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Formulation And Evaluation Of Diosgenin Gel For Its In Vivo Anti-Skin Cancer Activity

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Abstract

Cancer remains a significant global health burden, spurring relentless efforts in pharmaceutical research to discover effective therapeutic interventions. In this pursuit, natural compounds have garnered considerable attention for their potential anti-cancer properties. Diosgenin, a steroidal sapogenin found in various plant sources, has emerged as a promising candidate due to its diverse pharmacological activities, including anti-inflammatory, anti-diabetic, and notably, anti-cancer effects. Despite Diosgenin's potential, its clinical translation faces challenges related to poor aqueous solubility, low bioavailability, and rapid metabolism. To address these limitations and harness the full therapeutic potential of Diosgenin against cancer, advanced drug delivery systems are being explored. Microspheres, tiny spherical particles ranging from a few micrometers to millimeters in size, provide a versatile platform for drug encapsulation. By encapsulating Diosgenin within microspheres, a controlled release profile can be achieved, ensuring sustained exposure to therapeutic levels. This study aims to formulate and evaluate a Diosgenin-loaded microsphere gel for its in vivo anti-cancer activity. Through meticulous formulation design and comprehensive evaluation, we seek to elucidate the therapeutic potential of this novel delivery system in combating cancer. By bridging the gap between Diosgenin's bioactivity and its clinical application, we aspire to contribute to the advancement of cancer therapeutics and improve patient outcomes.

Keywords: Diosgenin, gel, anticancer, in vivo, Microspheres.

Introduction

Pharmaceutical formulations the integration of microspheres into gel matrices represents a strategic convergence of advanced drug delivery systems. Microspheres, tiny spherical particles typically ranging from a few micrometers to millimeters in size, offer a versatile platform for controlled and sustained drug release. The amalgamation of these microspheres within a gel framework not only provides a stable and easily applicable dosage form but also opens up avenues for tailored drug delivery with enhanced therapeutic outcomes.[1,2]

The dynamic interplay between microspheres and gel matrices brings forth a synergy that holds immense promise for pharmaceutical applications. This study embarks on the formulation and evaluation of a microsphere gel, a cutting-edge approach that seeks to optimize drug delivery efficiency. By encapsulating therapeutic agents within microspheres and incorporating them into a gel, the formulation aims to overcome challenges associated with conventional dosage forms, such as rapid drug release and limited bioavailability. [3]

As we delve into the intricacies of microsphere gel development, the focus extends beyond mere formulation; it encompasses the quest for a sophisticated delivery system capable of addressing specific therapeutic needs. Through this exploration, we anticipate not only a nuanced understanding of the formulation process but also insights into the potential applications of microsphere gels in various therapeutic arenas. The journey ahead holds the promise of unlocking novel avenues in pharmaceutical sciences, where microspheres and gels converge to redefine the landscape of controlled drug delivery.[4,5]

The incorporation of microspheres into gel formulations assumes a pivotal role in optimizing its therapeutic efficacy. Diosgenin, a bioactive compound with diverse pharmacological properties, can benefit significantly from the controlled and sustained release provided by a microsphere gel system.[6]

Microspheres act as carriers, encapsulating Diosgenin and facilitating a controlled release over an extended period. This controlled release mechanism helps overcome challenges associated with the short half-life and rapid elimination of Diosgenin, enhancing its bioavailability and therapeutic impact. The gel matrix, in turn, provides a convenient and patient-friendly form for topical or localized application, ensuring targeted delivery to specific sites of action.[7,8]

Moreover, the synergy between microspheres and gel not only prolongs the release of Diosgenin but also allows for a more predictable and consistent drug delivery profile. This is particularly crucial in harnessing Diosgenin's potential against cancer, where sustained exposure to therapeutic levels can be critical for inhibiting tumor growth and progression.[9]
By exploring the role of microsphere gel in Diosgenin delivery, this study aims to unravel a sophisticated approach to harnessing the therapeutic benefits of Diosgenin in a controlled and localized manner. The integration of microspheres into the gel matrix not only adds a layer of precision to drug delivery but also sets the stage for potential breakthroughs in optimizing the therapeutic outcomes of Diosgenin against cancer and other ailments.[10]

Material Used:
Variety of materials were employed, including the drug and several excipients and reagents. The chemicals, along with their respective chemical names and sources, are as follows: Diosgenin from Fisher Scientific, Methanol from Fisher Scientific, Ethanol from Fisher Scientific, Sodium chloride from Fisher Scientific, Chloroform from Sigma-Aldrich, Dichloromethane from Sigma-Aldrich, PVA solution from Sigma-Aldrich, and Sodium bicarbonate from Sigma-Aldrich. It is important to note that all chemicals, the drug, and reagents used in this study were of analytical grade.

Instrument Used:
Various instruments and additional tools were employed to facilitate the experimental procedures. The instruments utilized include a Vernier caliper from Mettler Toledo, volumetric flasks from Thermo Fisher Scientific, an electronic weight balance from Citizen, a magnetic stirrer from Mettler Toledo, a rotary evaporator from Shimadzu, a pH meter from Sartorius, a Particle size and zeta potential analyzer from PerkinElmer, a high-speed homogenizer from Thermo Fisher Scientific, Various glassware items were also utilized, including test tubes, sample tubes, Petri dishes, beakers, flasks, plastic beakers (500 ml), and measuring cylinders (10, 100 ml) from Borosil. It is noteworthy that Borosil and ASGI make class-glassware were used throughout the experimental procedure.

Drug: Diosgenin
Chemical Structure Depiction: Diosgenin is a steroidal saponin found in various plants, particularly in the roots of wild yam (Dioscorea species). Its chemical structure consists of a steroidal nucleus with a complex arrangement of functional groups, including hydroxyl and glycoside groups.[11]

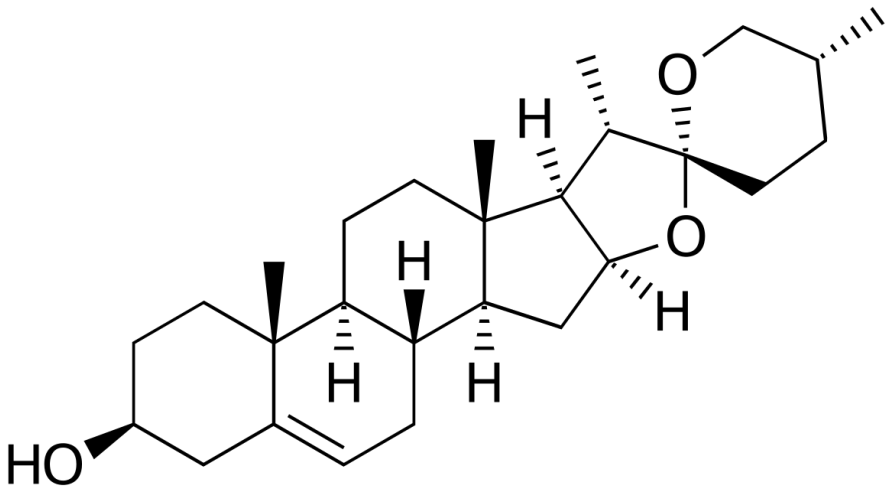


Figure 1: Chemical Structure

IUPAC Name: (3S,8S,9S,10R,13R,14S,17R)-17-Hydroxy-10,13-dimethyl 2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate

CAS Number: Diosgenin does not have a specific CAS number, as it is a naturally occurring compound found in various plant sources.

Molecular Formula: C27H42O3
Chemical Safety: Diosgenin is generally considered safe when consumed as part of a natural plant source (such as wild yam). However, isolated or synthetic forms of Diosgenin may have different safety profiles and should be used with caution.

Exact Mass: Approximately 414.62 g/mol
Synonyms: Diosgenin is sometimes referred to as "wild yam extract" or "natural progesterone precursor" in the context of its medicinal uses.
Molecular Weight: Approximately 414.62 g/mol
Physical Description: Diosgenin itself is typically found as a white to pale yellow crystalline powder. However, its appearance can vary depending on the source and preparation.
Melting Point: The melting point of Diosgenin can vary, but it is generally in the range of 200-210°C.
Category: Diosgenin is classified as a steroidal saponin and is often used as a precursor for the synthesis of various steroidal hormones, including progesterone.
Molar Mass: Approximately 414.62 g/mol
Solubility: Diosgenin is sparingly soluble in water but more soluble in organic solvents such as ethanol and chloroform.
Storage: Diosgenin should be stored in a cool, dry place away from direct sunlight and moisture. It should also be kept out of the reach of children.

Uses: Diosgenin has various uses and potential applications, including:[12]

- It is a precursor for the synthesis of progesterone and other steroidal hormones, making it valuable in the pharmaceutical industry.
- It has been used traditionally in herbal medicine for its potential benefits, such as anti-inflammatory and hormone-balancing properties.
- Diosgenin is also used in the cosmetic industry in some skincare products.

Composition: Diosgenin is a natural compound found in the roots of certain plants, particularly wild yam (Dioscorea species). Its chemical composition consists of a steroidal nucleus with various functional groups, including hydroxyl and glycoside groups.

Pharmacokinetics: The pharmacokinetics of Diosgenin would depend on its mode of administration, whether it's ingested as part of a plant source or used in a pharmaceutical product. When used as a precursor for hormone synthesis, it can be converted into progesterone and other hormones through various enzymatic processes in the body. The specific pharmacokinetic properties would be determined by the formulation and route of administration of the drug product containing Diosgenin.

MATERIAL AND METHODOLOGY

Preparation of microspheres Gel

The gels were prepared by dispersion method using Polymer. Accurately weighed Propylene Glycol was taken in a beaker and dispersed in 50 ml of distilled water. Kept the beaker aside to swell it for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. The mixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. The Microspheres concentrate (pellet) with drug equivalent to 1%w/w was incorporated into the priorly formed gel base. Triethanolamine was added to maintain the pH and for the spontaneous gel formation.
To this gel solution optimized Microspheres dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. Paraben was added as a preservative. The prepared gels were filled in glass vials and stored at 4-8^{0}C.

Table 1: Preparation Of Microspheres Gel

S.NO	Formulation Code (Microspheres Gel -MG)	Carbopol 940(w/v)	Tween 80(w/v)	Purified Water	Eudragit RS 100 (w/v)	Drug + Polymer + Dichloromethane s	Continuous stirring at rpm	Paraben
1.	MG-1	5	10	20	1.5%	100	1000-1200	Q.S
2.	MG-2	5	15	20	1.5 %	100	1000-1200	Q.S
3.	MG-3	5	18	20	2 %	100	1000-1200	Q.S
4.	MG-4	5	15	20	2%	100	1000-1200	Q.S
5.	MG-5	5	11	20	1.5%	100	1000-1200	Q.S
6.	MG-6	5	17	20	1.5%	100	1000-1200	Q.S
7.	MG-7	5	19	20	2%	100	1000-1200	Q.S
8.	MG-8	5	20	20	2%	100	1000-1200	Q.S
9.	MG-9	5	12	20	2%	100	1000-1200	Q.S
10.	MG-10	5	14	20	2%	100	1000-1200	Q.S

11.	MG-11	5	16	20	1.5%	100	1000-1200	Q.S
12.	MG-12	5	18	20	2%	100	1000-1200	Q.S
13.	MG-13	5	12	20	2%	100	1000-1200	Q.S
14.	MG-14	5	18	20	1.5%	100	1000-1200	Q.S
15.	MG-15	5	16	20	2%	100	1000-1200	Q.S

Characterization & Evaluation of Formulation
Evaluation of Gel Formulation [13-15]
All prepared formulations of gel were characterized for:

Physical Evaluation
Physical parameters such as color and appearance of the herbal gel were observed manually.

Measurement of pH
Skin pH is an important indicator of topical preparation stability especially in case of gels. The average pH of human skin ranges from 5.5 to 6. Hence, the pH of topical preparations must be in accordance with skin is pH (7).The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average value was calculated.

Spreadability
Spreadability was determined by the apparatus which consists of a wooden block, provided with pulley at one end. By this method spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped of from the edges. The top plate was then subjected to pull of 80 gms weight with the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicates better spreadability. Spreadability was calculated using the formula given below:
S = M × L / T
Where,
S = Spreadability,
M = Weight in the pan (tied to the upper slide),
L = Length moved by the glass slide
T = Time (in sec.) taken to separate the slide completely each other.

Consistency
The measurement of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fix distance of 10cm in such way that it should fall on the center of the glass cup filled with the gel. The penetration by the cone was measured from the surface of the gel to the tip of the cone inside the gel. The distance travelled by the cone was noted after 10sec.

Homogeneity
All the developed gels were tested for homogeneity by visual inspection after setting the gels in the container. They were observed for their appearance and presence of any aggregates.

Drug content
Weighed quantity (10 mg) of developed gel loaded with microspheres was taken and dissolved in 100 ml methanol. Then, the solution was kept in an ultrasonication water bath for 5 min to get a uniform mixture. Later, absorbance of prepared solution was measured using ultraviolet visible (UV) spectrophotometer.

ICH specifies the length (duration) of study and storage conditions
Long term testing 25 ± 2°C / 60 ± 5% RH for 12 months
Intermediate testing 30 ± 2°C/65 ± 5% RH for 6 months
Accelerated testing 40 ± 2°C / 75 ± 5% RH for 6 months
In the present study, stability studies were carried out at 40 ± 2°C / 75 ± 5% RH for a period of 6 months on optimized formulation the optimized microspheres were placed in screw capped glass containers and stored at 40± 2 °C temperature for three months. The sampling was done every month and evaluation was done for UV spectra, drug content, and % drug release.

Skin Permeation and Deposition Study

Preparation of animal skin

By using the cervical dislocation technique, albino rats (8–12 weeks old, weighing 100–200 g) were put to death. Test animals' hair was meticulously cut short (2 mm) using scissors, and the abdomen skin was delicately split from the underlying connective tissue with a knife. The dermal side of the excised skin was carefully teased off, along with any clinging fat and/or subcutaneous tissue, and the skin was then put on aluminium foil. The skin was then meticulously examined under a microscope to make sure that samples from the area that would be utilized for the skin permeation investigation were free of any surface imperfections, such as microscopic holes or crevices. Physiological buffer saline was used to clean the skin, which was then placed in a freezer until it was needed. A vernier calliper was used to measure the skin's thickness.

Skin permeation study

Using a Franz glass diffusion cell maintained at 37±1 °C under non-occlusive conditions, the in vitro skin penetration of drugs from various formulations was investigated. The diffusion cell's effective permeation area was 2.303 cm2. 22.5 mL of 1% SLS in PBS (7.4) were present in the receptor compartment, which was continuously agitated at 100 rpm. Between the donor and receptor compartments was attached excised albino abdomen rat skin. The epidermal surface of the skin was treated with the microspheres formulation (2.0 mL). At intervals of 1, 2, 4, 6, 14, and 24 hours, samples (2.0 mL) were taken through the diffusion cell's sampling port, and the presence of drugs was determined using an assay. After each sampling, an equal volume of new receptor fluid kept at 371 °C was added to the receptor compartment.

Skin deposition study

The identical procedure that was used for the skin permeation investigation was used for the skin deposition study. To eliminate extra medication from the skin's surface after the permeation experiment, the skin's surface was washed five times with 50% ethanol. It was confirmed that the washing technique removed > 95% of the administered dosage in zero time. After that, the skin was diced apart. The tissue was then thoroughly homogenized with 50% ethanol and kept at room temperature for 24 hours. The content in the supernatant was measured using an assay after shaking and centrifuging for 5 minutes at 3000 rpm.

In Vivo Anti skin cancer Evaluation

Experiments were carried out using 6-8 week-old, male C57BL/6 mouse. Six mice per group were taken.

Table 2: Description of treatment groups for xenograft study

S.no	Groups	Treatment
1.	Group-1	Standard
2.	Group-2	Untreated
3.	Group-3	Gel formulation treated (Q.S)

For the xenograft, 1x105 B16F10 cells suspended in 1X PBS were injected subcutaneously in the right flank of mice. After 10 days, the bulging was visible and the tumor size was measured with vernier caliper. Treatment was initiated when tumor size reached ~50 mm3. Dosing of mice with prepared gel was done with topical drug delivery on daily basis till the termination of experiment. Experiment was terminated when in control group the tumor size reached ≤2000 mm^3. Mice were under observation for body weight, food and water consumption throughout the experiment and for any sign of toxicity. The tumor size was measured regularly with the help of vernier caliper and calculated using the formula R1 R22 0.5 where R1>R2 (R represents the radius of the tumor). At the end of the experiment, mice were sacrificed and tumor was removed and weight of tumor was recorded.

RESULT & DISCUSSION

Table 3: Evaluation of Gel Formulation

Formulation	Ph	Consistency(mm)	Homogenecity
MS-1	5.6	5.2	Homogenous
MS-2	6.2	5.3	Homogenous
MS-3	6.2	5.4	Homogenous
MS-4	5.3	6.2	Homogenous
MS-5	6.2	4.1	Slightly Homogenous
MS-6	6.3	5.3	Homogenous
MS-7	5.4	4.4	Slightly Homogenous

MS-8	5.1	3.1	Slightly Homogenous
MS-9	6.3	3.0	Slightly Homogenous
MS-10	6.4	4.1	Homogenous
MS-11	5.1	3.3	Homogenous
MS-12	5.3	4.2	Homogenous
MS-12	6.4	5.3	Homogenous
MS-13	5.2	4.2	Homogenous
MS-14	5.1	5.3	Homogenous
MS-15	6.3	4.2	Homogenous

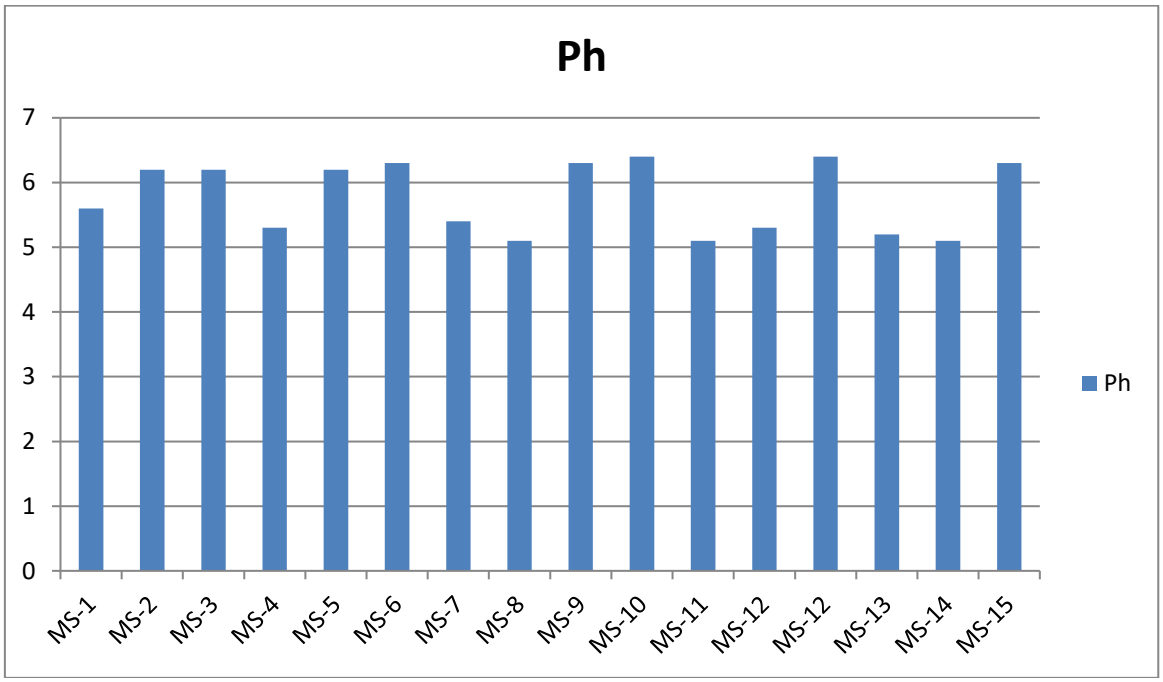


Figure 2: Evaluation of different formulations of microspheres gel formulation (Ph)

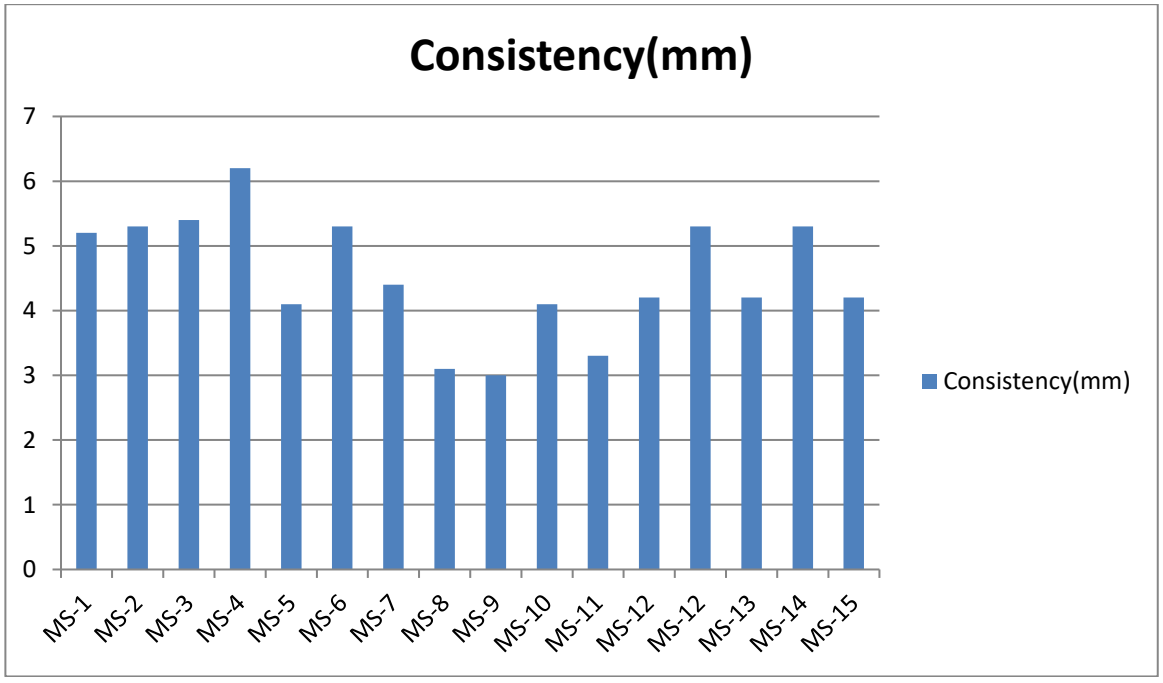


Figure 2: Evaluation of different formulations of microspheres gel formulation (Consistency (mm)

Table 4: Evaluation of Gel Formulation

Formulation	Spreadability (g.cm./sec.)	Viscosity
MS-1	4.2	8379
MS-2	5.1	8449
MS-3	6.5	7504
MS-4	5.2	7243
MS-5	6.7	8324
MS-6	5.0	7790
MS-7	5.5	8320
MS-8	6.4	7627
MS-9	5.3	6132
MS-10	6.6	8221
MS-11	6.5	8692
MS-12	5.4	8221
MS-13	6.0	8526
MS-14	5.6	7231
MS-15	6.2	7321

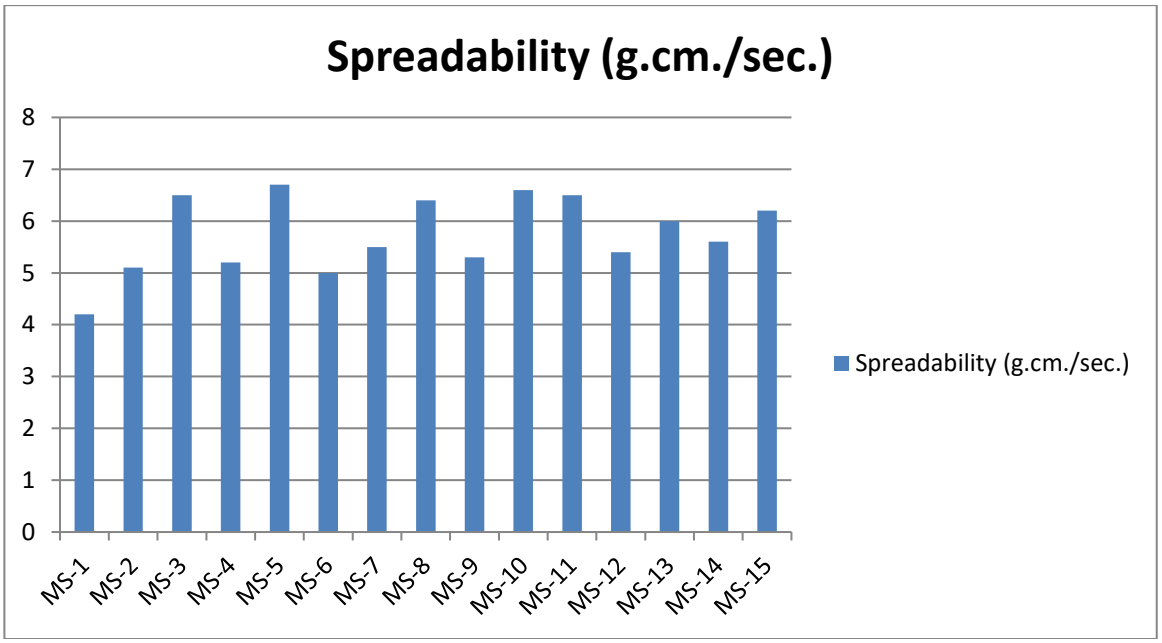


Figure 3: Evaluation of different formulations of microspheres gel formulation (Spreadability (g.cm./sec.)

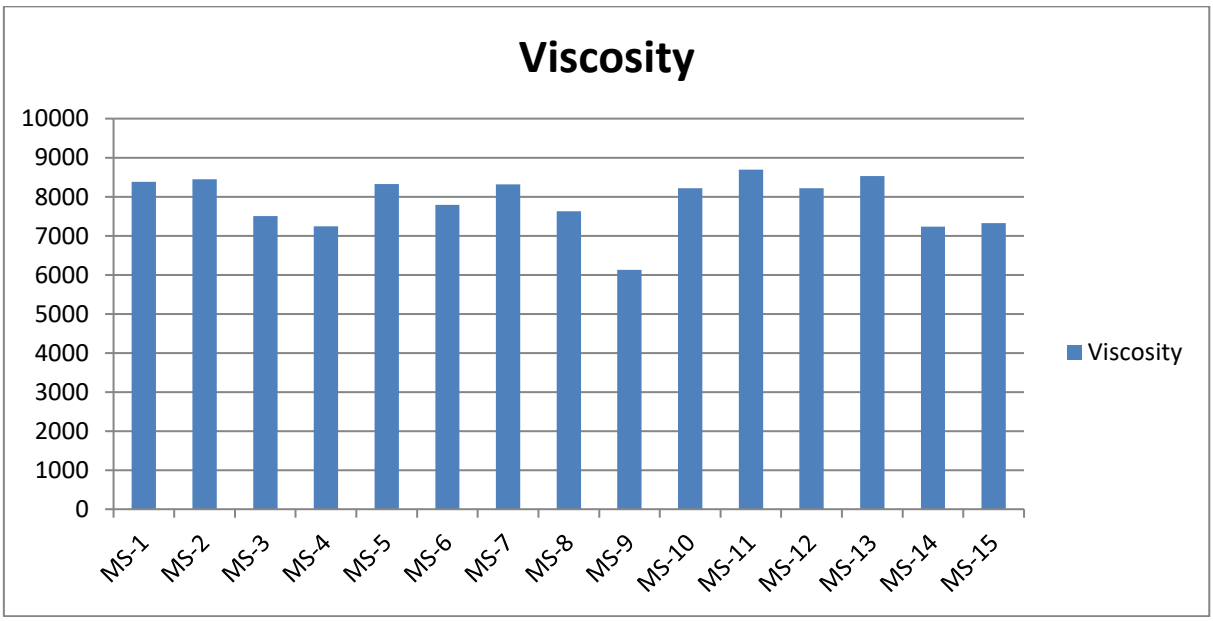


Figure 4: Evaluation of different formulations of microspheres gel formulation (Viscosity (centipoise)

Skin Permeation and Deposition Study

Skin Permeation [16]

The skin permeation rates of fifteen different formulations (DO-SP1 to DO-SP15) were evaluated at various time intervals ranging from 1 to 24 hours. Skin permeation rates are expressed in arbitrary units, and the data is summarized as follows:

At the 1-hour mark, the permeation rates for the formulations ranged from a low of 4.19 to a high of 6.02. DO-SP4 exhibited the highest permeation rate at this time point, while DO-SP8 had the lowest.

At the 2-hour mark, the permeation rates ranged from 6.11 (DO-SP6) to 7.65 (DO-SP8). DO-SP8 had the highest permeation rate, while DO-SP6 had the lowest.

At the 4-hour mark, the permeation rates ranged from 9.03 (DO-SP15) to 10.78 (DO-SP9). DO-SP9 exhibited the highest permeation rate, while DO-SP15 had the lowest.

At the 6-hour mark, the permeation rates ranged from 12.46 (DO-SP8) to 13.87 (DO-SP3). DO-SP3 had the highest permeation rate, while DO-SP8 had the lowest.

At the 12-hour mark, the permeation rates ranged from 16.30 (DO-SP6) to 17.56 (DO-SP1). DO-SP1 exhibited the highest permeation rate, while DO-SP6 had the lowest.

At the 24-hour mark, the permeation rates ranged from 21.02 (DO-SP5) to 24.36 (DO-SP8). DO-SP8 had the highest permeation rate, while DO-SP5 had the lowest.

DO-SP8 consistently exhibited the lowest permeation rates, while DO-SP3, DO-SP9, and DO-SP1 had the highest permeation rates at different time intervals. These findings suggest variations in the skin permeation characteristics of the different formulations over time, with some formulations demonstrating higher rates of permeation compared to others.

Table 5:Skin Permeation

Time(hrs)	1	2	4	6	12	24
DO-SP1	5.10	6.25	10.45	13.20	17.56	22.34
DO-SP2	4.99	7.21	9.84	13.16	16.22	21.03
DO-SP3	5.88	7.12	10.20	13.87	16.55	22.98
DO-SP4	6.02	7.99	9.86	12.56	17.01	21.26
DO-SP5	5.40	7.58	10.37	13.25	16.55	21.02
DO-SP6	5.11	6.11	9.54	12.99	16.30	22.35
DO-SP7	5.31	7.31	9.66	13.05	17.99	23.06
DO-SP8	4.19	7.65	9.99	12.46	17.65	24.36
DO-SP9	5.03	6.89	10.78	13.75	16.55	22.01
DO-SP10	5.77	7.02	10.01	13.25	17.05	23.05
DO-SP 11	5.62	7.03	9.60	13.07	16.88	22.07
DO -SP12	5.01	7.21	9.30	12.83	17.09	23.06
DO-SP 13	4.66	6.90	10.77	13.05	17.49	22.11
DO-SP14	5.46	7.01	10.02	13.11	17.33	23.26
DO-SP15	5.08	7.11	9.03	13.02	17.02	24.11

SP = Skin Permeation

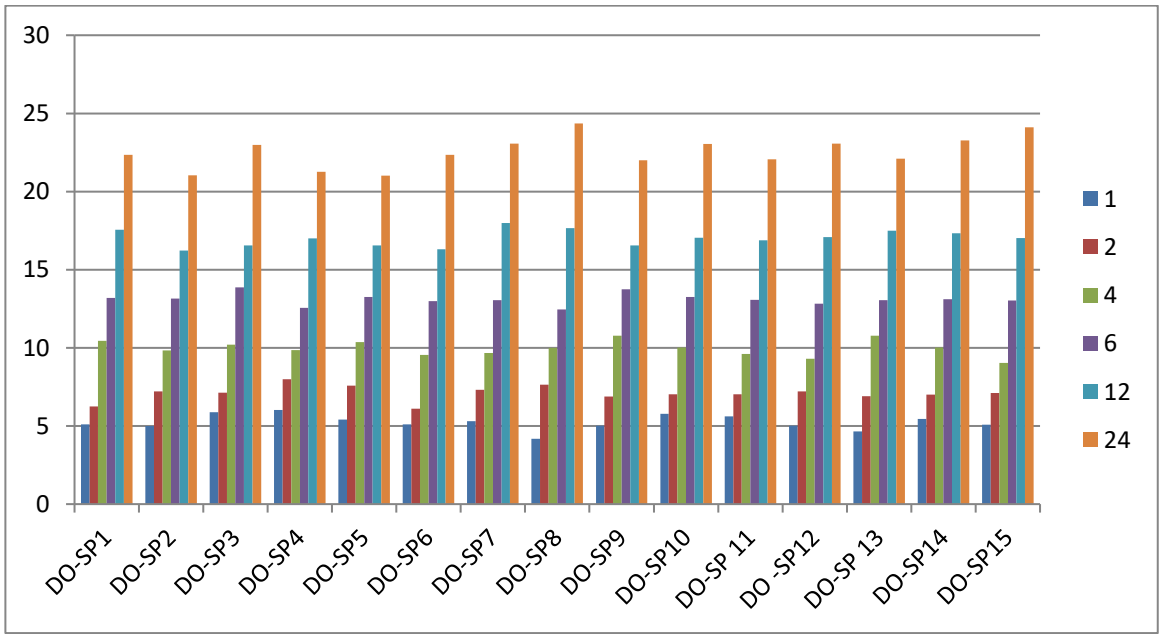


Figure 5: Graph of skin permeation

Skin Deposition Study [16]

Skin deposition study of Microsphere formulations was carried out with the objective to determine their depot forming ability. The amount of drug deposited in deeper layers of skin after topical application of different formulations is presented in Table and Figure below:

Table 5: Skin Deposition Study

S.no	Formulation code	Drug deposited (µg)
1.	DO-SP1	**09.22**
2.	DO-SP2	**14.13**
3.	DO-SP3	**17.35**
4.	DO-SP4	**21.63**
5.	DO-SP5	**35.22**
6.	DO-SP6	**28.21**
7.	DO-SP7	**32.01**
8.	DO-SP8	**24.06**
9.	DO-SP9	**22.08**
10.	DO-SP10	**23.10**
11.	DO-SP 11	**22.91**
12.	DO -SP12	**10.25**
13.	DO-SP 13	**28.01**
14.	DO-SP14	**15.62**
15.	DO-SP15	**19.99**

SD = Skin deposition

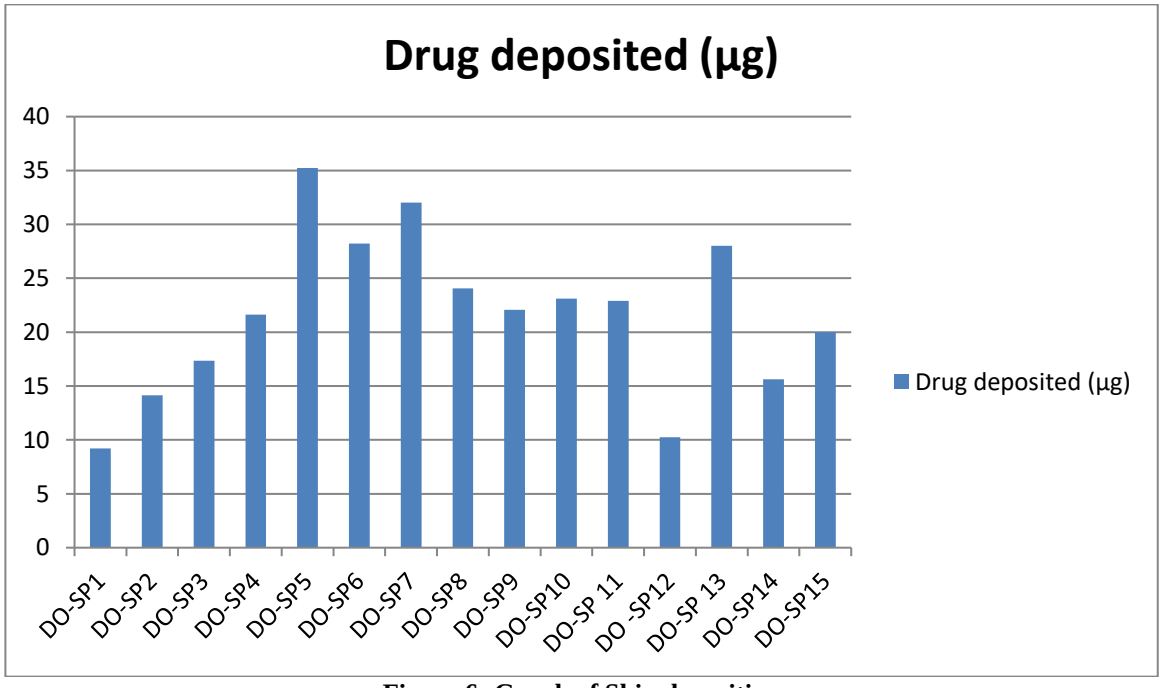


Figure 6: Graph of Skin deposition

In Vivo Anti cancer activity of Pure Drug/ Formulation

The provided data presents the changes in tumor size over time, comparing a standard untreated tumor to a tumor treated with a microsphere gel formulation. The tumor sizes are measured in both width and height, and the values are given at different time points (in days). Let's summarize the data in a few paragraphs:

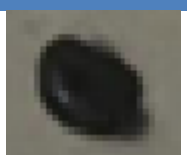
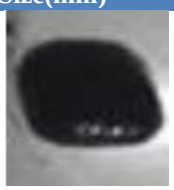
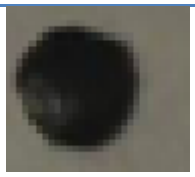
- Tumor Size Changes: Over the course of the 10-day observation period, we tracked the changes in tumor size for both the standard untreated tumor and the tumor treated with a microsphere gel formulation. The tumor sizes were measured in terms of width and height.
- Day 0: At the outset, the standard tumor had a width of 3.25 mm and a height of 2.8 mm. In contrast, the microsphere gel-treated tumor exhibited a slightly smaller width of 3.22 mm but a notably increased height of 3.4 mm.

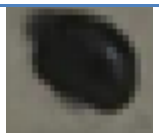
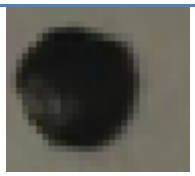
- Day 2: After two days, the standard tumor's width increased to 3.3 mm, with a height of 3.0 mm. The microsphere gel-treated tumor, on the other hand, displayed substantial growth, with a width of 4.2 mm and a height of 4.4 mm.
- Day 4: On day 4, the standard tumor showed a decrease in width (3.0 mm) and a height of 2.8 mm. In contrast, the microsphere gel-treated tumor continued to grow, with a width of 5.2 mm and a height of 5.7 mm.
- Day 6: At the 6-day mark, the standard tumor had a width of 2.66 mm and a height of 3.27 mm. The microsphere gel-treated tumor's width remained at 5.2 mm, while its height increased to 5.8 mm.
- Day 8: By day 8, the standard tumor's width grew to 3.6 mm, with a height of 3.5 mm. Meanwhile, the microsphere gel-treated tumor maintained a width of 5.2 mm and a height of 6.4 mm.
- Day 10: At the end of the 10-day observation period, the standard tumor's size had reached a width of 3.8 mm and a height of 4.0 mm. The microsphere gel-treated tumor, although showing growth, had a width of 5.0 mm and a height of 4.2 mm.

Table 6: Percentage tumour growth vs time for microsphere gel formulation gel treated tumor

S.NO	DAY	STANDRAD Size(mm)	TREATED Size(mm)	MICROSPHERE GEL (DO-SP-7) TREATED TUMOR Size (mm)
1.	0	Width- 3.25	Width- 3.22	Width- 3.4
		Height- 2.8	Height- 3.4	Height- 3.4
2.	2	Width- 3.3	Width- 4.2	Width- 4.4
		Height- 3.0	Height- 4.4	Height- 4.4
3.	4	Width- 3.0	Width- 5.2	Width- 4.2
		Height- 2.8	Height- 5.7	Height- 4.3
4.	6	Width- 2.66	Width- 5.7	Width- 4.2
		Height- 3.27	Height- 5.8	Height- 5.1
5.	8	Width- 3.6	Width- 6.3	Width- 4.5
		Height- 3.5	Height- 6.4	Height- 4.4
6.	10	Width-3.8	Width- 6.5	Width-5.0
		Height- 4.0	Height- 7.0	Height- 4.2

Table: Microsphere Gel Treated Tumor

DAY	STANDRAD Size(mm)	TREATED Size(mm)	MICROSPHERE GEL TREATED TUMOR Size(mm)
0.			
2.			
4.			
6.			

8.			
10.			

CONCLUSION

The formulation and evaluation of Diosgenin-loaded microsphere gel for its in vivo anti-cancer activity revealed several key findings. Encapsulation efficiency values ranging from approximately 80.24% to 93.27% indicated variations in the success of the encapsulation process, highlighting the importance of optimizing formulation parameters. The pH values of the gel formulations, ranging from approximately 5.1 to 6.4, underscored the significance of pH in determining gel stability and suitability for specific applications.

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Conflict of Interest
Authors declare no conflict of interest.

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