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Original Article

Evaluation of the effects of a dasatinib-containing, self-emulsifying, drug delivery system on HT29 and SW420 human colorectal carcinoma cells, and MCF7 human breast adenocarcinoma cells

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الملخص

أهداف البحث: داساتينيب هو مثبط تيروزين كيناز من الجيل الثاني. إنه يعمل كدواء جزيء صغير متعدد الأهداف من خلال استهداف كينازات التيروزين المختلفة التي تشارك في نمو الخلايا الورمية. وقد وجدت التحقيقات الأخيرة أنه يمنع تكاثر الخلايا السرطانية، والهجرة، ويحفز موت الخلايا المبرمج في مجموعة متنوعة من الأورام الصلبة. ومع ذلك، فهو ضعيف الذوبان في الماء تحت ظروف الرقم الهيدروجيني المختلفة، بالإضافة إلى نصف عمره القصير. لذلك، فإن تطوير نظام توصيل الدواء المحتوي على داساتينيب، ذاتي الاستحلاب يمكن أن يساعد في التغلب على هذه المشكلات في علاج الخلايا السرطانية.

طريقة البحث: تم تطوير تركيبات مختلفة لنظام توصيل الدواء ذاتي الاستحلاب ومحملة بداساتينيب باستخدام آيزوبروبيل ميريستات (مرحلة الزيت)، ولابرافيل (الخافض للتوتر السطحي)، والبولي إيثيلين جلايكول (الخافض للتوتر السطحي المشترك). تم تقييم الخواص الفيزيائية والكيميائية للتركيبات من حيث حجم القطرات، وكفاءة التغليف، وإطلاق الدواء في المختبر. تم أيضا تقييم السمية الخلوية للتركيبات على ثلاثة خطوط من الخلايا السرطانية، وسرطان القولون والمستقيم البشري وسرطان الثدي الغدي البشري، بالإضافة إلى خلايا الليفية الرئوية الجنينية البشرية الطبيعية من أجل الانتقائية.

النتائج: داساتينيب-تركيبة نظام توصيل الدواء ذاتي الاستحلاب، تتميز بحجم جسيم جيد، وكفاءة تغليف، وإطلاق دواء في المختبر. في فهمنا، أظهرت هذه الدراسة أن فعالية داساتينيب المضادة للسرطان - نظام الاستحلاب الذاتي، ونظام توصيل الدواء كان لها تأثيرات أفضل على السمية الخلوية على خطوط الخلايا السرطانية الثلاثة، وسرطان الثدي الغدي البشري، وسرطان القولون والمستقيم البشري، مقارنة مع داساتينيب النقي.

الاستنتاجات: من المحتمل أن تكون تركيبات نظام توصيل الدواء داساتينيب-الاستحلاب الذاتي، فعالة كطريقة مستدامة لتوصيل الدواء لعلاج السرطان.

الكلمات المفتاحية: داساتينيب؛ الاستحلاب الذاتي؛ نظام توصيل الدواء؛ إطلاق الدواء؛ الانحباس؛ السمية الخلوية

Abstract

Background/Aim: Dasatinib (DS), a second-generation tyrosine kinase inhibitor, functions as a multi-target small-molecule drug via targeting various tyrosine kinases involved in neoplastic cell growth. DS inhibits cancer cell replication and migration, and induces tumor cell apoptosis in a variety of solid tumors. However, it is poorly soluble in water under some pH values. Therefore, the development of a DS-containing, self-emulsifying, drug delivery system (SeDDs) could help overcome these problems in treating cancer cells.

Methods: Various SeDD formulations loaded with DS were developed with isopropyl myristate (oil phase), Labrafil (surfactant), and polyethylene glycol (co-surfactant). The physicochemical properties of the formulations were assessed according to droplet size, encapsulation efficiency, and in vitro drug release. The cytotoxicity of the formulations on the cancer cell lines HT29 and SW420 (human colorectal carcinoma), and MCF7 (human breast adenocarcinoma), in addition to MRC5 normal human fetal lung fibroblasts, was evaluated to assess selectivity.

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Results: The DS-SeDD formulation showed favorable particle size, encapsulation efficiency, and in vitro drug release. The anti-cancer potency of DS-SeDDs had greater cytotoxicity effects than pure DA on the three cancer cell lines, MCF7, HT29, and SW420l.

Conclusion: The developed DS-SeDD formulations may potentially be an effective sustained drug delivery method for cancer therapy.

Keywords: Cytotoxicity; Dasatinib; Drug release; Entrapment; SeDDs; Self-emulsifying

Introduction

Cancer is the second leading cause of death worldwide, following cardiovascular disease.[1] This complicated illness can result from a variety of spatiotemporal alterations in cellular physiology, and the disease's biological endpoint is neoplasia, i.e., abnormal cell growth.[2] For most patients with cancer, tumor cell invasion into nearby tissues and distant organs is a major cause of morbidity and death.[3] The biological process through which normal cells transform into cancer cells has been a subject of biomedical scientific research for decades.[2] Despite these research efforts, curative or long-term treatment strategies for metastatic cancer remain challenging, because of the contradictions and paradoxes that have long plagued the field. Cancer treatments include radiation, surgery, and chemotherapy.[4,5] Targeting tumor cells ubiquitously is not always possible, because some medications do not diffuse well, and the randomness of the diffusion process is difficult to regulate.[6] This lack of control can lead to multiple drug resistance, a condition in which patients do not respond to chemotherapy treatments because their cancer cells are resistant to one or more drugs.[7–9] Most chemotherapy drugs are administered intravenously or orally; however, because of first-pass metabolism, oral administration is frequently limited and requires higher doses.[10–12] Moreover, most chemotherapeutic agents also act on normal tissues, and their non-targeting nature may lead to adverse effects.[13] Consequently, creating novel medications that may be used in cancer treatment is critical.

Oral dasatinib (DS) is a tyrosine kinase inhibitor used to treat lymphoid leukemia with Philadelphia chromosome positivity and chronic myelogenous leukemia.[6] The Food and Drug Administration (FDA) authorized its use in 2006 for patients intolerant or resistant to Ph+ CML. Src family kinases, which are involved in a variety of signaling pathways, are another target of DS.[6,14] A variety of human cancers, including those of the breast, colon, and prostate, are more likely to survive, proliferate, and spread in the presence of abnormal Src family kinase activity.[14] Recent studies have indicated that DS inhibits cancer cell replication, migration, and invasion, and induces tumor cell apoptosis.[15] Two commercial DS tablets are currently authorized for clinical use: (1) SPRYCEL®, which is manufactured by Bristol-Myers Squibb (BMS) and was approved by the FDA in 2006 in the crystal form of MH H1-7, and (2) YINISHU®, which is made by CHIA TAI TIANQING, has AH N-6 crystal form,[16] and was approved in 2013 by the FDA.[17] DS has high permeability and low solubility, and therefore is categorized as a class II drug according to biopharmaceutical classification.[16] DS has low water solubility, is sensitive to pH, and has a short half-life.[18] In mammals, DS has a bioavailability of only 14–34%,[19] because of its low solubility in the small intestine, thus causing a high first-pass effect and incomplete absorption.[20–22] To increase the efficacy of DS and decrease adverse reactions, redesigning the drug by using nanotechnology could achieve the goal of providing an effective new drug for cancer treatment.

Nanotechnology in drug development has several advantages including (1) prolonging drug stability, (2) increasing the capacity to carry highly toxic drugs with a very narrow therapeutic margin, (3) targeting cancer cells, and (4) circumventing drug-resistance mechanisms.[23] Self-emulsifying drug delivery systems (SeDDs), an advanced drug formulation method, have demonstrated excellent performance in achieving therapeutic advances.[15] Niza et al. and Singh et al. have both reported that the SeDD formulation method can help control drug delivery and enhance bioavailability in breast cancer therapy,[24,25] and overcome the low solubility and irreversible precipitation observed with conventional formulations.[26] In another approach, DS-loaded albumin nanoparticles have exhibited more potent anti-leukemia efficacy than free DS treatment, and have shown enhanced specificity of tumor activity in lung, cervical, breast, and ovarian cell cancers.[27] Therefore, this study was aimed at increasing DS solubility for cancer treatment through SeDD technology. The developed formulations were characterized in terms of physicochemical properties and cytotoxicity activity toward the HT29, SW420, and MCF7 cancer cell lines, in addition to the normal MRC5 cell line.

Materials and Methods

Materials

DS was obtained from Xián Lukee Bio-Tech Co., Ltd China, and re-wetted. The RC tubing was 10 Kd MWCO Spectra/por 6-Dialysis Membrane. Isopropyl myristate was obtained from Manufacture Scharlau, HUANG, 2008 #79. Lemon oil (Argentinean origin, ISOlab), oleic acid (M.wt 282047), polyethylene glycol (PEG; average Mn 400), and Tween 80-Span 80 (viscosity 1200–000 m Pa s) were purchased from Sigma Aldrich. Labrafil M1977CS, Labrafil M1944cs, and Miglyol 812 N were purchased from UFC Biotechnology. Monobasic sodium phosphate (Fluka Analytical), diethylene glycol monoethyl ether 98% (Alfa Aesar) transcutol, and hydrochloric acid (Honeywell Fluka) were used. Water was deionized with a Milli-Q filtration system.

Cell lines

HT29 and SW420 (human colorectal carcinoma), MCF7 (human breast adenocarcinoma), and MRC5 (normal

human fetal lung fibroblast) cells were sourced from the American Type Culture Collection (ATCC, Rockville, USA). Roswell Park Memorial Institute (RPMI) 1640 medium (with 10% fetal bovine serum) were used to subculture the three cancer cell lines. Eagle's minimum essential medium (with 10% fetal bovine serum) was used to maintain MRC5 cells. All cells were cultured at 37 °C, 5% CO_2, and 100% relative humidity, for a maximum of five to ten passages.

Determination of maximum absorption of dasatinib

A UV−visible spectrophotometer (Cary 60 UV−Vis; Aglient Technology) was used to scan various DS-SeDDs and pure drug samples in the 200−800 nm range. The wavelength corresponded to the maximum absorbance (λ max). The absorbance of the final solution was compared against a blank at 324 nm. The calibration curve was created by plotting the absorbance against the DS concentration.

Pre-formulation study

Solubility assessment

Because DS is insoluble in water, the choice of the optimum oil, surfactant, and co-surfactant is crucial in increasing solubility and achieving high loading capacity in SeDD formulations. The equilibrium solubility of DS was measured in various oils (oleic acid, lemon oil, Miglyol, and isopropyl myristate), water, and pH 3 buffer. After selecting the oil, we selected the surfactant and co-surfactant according to their solubility in the oil. The solubility of oil in various surfactants (Tween 80, Labrafil, and Kolliphor) and various cosurfactants (PEG and Transcutol) was determined. The constituents of the SeDDs were chosen according to the maximum solubility of DS while achieving high miscibility with each constituent, to enable spontaneous formation of a stable emulsion after dilution with a buffer.

An excess of DS was mixed with 2 ml of each oil, pH 3 buffer, or water. Before the mixtures were allowed to reach equilibrium in a shaking water bath at 37 ± 1 °C for 24 h, the mixtures were vortexed to aid in the mixing of DS with the vehicle. After 1 day, the mixtures were centrifuged at 10,000 rpm for 10 min to separate un-dissolved DS, then filtered through a 0.45 μm membrane filter (M/s mdi Membrane Technologies LLC, California, USA). The filtered samples were diluted with methanol, and the concentration of DS was assessed with a UV/vis spectrophotometer at 324 nm and use of a standard calibration curve of known DS concentrations. For selection of the surfactant, 15 w/v% surfactant aqueous solutions were prepared, and 4 μL oil was added and mixed through vigorous vortexing. If a clear one-phase solution was obtained, addition of the oil was repeated until the solution became cloudy.[28]

Experimental design

After the optimum types of oil, surfactant, and co-surfactant were selected, a literature review and experimental study were conducted to select the optimum ratios and concentrations of oil, surfactant, and co-surfactant.[29−33] Various formulations were prepared with different concentrations of oil (1−2%), surfactant (4.5−6%), and co-surfactant (3−3.5%), as shown in Table 1. Visual observation (phase separation or turbidity), droplet size, zeta potential, and entrapment efficiency (EE) were measured as dependent variables to properly determine the boundaries of emulsion and microemulsion phases.

Preparation of DS-SeDDs

Various DS-SeDD formulations were prepared with the standard admixture method.[34,35,36] First, DS was dissolved in oil and stirred for 20−30 min, thus yielding a clear mixture. Second, the surfactant was blended with co-surfactant and stirred for 1 min. This mixture was added to the oil-DS solution under stirring at high speed with a magnetic stirrer (Parfitt, Ambala, India) for 20−30 min. The oily phase was vortexed with a mixer for 5−10 min. Subsequently, pH 3.0 buffer was prepared and added dropwise to the oil phase. The emulsion was homogenized in a high-shear homogenizer for 5−15 min at 80−90 rpm. The resulting emulsion was stored at 4 °C.

Physio-chemical characterization of DAS formulations

Droplet size, polydispersity index, and zeta potential

With a dynamic light scattering technique, the droplet size, PDI polydispersity index value, and zeta potential at room temperature (25 °C ± 0.5 °C) with a Zetasizer instrument (Nano ZS, Malvern Instrument Ltd., UK) were determined for the optimized DS-SeDDs. For measurement, samples were diluted with deionized water (1:100) and injected into the measuring cell to assess the globule size distribution.[37] Each sample was prepared in triplicate to ensure replicability.

Drug entrapment efficiency

To determine the amount of drug entrapped in the formula, we diluted DS-SeDD samples with dimethylformamide in pH 3 buffer; the mixture was stirred for 30 min and centrifuged at 10,000 rpm for 20 min. The absorbance of the supernatant was measured with UV spectroscopy at 324 nm, the wavelength at which DS absorbs maximum light. After centrifugation, DS and supernatant particles were used to create a standard curve, as previously described.[38,39]

Calculations were performed with the following formula:

$$\text{Drug EE (\%)} = \frac{\text{Amount of DS in SneDD}}{\text{Total amount of drug}} \times 100$$

In vitro drug release study

The drug release profile can aid in confirming drug dispersion in a self-emulsion system. Using the dialysis bag diffusion method, we investigated the diffusion of formulations through a cellulose acetate membrane (molecular weight cut-off 12,000−14,000 Da). Three milliliters of sample was injected into the cellulose membrane, and both ends of each dialysis bag were tied carefully. Subsequently, the dialysis bags were carefully immersed in beakers containing a mixture of phosphate buffer solution and ethanol (ratio 7:3 v/v, pH: 7.4).

A magnetic bar was used to agitate the elution medium at 100 rpm. To maintain the sink conditions, 3 ml receptor medium was removed at various intervals (15, 30, 45, 60, 90,

and 24 h) and replenished with an equal volume of new medium. These samples were analyzed with a UV−Vis spectrophotometer at 324 nm to determine the amount of drug released, as previously described.[34,37]

Cytotoxicity assays

The cytotoxicity of two compounds was evaluated with MTT assays, as previously described.[40,41] Each cell line was separately cultured in 96-well plates (3×10^3/well) and incubated with each compound at a final concentration of 0−50 μM, at 37 °C (0.1% dimethyl sulfoxide; n = 3 independent experiments). After 72 h of incubation, the cytotoxicity of each drug was calculated with MTT assays. In brief, MTT in the culture medium at a concentration of 0.5 mg/ml was added to each well and incubated at 37 °C for 3 h. Dimethyl sulfoxide was used to break down the formazan granules after MTT solution was withdrawn. Absorbance was measured with BIO RAD PR4100 and Hercules multi-plate readers (CA, USA)\. The number of live cells was correlated with the optical density of the purple formazan A_{550}, and the removal concentration causing 50% inhibition (IC_{50}), with respect to blank cells (100% growth), was examined via GraphPad Prism.

Statistical analysis

IBM's SPSS 22 (USA, P 0.05) was used to perform one-way analysis of variance and unpaired two-tailed t-tests. The results are shown as mean ± standard deviation (SD), and each test was performed in triplicate. Student's T-test was used in data analysis. According to statistical standards, a p-value <0.05 was considered significant.

Results and discussion

The purpose of this study was to evaluate the production, physicochemical characteristics, in vitro drug release spectrum, and drug EE of DAS-loaded self-emulsions. In addition, the self-emulsions were investigated for their efficacy and effects in vitro on three cancer cell lines, HT29, SW420, and MCF7, and one normal cell line, MRC5.

Determination of maximum absorption

Using a UV−Vis spectrophotometer, we successfully analyzed various DS-SeDDs and pure DS over a wavelength range of 200−400 nm. The wavelength corresponding to the maximum absorbance (λ max) was noted. The wavelength at which the sample's absorbance peaked was 324 nm. The absorbance of the final solution was compared against that of a blank sample at 324 nm. An R^2 value of 0.9733 was obtained by plotting the absorbance against the DS concentration to create a calibration curve. The DS standard concentrations showed excellent linearity.

Pre-formulation study

Solubility assessment

Lipophilic drugs are preferably solubilized in o/w nanoemulsions, whereas w/o systems appear to be a better choice for hydrophilic drugs. Various types of oils, surfactants, and co-surfactants were selected according to a literature review.[29−33] The optimum types of oils, co-surfactants, and surfactants (Table 1) were used to ensure a suitable mixture to solubilize the drug and develop the DS-SeDD formulation. Solubility of DS in the oil phase is an important criterion for the selection of oil. This aspect is particularly important in oral formulation development, because the ability of a nanoemulsion to maintain the drug in solubilized form is greatly influenced by the drug's solubility in the oil phase. Moreover, formulation of a nanoemulsion with an oil with low drug solubility would require incorporation of more oil to achieve the target drug dose, and consequently a higher surfactant concentration to achieve oil solubilization, thus potentially increasing the toxicity of the system. Solubility tests indicated that DS had a maximum solubility in isopropyl myristate as the oil, similarly to previous findings.[42,43]

The most critical problem associated with nanoemulsion-based systems is the toxicity of the components. Large amounts of surfactants may cause gastrointestinal symptoms when administered orally. Therefore, proper selection of surfactants is necessary.[28,43] Determining the proper surfactant concentration and using the minimum concentration in the formulation are important. Nonionic surfactants are less toxic than their ionic counterparts and typically have lower CMCs. In addition, o/w nanoemulsion dosage forms for oral use based on nonionic surfactants are likely to provide in vivo stability.[28,43] After selecting the oil, we selected the surfactant and co-surfactant according to their solubilization with the oil. The surfactant does not require good drug solubilizing power, because the dilution of the nanoemulsion in the gastrointestinal tract lowers the solvent capacity of the surfactant or cosurfactant, which could be a risk of precipitation.[28,44] Surfactant−oil miscibility can thus provide an initial indication of the possibility of nanoemulsion formation with this system. Here, we selected the surfactant providing the maximum nanoemulsion area alone without the addition of the cosurfactant. The greater the nanoemulsion area, the greater the nanoemulsification capacity of the surfactant. Labrafil solubilized the maximum amount of isopropyl myristate and consequently was chosen as the surfactant for nanoemulsion development.

Cosurfactants are added to obtain nanoemulsion systems at low surfactant concentrations.[28,45] The cosurfactants

Table 1: Preparation of DS-SeDDs.

Sample	Oil-isopropyl myristate	Labrafil surfactant	Drug-DAS	Co-surfactant poly (ethylene glycol)
DS-SeDD 1	1 ml	6 ml	25 mg	3 ml
DS-SeDD 2	2 ml	5 ml	25 mg	3 ml
DS-SeDD 3	1.5 ml	5 ml	25 mg	3.5 ml
DS-SeDD 4	2 ml	4.5 ml	25 mg	3.5 ml

decrease the interfacial tension, increase the fluidity of the interface, and allow for greater oil penetration into this region.[28,45] Hydrophilic co-surfactants are considered to prefer the interface and lower the energy necessary to form nanoemulsions, thereby increasing stability. Nanoemulsion area was used as the assessment criterion for the evaluation of cosurfactants. The use of a long chain, branched co-surfactant generally resulted in the highest nanoemulsion area and consequently the highest nanoemulsification efficiency. Therefore, PEG was selected as a co-surfactant. Accordingly, we used isopropyl myristate, Labrafil, and PEG-400 to prepare the DS-SeDD formulations. For lipophilic drugs, such as DS, that exhibit poor water solubility and rate-limited dissolution, SeDDs may increase the rate and extent of absorption and result in more reproducible blood−time profiles.

Experimental design

According to a literature review, nanoemulsion area is frequently used as the assessment criterion for the evaluation of nanoemulsion systems. The system yielding the highest nanoemulsion area achieves the highest nanoemulsification efficiency.[28,43] Using 2:1 ratio of surfactant:co-surfactant, rather than other ratios, has been found to achieve the maximum area.[28,43] This effect is attributable to differences in surfactant and cosurfactant packing at the o/w interface. Moreover, decreasing the oil level increases the nanoemulsion formation area,[28,43] thereby suggesting that the oil constitutes the inner phase of the nanoemulsion droplets, in agreement with a direct o/w-type structure. Accordingly, we performed preformulation studies to determine the concentrations of isopropyl myristate, Labrafil, and PEG. Different concentrations of oil (1−2%), surfactant (4.5−6%), and co-surfactant (3−3.5%) were evaluated in terms of droplet size, zeta potential, and EE.

Physio-chemical characterization of prepared DS-SeDDs

Droplet size, polydispersity index, and zeta potential

All DS-SeDD formulations were evaluated with a Zetasizer instrument and demonstrated a different range of droplet sizes (diameters), as indicated in Table 2. We used a straightforward method of formulating DS-SeDDs in a wide range of sizes via modulation of the oil, surfactant, and co-surfactant. With concentrations of 1%, 6%, and 3% (wt./vol) of oil, surfactant, and co-surfactant, respectively, DS-SeDD1 had the smallest particle size, and had an average size of 2040.7 ± 204.7 nm and a PI value of 0.3 ± 0.005. An increase in the oil and co-surfactant concentrations to 1.5 and 3.5 (wt./vol) yielded DS-SeDD3, which had the largest particle size, 7118.0 ± 398.6 nm, and a PI value of 0.4 ± 0.062. Additionally, use of a 2% of oil concentration with 4.5% and 3.5% surfactant and co-surfactant, respectively, yielded DS-SeDD4, which had an average particle size of 5016.0 ± 24.0 nm and a PI value of 0.5 ± 0.009. The dispersed phase in the SeDDs droplets were emulsions with oil, typically had a particle size of 20−200 nm. The minuscule sizes of the droplets in nanoemulsions are the primary distinction between these formulations and ordinary SeDDs. This compact size confers various benefits.[46,47,48] The small droplet size of SeDDs enhances drug stability and confers higher kinetic stability. This stability is frequently ascribed to the tiny droplets' lower gravitational pull, which decreases coalescence, creaming, and sedimentation.[49] Unlike conventional formulas, which may appear opaque because of larger droplet sizes, SeDDs have greater transparency, and are typically translucent or transparent. This optical clarity has benefits in many industries, including medicines. Moreover, SeDDs' small droplet sizes enhance bioavailability by expanding the surface area for interaction with biological tissues or membranes, thus potentially increasing the bioavailability of encapsulated substances (such as medications, nutrients, or flavorings), and consequently increasing efficacy and absorption.[50] Targeted delivery is enabled by the ability to enclose bioactive substances and medications in SeDDs for delivery specifically to particular tissues or cells. Nanoemulsions can increase the effectiveness of drug administration and decrease off-target effects by altering the droplet surface characteristics or adding targeted ligands. Overall, SeDDs' distinct qualities and benefits over traditional emulsions stem from their smaller particle sizes, which make them appealing for a range of drug formulations.[51]

Most designed formulations are positively charged. Using positively charged microemulsions (also referred to as cationic microemulsions) in cancer treatment may have potential advantages[52−54] by facilitating favorable interactions with negatively charged cancer cell membranes. This interaction could potentially increase treatment efficacy by facilitating better cellular uptake and enhanced delivery of therapeutic agents directly to tumor cells. Additionally, the ability of cationic microemulsions to carry a positive charge may facilitate internalization of the drug-loaded microemulsion into cancer cells. Subsequent release of the payload inside the cells increases the drug concentrations at sites of therapeutic action.[55,56] In general, microemulsions can increase the stability and solubility of poorly soluble medications, thus increasing drug availability and effectiveness. These characteristics can be further improved by the positive charge in cationic microemulsions, particularly for drugs with poor solubility.[57,58]

Table 2: Physical characteristics of the developed DS-SeDD formulations.

Formulas	Z-Average (mean, d. nm) ± *SD*	PdI (mean) ± *SD*	Intercept (mean) ± *SD*	Zeta potential (mean, mV) ± *SD*	EE% ± *SD*
$DS\text{-}SeDD_1$	2040.7 ± 204.7	0.3 ± 0.005	0.78 ± 0.013	1.85 ± 0.106	91.73 ± 0.84
$DS\text{-}SeDD_2$	6251.0 ± 919.2	0.5 ± 0.017	0.59 ± 0.025	0.84 ± 0.051	95.64 ± 0.76
$DS\text{-}SeDD_3$	7118.0 ± 398.6	0.4 ± 0.062	0.70 ± 0.054	2.48 ± 0.276	97.53 ± 1.02
$DS\text{-}SeDD_4$	5016.0 ± 24.0	0.5 ± 0.009	0.83 ± 0.014	0.39 ± 0.013	96.12 ± 0.64

3.3.2. Drug entrapment efficiency tests

High-drug encapsulation microemulsions provide several potential advantages in the context of cancer treatment.[54] First, these microemulsions can help target delivery to tumor cells and overcome drug resistance.[59,60] High drug encapsulation microemulsions' targeted drug delivery to tumors enables specific targeting of cancer cells. Because of their small sizes and customizable surface characteristics, these systems may decrease off-target effects and improve the therapeutic index by increasing accumulation at tumor sites through the enhanced permeability and retention effect.[60] Microemulsions, with the use of various drug delivery methods or drug uptake pathways, may overcome the resistance mechanisms observed in some cancer cells, thus increasing treatment efficacy.[59]

One method to measure the amount of free drug in the medium containing the dispersion is determining the DAS drug's EE. The self-emulsion system requires a high EE to ensure that the encapsulated drug remains inside the droplets. DAS's limited aqueous solubility and lipophilic characteristics prolong its retention period during the formulation's disperse phase. All SeDD formulations were used to assess DA encapsulation effectiveness. The results of the EE tests are shown in Table 2 for all DS-SeDD formulations. Although the concentration of DS did not change among formulas, the efficiency of DS encapsulation differed among SeDD formulations 1−4, ranging from 91.73% to 97.53%.

The encapsulation efficiencies for the engineered 5016.0 ± 24.0 nm and 6251.0 ± 919.2 nm formulations were 96.12 ± 0.64%, and 95.64 ± 0.76%, respectively. For DS-SeDDs with sizes of 2040.7 ± 204.7 nm (DS-SeDDs1), as determined by DLS, the EE% was 91.73 ± 0.84, the lowest value among all formulations. In contrast, the highest encapsulation efficiency (97.53 ± 1.02) was obtained for the DS-SeDD3 formula, when the amounts of oil, surfactant, and co-surfactant were 1.5 ml, 5 ml, and 3.5 ml, respectively. The retention efficiency of DS-SeDD3 and DS-SeDD4 exhibited maximum drug trapping efficiency, possibly because of the use of the SeDD technique, involving a broad particle size distribution within a microemulsion drug delivery system. Therefore, both formulations were selected for further investigation.

In pharmaceutical applications, high drug encapsulation with a broad particle size distribution may offer several benefits in cancer treatment.[57] Improved solubility of poorly water-soluble medications can increase absorption and bioavailability in the body through efficient encapsulation. Better therapeutic results may be achieved because more of the medication can be used efficiently; moreover, drugs can be released from encapsulation systems in a controlled or sustained manner, thereby enabling consistent and extended release over time. By maintaining therapeutic drug levels in the body, this controlled delivery may minimize adverse effects and enable a lower dosing frequency. These observations are consistent with the results presented by Deshmukh et al., which have confirmed that the EE% of furosemide, when loaded as SNEDS, ranges between 90.08% and 102.45%.[61]

In vitro drug release study

Treating cancer with microemulsions achieving 100% drug release in less than 24 h can be advantageous. Studies[35,62,63] assessing total drug delivery have found that 100% drug release from microemulsions within 1 day ensures maximal delivery of therapeutic agents to intended sites, thus potentially increasing the drug concentrations at tumor sites and boosting treatment effectiveness against cancer cells. Additionally, if the drug is released completely within a given time frame, it may achieve optimal therapeutic effects by reaching target cells at sufficient concentrations to achieve anticancer activity.

Cumulative DS release profiles were acquired from differently sized DS-SeDDs over 24 h (Figure 1). We observed slow release of DS (approximately 23%) after 24 h for pure DS. The release of DS from DS-SeDD3 and DS-SeDD4 after 24 h was 100 ± 5% and 88 ± 3%, respectively. The release of DS from DS-SeDD3 was higher than that from DS-SeDD4. In DS-SeDD3, the lipid carrier shell protected the drug's heterogeneous large particles from degradation over an extended period. The EE% results indicated that the DS-SeDD3 formulation had a higher DAS encapsulation efficiency in the lipid carrier at

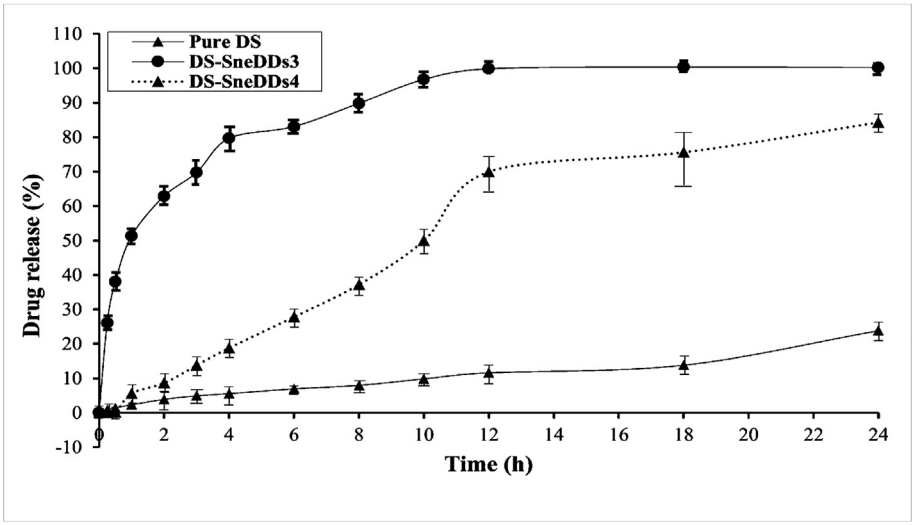

Figure 1: In vitro permeation profiles of pure DAS and DS-SeDD formulations.

97.53 ± 1.02%, compared with DS-SeDD4, at 96.12 ± 0.64%. Additionally, the release of DS from DS-SeDD3 after 24 h, with a release rate of approximately 97.53 ± 1.02%, indicated that the optimized self-emulsion's modal release showed delayed-release characteristics. The SEDDS preparation also enhanced DS solubility in the emulsion, maintained appropriate concentrations for longer times, and decreased drug release. Previous research has linked drug release delays to DAS dissolution and encapsulation in oily emulsion phase systems, wherein drug molecules must overcome diffusion through the oil droplet core, as well as diffusion across surface-active membranes. Before entering the aqueous phase, the medication is released slowly.[33] For all Das-SNEDDS formulations, as shown in Table 2, EE% ranged from 90.26% (F5) to 97.53% (F3), and the absence of significant variations in drug content among formulations indicated that the drug was distributed uniformly. Formulations F3 and D4 had the highest EE%, possibly because of the higher surfactant and co-surfactant concentrations in these two formulas, which had high drug dissolving capacity.

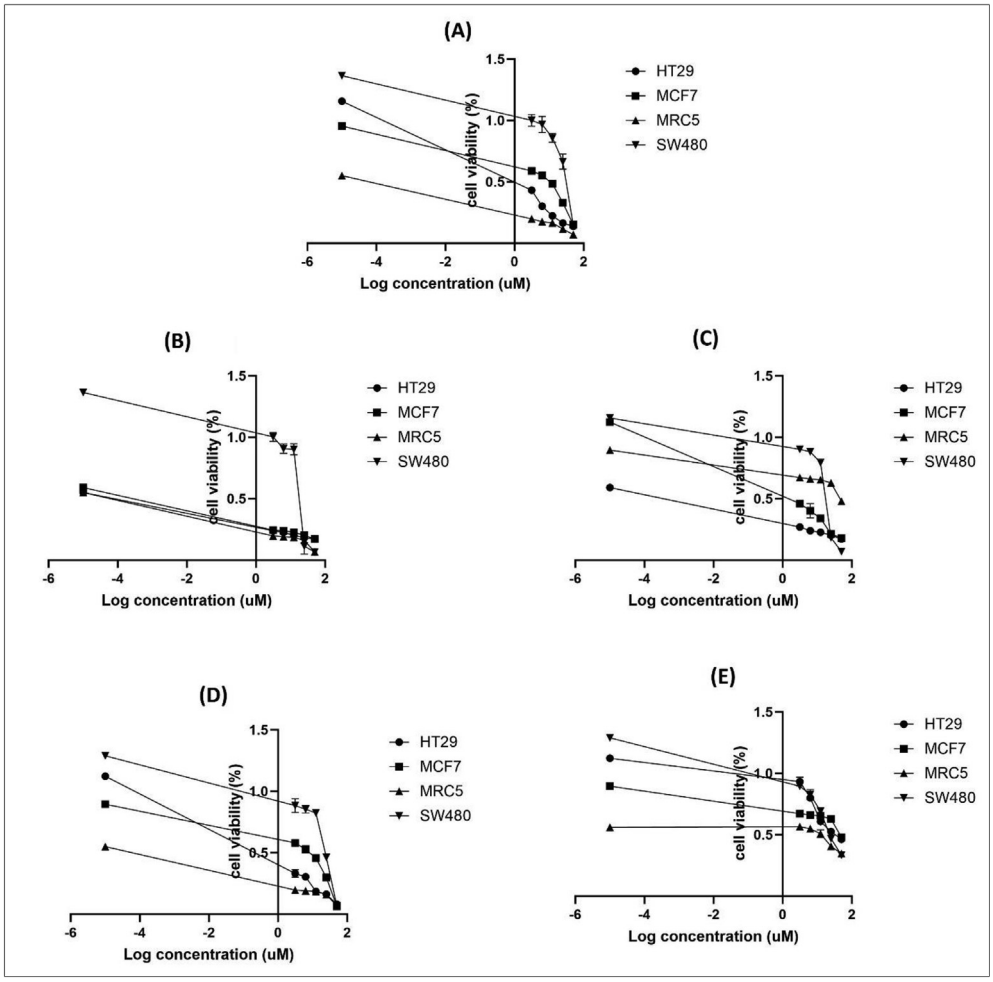

Figure 2: Concentration-dependent cytotoxicity of **(A)** pure DS, **(B)** DS-SeDD3, **(C)** SeDDs3-blank, **(D)** DS-SeDD4, and **(E)** SeDDs4-blank in three cancer cell lines, as determined with MTT assays. The information is presented as the mean ± SD of three separate replicates.

Table 3: Cytotoxic activity of DS-SeDD3, DS-SeDD4, and pure DS toward three cancer cells and normal fibroblasts (MTT 72 h, IC_{50} (μM) ± SD, n = 3).

Formulation	MCF7	HT29	SW820	MRC5
Pure DS	5.37 ± 0.05	1.46 ± 0.11	12.38 ± 1.40	0.78 ± 0.01
DS-SeDD3	0.23 ± 0.06	0.56 ± 0.01	10.60 ± 2.16	0.59 ± 0.06
SeDD3-blank	1.39 ± 0.04	0.62 ± 0.06	12.62 ± 2.25	3.17 ± 0.10
DS-SeDD4	7.50 ± 0.59	0.81 ± 0.21	10.97 ± 1.09	0.58 ± 0.06
SeDD4-blank	3.83 ± 0.19	5.92 ± 0.36	5.64 ± 0.52	17.30 ± 1.64

Table 4: Selectivity index (SI) of compound/formulations compared with normal MRC5 cells.

Formulation	MCF7	HT29	SW820
Pure DS	0.14	0.53	0.06
DS-SeDD3	2.54	1.05	0.06
SeDD3-blank	2.28	5.11	0.25
DS-SeDD4	0.07	0.71	0.05
SeDD4-blank	4.51	2.92	3.06

In vitro bio-characterization of DS-SeDDs

Cytotoxicity assays

The in vitro cytotoxicity of DS-SeDDs, pure DS, and SeDD-blank was evaluated with MTT assays on the three cancer cell lines (Figure 2 and Tables 3 and 4). MRC5 cells (normal human fetal lung fibroblasts) were used as the non-malignant control group.

In all examined cell lines, cell viability declined with increasing treatment concentration, regardless of the type of neoplastic cell line (Figure 2).

The sigmoidal curve for each studied cell type was used to determine the IC_{50} values. Compared with pure DS, DS-SeDD3 and DS-SeDD4 were more effective in killing cancer cells: in HT29 cells, the IC_{50} values of DS-SeDDs3 and DS-SeDDs4 were 0.56 ± 0.01 and 0.81 ± 0.21 μM, respectively; in SW820 cells, the values were 10.60 ± 2.16, and 10.97 ± 1.09 μM, respectively (Table 3). In comparison to observations in HT29 cells, the IC_{50} concentrations of DS-SeDD3 were lower than those of pure DS by approximately 1.46 fold, whereas the DS-SeDD4 IC_{50} were lower than those of pure DS by approximately 1.79 fold. For SW820, the DS-SeDD3 and DS-SeDD4 IC_{50} concentrations were lower than those of pure DS by 1.17 and 1.13 fold, respectively.

DS-SeDD4 was marginally less efficient against MCF-7 than against HT29 and SW820 (Figure 2 and Table 2). Interestingly, DS-SeDD3 appeared to show the highest efficiency against MCF7 and HT29 cells, rather than against normal MRC5 cells (Table 3). The IC_{50} of pure DS in SW820 cells was 12.38 ± 1.40 μM, a value higher than its IC_{50} in the other types of cancer cells (Table 2 and Figure 2-A). The IC_{50} values of DS-SeDD3 and DS-SeDD4 in SW820 cells were 10.60 ± 2.16 and 10.97 ± 1.09 μM, respectively, values higher than the IC_{50} values in the other cancer cell lines tested. Additionally, in MCF7, HT29, and SW820 cells, DS-SeDD3 had IC_{50} values 23.35, 2.61, and 1.17 times lower, respectively, than that of pure DS.

To avoid the possibility of toxic effects of SeDD ingredients (isopropyl myristate, Labrafil, and PEG), we used the aforementioned cell lines for cytotoxicity experiments with SeDD-blank, particles lacking DS. Neither cancer cell lines nor normal fibroblasts were harmed by the SeDD-blank.

The in vitro cytotoxicity of SeDD-blank was examined to demonstrate that the empty microemulsion was not harmful to cells. According to published research, apoptosis, manifesting as chromatin condensation and nuclear blebbing, is an indicator of emulsion toxicity.[51] Our MTT assay results demonstrated that cells treated with SeDD-blank did not show a decline in viability over 3 days.

As anticipated, pure DS exhibited less potency against the tested cancer cell lines than DS-SeDDs. Other likely explanations for the low toxicity of pure DS in cancerous cells might be its lower solubility than DS-SeDDs and its minimal intracellular retention.

DS is well known to have low solubility in water, which may restrict its ability to dissolve in aqueous solutions. DS can also be hydrolyzed in solutions containing water, thus potentially decreasing its availability and stability in aqueous conditions.

In scenarios in which DS has low solubility and is prone to hydrolysis in water-based solutions, only a small fraction of the drug may dissolve and remain intact, whereas the rest may precipitate or degrade. This limited dissolution and stability in water-based environments might influence its bioavailability and cellular uptake. However, even a small portion of DS that is dissolved and remains non-degraded in the solution could potentially be internalized by cells. Cell uptake mechanisms vary according to the physicochemical properties of the drug and the cellular environment.

DS-SeDDs, in contrast, were much more effective against cancer cells than the pure DS, according to our MTT assay data, and the potency was size-dependent: larger DS-SeDDs resulted in lower IC_{50} values against cancer cells. Ying et al. have reported noticeable drug accumulation in cells treated with nanocarriers, possibly as a result of the formulations' enhanced penetration and retention.[64]

Conclusion

In recent years, self-emulsifying formulations have emerged as promising drug delivery systems because of their potential to improve lipophilic drugs. In this study, DS-SeDDs were successfully prepared, and their relative in vitro drug release efficacy and cytotoxicity were demonstrated against the cancer cell lines HT29/SW420 (human colorectal carcinoma) and MCF7 (human breast adenocarcinoma), as well as MRC5 normal human fetal lung fibroblasts. The developed DS-SeDDs may provide substantial advantages as potential DS delivery systems achieving more effective and sustained drug treatments for cancers.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

There are no ethical issues associated with this work. The authors declare that no ethical approval was needed, and no animals or patients were included in the study.

Authors contributions

RB, AT, AA, and MA designed and conceived the study, conducted research, provided research materials, and organized and collected data. AT and RB analyzed and interpreted data. RB and AT wrote the initial and final drafts of the article, and provided logistic support. The final draft of the manuscript has been critically reviewed and approved by all authors, who also bear responsibility for its content and similarity index. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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