culture and maintain the cells, followed by incubation of the cells at 37°C in a humidified atmosphere with CO_2 (5%).

Cell proliferation by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay

The MTT reduction assay, which was used to measure the cellular vitality of cancer cells as described by Deshmukh and Trivedi [24] with some modifications. HT 29 colon cancer cells were used for the study. A 96-well plate was seeded with 10,000 cells per well, and the cells were incubated for 24 hours. Following incubation, specific Candidone dosages were prepared using dimethyl sulfoxide (DMSO) (0–800 µg/ml) and cells were treated with the specific dose for 24 hours. Following a 24 hours drug treatment, the cells were twice cleaned with phosphate buffered saline and incubated for 4 hours at 37°C with 100 µl of MTT (0.5 mg/ml). Subsequently, 100 µl DMSO was added to each well to dissolve the formazan crystal after the entire MTT was withdrawn. A spectrophotometer was used to measure the optical density of the solubilized formazan product (with a reference 570 and 660 nm filter). Cell survival percentages were used to express the results. Cancer cells that were given serum-free medium were regarded as 100% viable and were utilized to demonstrate viability. The Nikon Eclipse TS 100 inverted microscope was used to observe the drug-treated and untreated cells. Nikon Eclipse TS-100F inverted microscope was used to see the morphological changes of cancer cells and photos of the cells were captured using a high-resolution Nikon L22 camera.

Table 1. Coded value of variables in full factorial design layout.

Independent variables	Level		
	Low (−1)	Intermediate (0)	High (1)
X1 concentration of Eudragit RS 100 (mg)	400	600	800
X2 Stirring rate	500	1,000	1,500

Method of preparation microsponges

Eudragit RS-100 microsponges loaded with Candidone were formulated by quasi-emulsion solvent diffusion method [25]. Eudragit RS-100 and triethyl citrate dissolved in 5 ml ethyl alcohol and formed the internal phase. Triethyl citrate was used as a plasticizer. The drug was added with gradual stirring and formed one phase (internal phase). The outer phase was prepared with the help of 5 g PVA dissolved in 100 ml of water. The inner phase was poured into the external phase with gradual stirring. After 2 hours of stirring due to the removal of ethyl alcohol, the microsponges were formed. The microsponges were filtered and dried at 40°C for 12 hours.

A 3^2 full factorial design was employed to investigate the effect of independent variables (Eudragit RS 100 and Stirring rate) on the dependent variables (PS and Production yield and percentage drug release) for optimizing the candidone microsponges using design expert software (trial version). The concentration of Eudragit RS 100 (X1) and Stirring speed (X2) were optimized at different levels low (−1), intermediate (0), and high (1). PS (µm) (Y1), Production yield (%) (Y2), and % CDR (Y3) were selected as response variables (Table 1). Triethyl citrate, Ethyl alcohol, and PVA were kept constant for all the formulations. All the data regarding the experimental design matrix is given in Table 2, which consists of 09 experiments. The experimental data were fitted to the selected experimental design using the statistical module, and the statistical parameters were calculated. The partial least squares regression method was utilized to generate the prediction models.

Characterization on microsponges

The following evaluation parameters on microsponges were evaluated.

PS and surface morphology

Microsponge samples were analyzed by optical microscopy. A trinocular microscope equipped with a camera was used for the purpose. The samples were analyzed at 45×. In addition, the surface analysis of the optimized formulation was done by scanning electron microscopy.

Table 2. 3-level 2-factor full factorial design layout.

S. No	Formulation code	Drug (mg)	PVA (g)	Eudragit RS 100	Stirring rate	Dependent variables
1	F1	100	5	400	500	PS (Y1)
2	F2	100	5	600	500	%EE (Y2)
3	F3	100	5	800	500	%CDR after 24 hours (Y3)
4	F4	100	5	400	1,000	
5	F5	100	5	800	1,000	
6	F6	100	5	800	1,000	
7	F7	100	5	400	1,500	
8	F8	100	5	600	1,500	
9	F9	100	5	800	1,500	
10	F10[a]	100	5	768	1,500	

[a]Extra design checkpoint.

Encapsulation efficiency, actual drug content, production yield, and drug loading (DL)

It is determined by taking 10 mg of microsponges, dissolved in 100 ml of phosphate buffer, pH 7.4, and sonicated for 4 hours at 30°C. The 0.2 µm membrane filter has been used for the filtration of the sample. After filtration, the sample was analyzed in a UV-Vis spectrophotometer at two wavelengths, 290 nm, and 420 nm, against the blank, as reported by Sareen *et al.* [25]. Production yield was calculated by taking the raw material's initial weight and the microsponges final weight.

$$\text{Actual drug content (\%)} = \frac{\text{The actual drug content in microsponge}}{\text{The total amount of microsponge}} \times 100$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{The actual drug content in microsponge}}{\text{The amount of drug added to the microsponge}} \times 100$$

$$\text{DL efficiency (\%)} = \frac{\text{The actual drug content in microsponge}}{\text{The total amount of polymer and drug}} \times 100$$

In vitro drug release study and kinetic of release

Studies of the *in vitro* release of drugs were performed in the basket apparatus of the United States Pharmacopeia, maintaining the stirring rate (50 rpm) and the temp. 37°C ± 0.5°C Drug release was initially performed in 900 ml of 0.1N HCl, pH 1.2, for 2 hours, accompanied by phosphate buffer, pH 6.8, for the next 6 hours and finally for 6 hours in simulated colonic fluid (SCF) pH 7.4 (containing 120 IU of pectinase). To keep the sink condition, aliquots were taken out at regular intervals and replenished with an equal amount of the fresh medium.

The *in vitro* release data were analyzed to other kinetic equations to evaluate the drug release process and compare the release profile of different formulations of microsponge. The kinetic models contained zero and first-order model Higuchi and Korsmeyer-Peppas plot.

RESULT AND DISCUSSION

Molecular docking

The molecular docking method was employed to explore the anticancer plant-derived compounds against the structure of 1SVC (NF-kappa B p50 homodimer bound to DNA). Dockthor and Cygwin scores were used to identify the suitable candidate for further study. From the results of molecular docking, the highest binding affinity was found to be candidone (−6.921 kcal mol^{-1}) out of 26 compounds. It also possesses a ligand efficiency of 0.2615 kcal mol^{-1}. The complete list of compounds with their binding energies is shown in Table 3. The binding site of candidone in 2D format and the interaction of amino acid residue with hydrogen bonding against NF-kaffa B is shown in Figure 1. Hence, candidone has been chosen as the herbal phytochemical to perform further studies against CRC.

Validation by *in-vitro* screening against cancer cell line

The drug molecules obtained through the computational screening were further validated through an *in-vitro* cytotoxicity study. MTT assay was used to assess the cytotoxicity of drugs against HT 29 cancer cell lines. The overnight cultured cells (10,000) were treated with different concentrations of Candidone (0–800 µg/ml) for 24 hours. There is a dose-dependent reduction in the viability of the treated cells. In Figure 2A, the vitality of the Candidone-treated cells decreased from 100% to 12% in contrast to the cells cultured on a serum-free (incomplete) medium. Using light microscopy with 20× and 40× objectives, the cellular morphology of the treated cells was also examined. The MTT assay results, which showed a considerable decrease in the number of cancer cells in the field as compared to the control, are consistent with the observations made using microscopy (Fig. 2B). The treated cells exhibit an aberrant shape, an unhealthy appearance, and various

Table 3. List of anticancer plant-derived compounds obtained from computational molecular docking method against the structure of NF-kappa B p50 homodimer bound to DNA.

S. No	Name	Pubchem Id	Dockthor	Cygwin
1	Candenatenin A	44254875	−6.212	−3.25
2	2′,3′,5′,7- Tetrahydroxy Flavone	5321864	−6.71	−3.88
3	2′,5′,6′,7- Tetrahydroxy Flavanone	42607849	−6.272	−3.86
4	2′,5′,6′,7- Tetrahydroxy Flavone	5321865	−5.739	−3.67
5	3′- Formlyl-2′4′,6′- Trihydorxy-5′, Methyldihydrochalcone	11033908	−6.029	−2.74
6	4;- Bromoflavone	1686	−6.737	−4.1
7	Candenatenin C	44254877	−6.431	−3.44
8	Duartin	3666064	−6.179	−3.37
9	Emcrydone	10063979	−7.163	−4.16
10	Eupafolin	5317284	−6.424	−4.6
11	Hesperetin	72281	−5.971	−4.53
12	Isccarthamidin	5315720	−5.947	−4.7
13	Kaempferol	5280863	−6.318	−4.32
14	Leteolin	5280445	−6.015	−4.75
15	Maividin	159287	−5.946	−3.71
16	Mucronulatol	442811	−6.349	−3.83
17	Neccalyxins A	21588173	−6.041	−4.58
18	Pelargonidin	440832	−6.379	−3.53
19	Peonidin	441773	−5.917	−2.87
20	Scutellarein	5281665	−6.061	−4.53
21	3,8-Dihydroxy-9-Methoxypterccarpan	5318889	−6.02	−4.2
22	**Candidione**	**14033978**	**−6.921**	**−4.76**
23	Isoneorautenol	73649	−6.427	−3.87
24	Neorautenol	11500744	−6.265	−4.25
25	Phasecllin	91572	−6.621	−4.69
26	5,7,4′-Trihydroxy,6-Methoxyhomoisoflavanone	638288	−6.482	−4.33

Bold value denote molecule taken for the study.

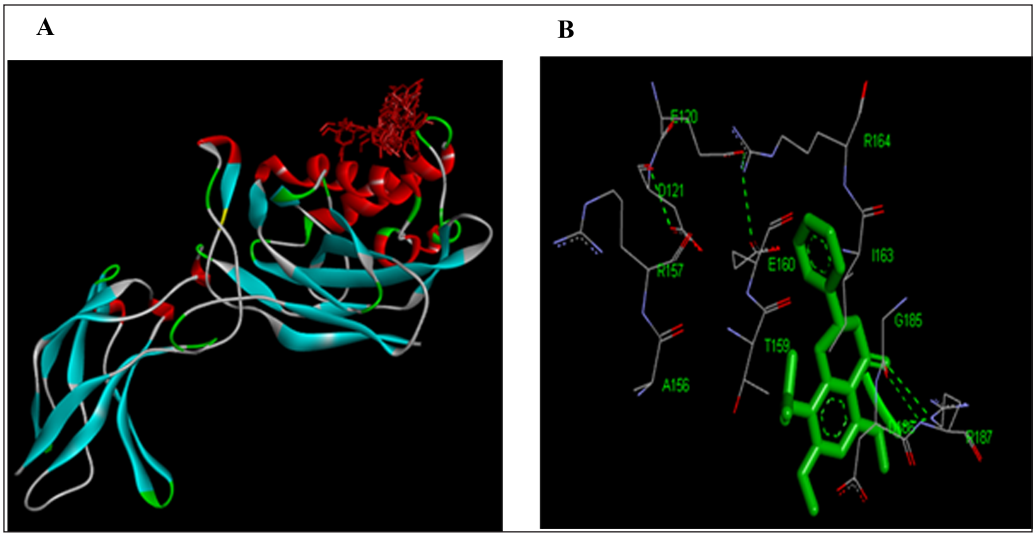

Figure 1. The target and compound binding.

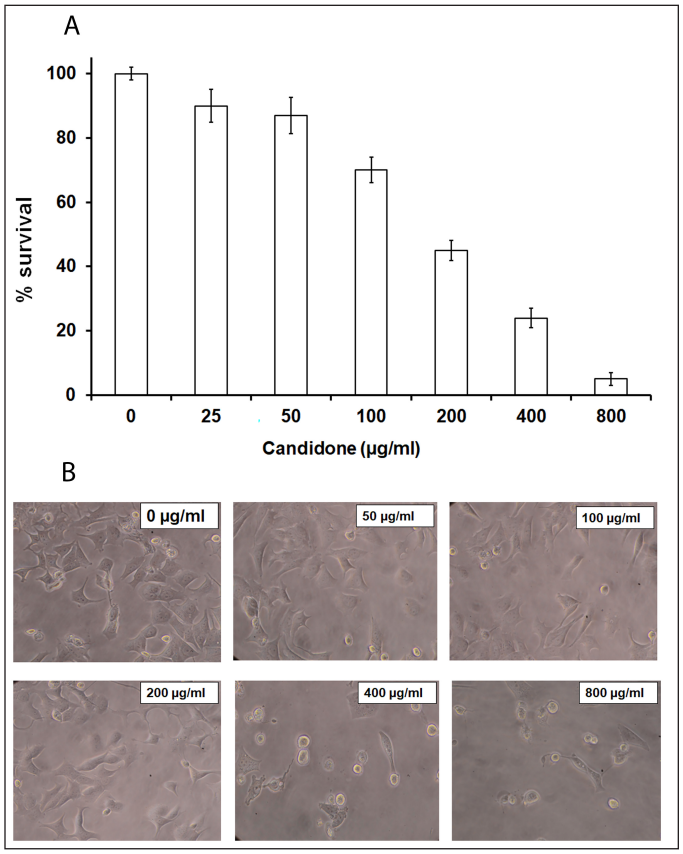

Figure 2. MTT assay of the candidone in HT 29 cell lines (A). Microscopic image of the HT 29 cell lines at 40× (B).

apoptotic symptoms such as blebbing and shrinking of the cell body. The untreated, on the other hand, appears healthy and has normal morphology. Together, the data in Figure 2 indicates that the candidone exhibits toxicity against HT29 colon cancer cells. Similar results were also reported by Boonyarat *et al.* [13] where candidone inhibits the proliferation, migration, and invasive potential of human hepatoblastoma (HepG2) cells. Similarly reported that the candidone can sensitize multidrug-resistant cells to daunorubicin and mitoxantrone and could be considered as attractive multiple drug resistance modulators for cancer [26]. Therefore, it is assumed that candidone exhibits the death of cancer cells following the apoptotic pathway.

Fourier Transform Infrared Spectroscopy (FTIR) studies

FTIR studies were conducted for the actual medication, excipient, and product and excipient combination. They have been tested within 400–4,000 cm^{-1} range. KBr press was used and medication, excipient, and medication plus excipient pellets were created. The spectra of candidone and formulation are shown in Figure 3A and B. The spectral data shows that there was no change in the peak of formulation as compared to the candidone. Thus there was no interaction among the components of the formulation.

Compatibility studies

No visual change is observed in the drug and excipients after storage. The results have been summarized in Table 4.

Evaluation parameters

The results of various evaluation parameters are as follows.

PS, actual drug content, DL, and EE

The PS of a drug formulation affects how well it dissolves and absorbs. The formulations of Candidone-loaded microsponges were examined for their PS with the help of zetasizer. The size of the microsponges (F1–F9) was found to be in the size range from 44.26 to 55.77 µm (Table 5). It is well established that as PS decreases, surface area rises. This may make it easier for drugs to be absorbed from formulations. Therefore, the optimized formulation should have smaller particles that release the drug from the formulation. The formed microsponges are negatively charged, which means they will

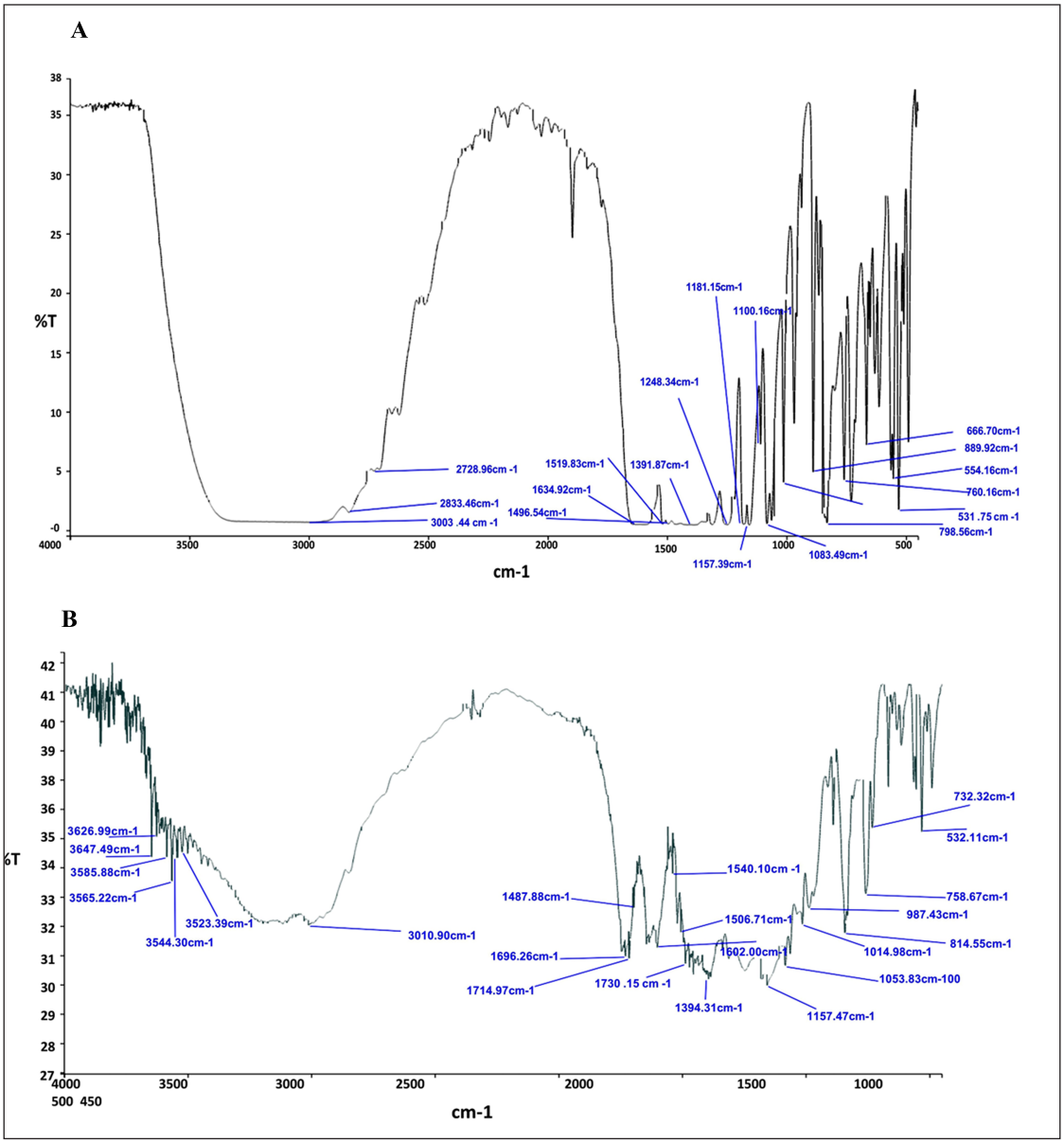

Figure 3. FTIR study. A. Candidone, B. Formulation.

have excellent stability, according to a zeta potential result (data not shown). The results of actual drug content, DL, and encapsulation efficiency of different microsponge formulations (F1–F9) are shown in Table 5. The range of the actual drug content values is 71.23% to 78.34%. The EE values are found to differ between 65.44% and 76.57% across the various formulations. The % DL of the formulations is in the range from 32.24% to 49.67% and the production yield ranges from 70.68% to 86.42%.

SEM and microscopy

Using scanning electron microscopy, the F7 formulation surface was examined; the results were displayed in Figure 4. Because of the spherical shape and sponge-like structure of the microsponge formula, it can be seen from the surface topography that the microsponges had microscopic pores. The inner phase solvent diffused from the microparticle surface, creating the pores. The particles were called microsponges because of the way they looked. The optimal microsponge formulae prepared with a higher polymer amount (800 mg) with high stirring speed (1,500 RPM) result in the production of drug crystals over the particle surfaces, as revealed by examination of the outer surface. This result can be readily explained by the previous work, which states that more drugs will diffuse to the surface of the microsponges dissolved in the solvents at greater drug/polymer ratios [27]. The existence of micropores increases the dissolution of the drug by increasing the penetration of the dissolving solvent. The figure makes it evident that microsponges were spherical and contained pores. The prepared microsponges were also

Table 4. Physical compatibility studies for microsponges.

Sr. No.	Parameter	Ratio	Initial	Contr ol	40°C/75 % RH - open	40°C/75 % RH - closed	60°C (close)	60°C (open)
1	Candidone	Contr ol	Light yellowish	NC	NC	NC	NC	NC
3	Eudragit RS 100	Contr ol	Cloudy colour	NC	NC	NC	NC	NC
4	PVA	Contr ol	White colour	NC	NC	NC	NC	NC
5	Triethyl citrate	Contro l	Transparent	NC	NC	NC	NC	NC
7	Candidone + Eudragit RS 100	1:1	Light yellowish and cloudy yellow	NC	NC	NC	NC	NC
8	Candidone + PVA	1:1	Light yellowish and white	NC	NC	NC	NC	NC
9	Candidone + triethyl citrate	1:1	Light yellowish with a drop of triethyl citrate on it	NC	NC	NC	NC	NC
10	Candidone + + Eudragit RS 100 + PVA	1:1:1:1	Yellow and white powder with a drop of triethyl citrate on it	NC	NC	NC	NC	NC

NC: no change.

Table 5. PS, actual drug content, DL, and encapsulation efficiency of different formulations.

S. No.	Formulation code	PS (µm)	Actual drug content	EE (%)	DL (%)	Production yield (%)
1.	F1	55.71 ± 1.25	71.23 ± 0.25	69.99 ± 0.51	32.24 ± 0.58	72.36 ± 2.12
2.	F2	57.77 ± 2.98	72.46 ± 0.59	67.78 ± 0.28	30.26 ± 0.45	71.98 ± 3.28
3.	F3	51.48 ± 1.64	74.34 ± 0.42	65.44 ± 0.84	31.41 ± 0.36	70.68 ± 2.84
4.	F4	52.76 ± 1.54	73.24 ± 0.22	74.68 ± 0.24	41.51 ± 0.72	78.05 ± 3.14
5.	F5	54.57 ± 1.72	73.79 ± 0.37	72.74 ± 0.53	43.39 ± 0.52	75.10 ± 2.86
6.	F6	47.89 ± 0.86	75.42 ± 0.26	70.75 ± 0.32	41.27 ± 0.86	75.04 ± 1.88
7.	**F7**	**44.26 ± 0.84**	**78.34 ± 0.44**	**76.57 ± 0.26**	**49.67 ± 0.94**	**86.42 ± 1.16**
8.	F8	48.32 ± 0.62	74.46 ±0.31	74.34 ± 0.54	36.47 ± 0.28	84.25 ± 1.12
9.	F9	54.81 ± 0.25	72.35 ± 0.47	71.84 ± 0.25	42.45 ± 0.76	83.91 ± 0.58

Bold value denote optimize formulation.

observed under a compound microscope (45× and 100×) to observe the shape and size of the formulation. The prepared microsponges were found to be spherical but the pore was not visualized properly (Fig. 5).

Drug release study and release kinetics

To replicate the gastrointestinal tract (GIT) environment, an *in vitro* release study of candidone from microsponges was conducted at three distinct pH values: pH 1.2 for 2 hours, pH 7.4 for the following 6 hours, and in SCF (containing 120 IU of pectinase) for the next 6 hours. The investigations were conducted at 37°C ± 0.5°C in 900 ml of the dissolving media. Figure 6 illustrates the drug release from microsponges *in vitro*. 3.35%–8.12% of cumulative drug release was released from the micro sponges in the first 2 hours, while 8.03%–16.25% of the drug was released from phosphate buffer 6.8 pH in the next 4 hours. As the pH increases to 7.4 and media is converted to SCF the release of drug from microsponges increases from 16.25% to 94.25% in the next 6 hours. Results show that the candidione was released from the formulation in SCF as the polymer employed for the fabrication of the microsponge was a pH-sensitive polymer and only dissolved in the 7.4 pH. Thus, the drug release suggested that Eudragit RS100 inhibited the premature release of the drug in the upper GIT.

The results of the kinetic drug release of various microsponge formulations are shown in Table 6. After putting all the formulation's drug release profiles through different kinetic models, the best correlation coefficient (r^2 = 0.9921) indicated that the release profile followed the Higuchi model, which proposed diffusion as the primary mechanism of drug release.

Optimization of experimental design

Using the Design Expert software version 7.0.0, optimization studies were carried out, and after variables were transformed, a second-order polynomial equation was obtained. The amount of polymer and stirring speed has an impact on the PS, %EE, and *in vitro* drug release (Fig. 7). The equation's results show that the polymer amount (X1) has a greater impact

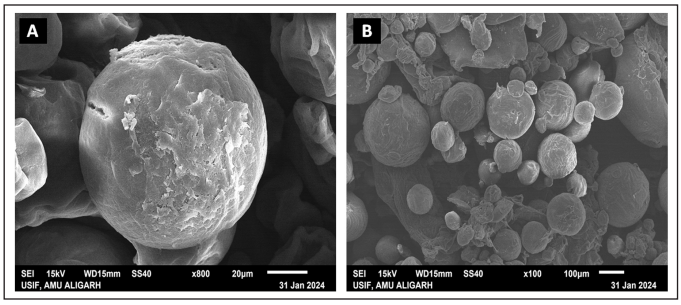

Figure 4. SEM photomicrographs (A) Microsponges of formulation F9, (B) Microsponges of formulation F9 without drug.

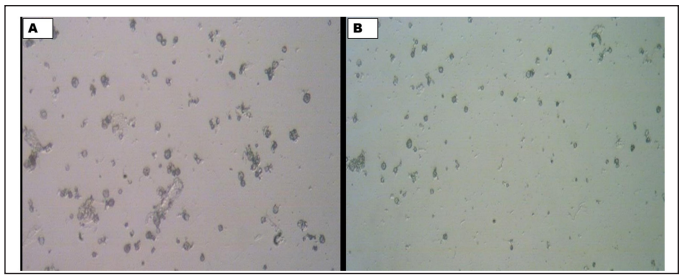

Figure 5. Microscopic photomicrographs (A) Microsponges of formulation F9, (B) Microsponges of formulation F9 without drug at 45× magnification.

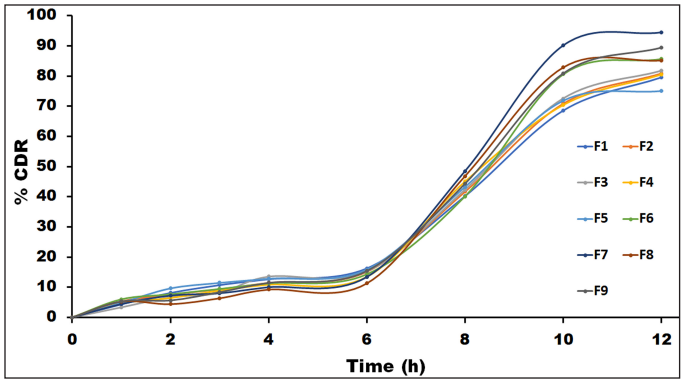

Figure 6. Release study of different microsponges formulations.

than the stirring speed (X2). In addition, the PS was negatively impacted by the stirring speed (i.e., the PS dropped as the stirring speed increased). Smaller sized microsponges were formed as a result of the emulsion's microdroplet size decreasing as the stirring speed increased. These results are consistent with earlier reports. A linear trend of the mean PS in ascending order with an increase in each variable is shown in Figure 7a.

An essential factor in determining the DL capacity of microsponges is the drug EE. This metric depends on the drug's physicochemical characteristics, the synthesis method, and formulation factors. The equation's result shows that the polymer (X1) has a greater impact than the stirring speed (X2). In addition, drug EE was positively impacted by stirring speed (that is, when stirring speed increased, PS decreased and drug EEincreased) as shown in Figure 7b. This is because larger droplets with less surface area are produced by a higher amount of polymer, which slows the diffusion of the drug from such microsponge and increases encapsulation efficiency.

The results shown in Figure 4c show that the release of drug from the microsponge is affected by both the amount of polymer (X1) and stirring speed (X2). The drug release was impacted by the stirring speed because an increase in stirring speed causes the formation of small PS of the microsponge. On the other hand, drug release from microsponge is reduced as the polymer amount is increased due to a decrease in DL. A greater polymer matrix content within the microsponge results in a longer diffusional channel, which in turn reduces the total drug release from the microspheres.

Additional theoretical formulation F10 was created as an extra checkpoint. The factors for the F10 formulation were generated by the software using polynomial equations. When the predicted and experimental values of the percent CDR were compared, there was no discernible difference between them, supporting the validity of optimization. The transformed equation generated by design expert software for PS, EE, and CDR are as follows:

PS = 53.34 -2.43*A -4.73*B +3.70*A*B -2.40*A2 +0.32*B2 +2.70*A2 *B +4.01*A*B2

% EE = +72.77 -1.97*A +3.28*B -0.045*A*B -0.075*A2 -1.73*B2 -0.035*A2*B -0.35*A*B2

CDR = +73.78 +6.09*A +15.78*B -4.22*A*B +1.54*A2 +0.62*B2 -2.66*A2*B -6.74*A*B2

Table 6. Kinetic release study of microsponge different formulations.

Formulations	Zero order		First order		Higuchi square model		Korsmeyer peppas model	
	r^2	K	r^2	k	r^2	K	r^2	k
F1	0.7242	3.0145	0.9513	0.0107	0.9800	18.67	0.7601	1.3412
F2	0.7338	2.8445	0.9640	0.0079	0.9732	17.22	0.7805	1.3630
F3	0.7304	2.9256	0.9687	0.0098	0.9754	17.63	0.7738	1.3362
F4	0.7359	2.5563	0.8916	0.0045	0.9678	16.73	0.7861	1.2438
F5	0.7410	3.1078	0.9564	0.0124	0.9519	15.64	0.8781	1.3859
F6	0.6754	2.3673	0.9681	0.0038	0.9703	16.98	0.8856	1.4054
F7	**0.7956**	**2.5441**	**0.8744**	**0.0047**	**0.9921**	**19.72**	**0.6964**	**1.5103**
F8	0.7867	4.3472	0.8778	0.0152	0.9242	13.02	0.8427	1.7082
F9	0.7368	3.1065	0.9113	0.0262	0.9475	14.27	0.9147	1.3743

Bold value denote optimize formulation.

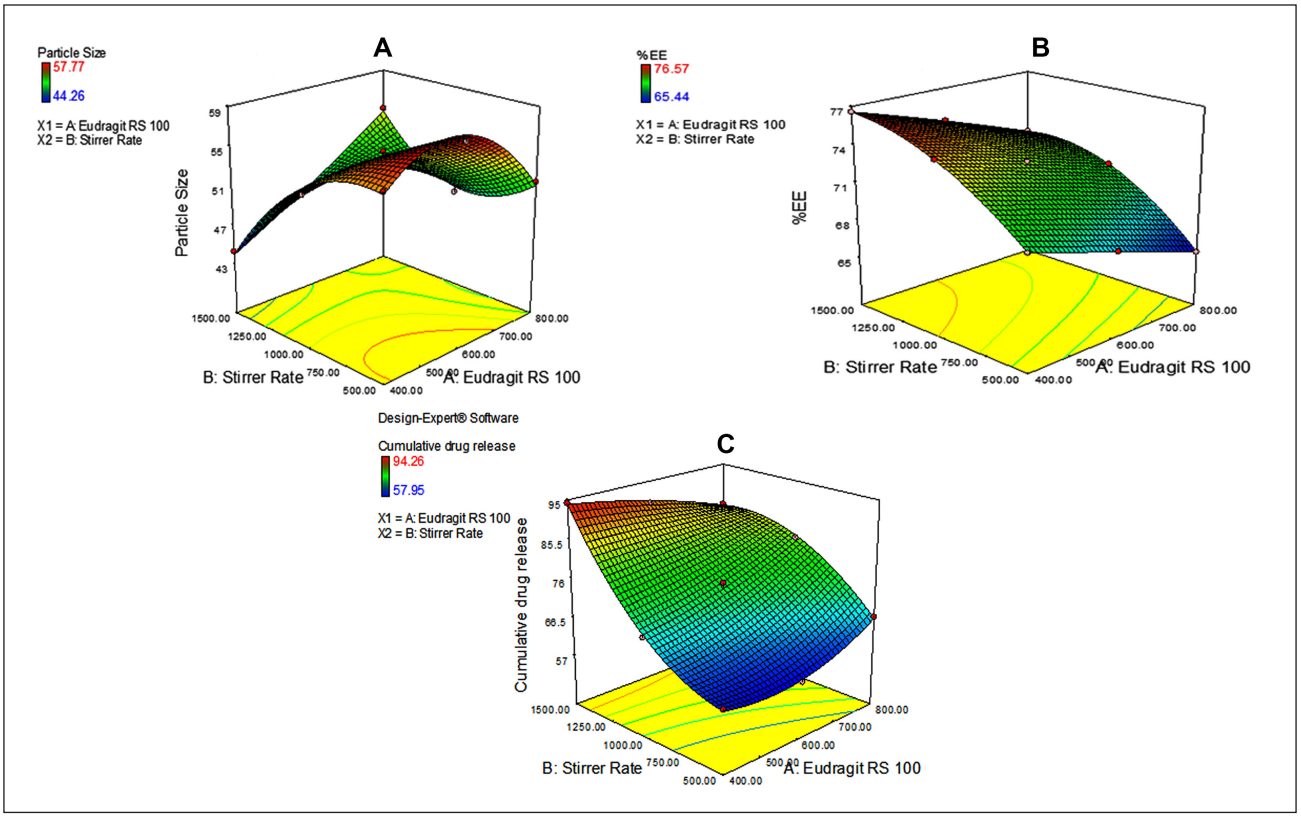

Figure 7. Response surface plots illustrating the impact of independent variables on dependent variables.

Based on the lowest PS (44.26 ± 0.84 µm), highest EE (76.57% ± 0.26%), and highest drug release (94.26% ± 2.64%), the formulation F7 was determined to be the optimal formulation. After putting the F7 formulation's drug release profile through different kinetic models, the best correlation coefficient (r^2 = 0.9921) indicated that the release followed the Higuchi model, which proposed diffusion as the primary mechanism of drug release.

CONCLUSION

The second most frequent cause of cancer-related death is colon cancer. Chemotherapy, a treatment of cancer by conventional therapy has lots of adverse effects. Phytopharmaceuticals can offer good therapeutic efficacy with minimal side effects. Therefore, the study's goal was to search for a novel phytopharmaceutical that can bind to NF-kappa B p50 homodimer bound to DNA and exhibit anticancer properties. Out of 26 compounds, Candidione was found to be a suitable candidate based on binding energy studies. Furthermore, it was checked for its anticancer properties against HT 29 colon cancer cells and it was observed that candidone exhibits death of cancer cells in a dose-dependent manner. For the candidone to deliver in the colon and to release in a sustained manner its microsponges were prepared successfully using the quasi-emulsion solvent diffusion method and optimized through factorial design. The concentration of the different polymers significantly impacted the size of microsponges. The 3^2 full factorial design results revealed that the stirring speed and concentration of polymer significantly affect the dependent variables such as PS, % EE, and drug release. It was discovered that the developed formulation had microscopic pores on its surface and was spherical. According to an *in vitro* drug release investigation, eudragit RS100 was utilized as a pH-sensitive polymer with a threshold pH value over six to avoid the upper gastrointestinal tract and exhibit targeted and controlled release at colonic pH. The kinetic models indicated that the release of candidone from the formulation follows the Higuchi model, which proposed diffusion as the primary mechanism of drug release. According to the findings, candidone-loaded microsponges could be a potential medication delivery method for the treatment of colon cancer. Further, the outcome of the study further needs to be validated through *in vivo* studies utilizing an appropriate animal model.

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This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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