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Development and Evaluation of Hesperetin- Loaded Solid Lipid Nano-particles

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ABSTRACT

The present research was based on the Development and Evaluation of Hesperetin- Loaded Solid Lipid Nano-particle. The hesperitin was obtained from the Sigma-Aldrich, India. Stearic acid, Span 20, Tweens 80 (Sigma Aldrich, India), distilled water, ethanol were procured from the local chemical shop in Haridwar, UK. In Preformulation studies, various parameters i.e., organoleptic properties, melting point, solubility, Drug excipients compatibility studies and Preparation of Standard Calibration Curve. Preparation of gallic acid- based solid lipid nanoparticles was done using spray-drying method. It was characterized for various parameters i.e., Physical appearance, Entrapment efficiency, Drug content determination, pH determination, Determination of particles size & PDI, SEM analysis, In vitro drug release and Stability studies. Hesperitin showed very poor solubility in distilled water and ethanol. Both stearic acid and cholesterol dissolved it freely. Formulations F1-F6 were observed to be clear, white and homogenous in appearance. After 6 hours, SLN 1, SLN 2, SLN 3 showed % drug release as 89.3±0.4, 92.6±0.3 and 91.4±0.2, respectively. While SLN 4 demonstrated increased % release as 92.9±0.2 %. In conclusion, among the several forms of solid lipid nanoparticles of hesperitin, F5 was found to be the most significant formulation in terms of in-vitro drug release, droplet size, and drug content. It also showed improved stability, with no significant change in pH, % drug release and physical appearances after being stored for a month.

Keywords: *Solid lipid nanoparticles, Hesperetin, in-vitro drug release, Drug excipients compatibility.*

1. INTRODUCTION

Nanoparticles are solid particles having a size b/w 10-1000nm. To entrap/ attach the drug, a nanoparticle matrix is used. Biodegradable nanoparticles, especially those wrapped in hydrophilic polymers such as polyethylene glycol [1][2]. Nanoparticles are appealing for such applications because of their valuable & unique features i.e., surface to mass ratio which is greater than other particles and allowing for catalytic reaction with power to adsorb other compounds [3]. Liposomes were used as carriers with specific usefulness i.e., protecting from degradation/targeting to the action site, and reducing toxicity or adverse reactions, but their techniques are limited due to inherent issues such as low encapsulation efficiency, quick spillages of liquid drugs, and poor storage stability [4].

The medication is encased in polymeric nanocarriers in a spherical polymeric matrix. Polymer-based particles made of poly are a nice example. On the other hand, various forms of nanoparticles have been investigated for medical targeting. For instance, dendrimers are now being created [5].

Chemically, hesperetin is a trihydroxyflavone with three hydroxy groups located at positions 3, 5, and 7, with an additional methoxy substitute also present at position 4. Hesperetin is a trihydroxyflavanone having the three hydroxy gropus located at the 3', 5 and 7-positions and an additional methoxy substituent at the 4'-position. It has a role as an antioxidant, an antineoplastic agent and a plant metabolite. It is a monomethoxyflavanone, a trihydroxyflavanone, a member of 3'-hydroxyflavanones and a member of 4'-methoxyflavanones. It is a conjugate acid of a hesperetin(1-). Hesperetin belongs to the flavanone class of flavonoids. Hesperetin, in the form of its glycoside [hesperidin], is the predominant flavonoid in lemons and oranges [6].

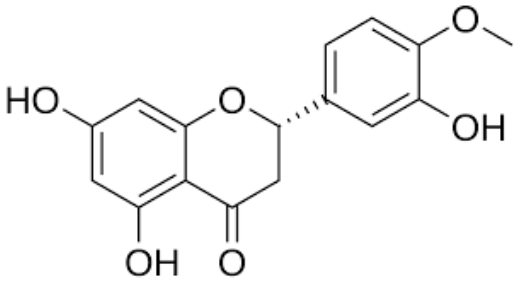


Fig 1. Structure of hesperetin

Physical state: Solid

IUPAC: (2*S*)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydrochromen-4-one

Log P: 2.6

Solubility: 273mg/l

Melting point: 227.5 °C

Chemical formula: $C_{16}H_{14}O_6$

Molecular weight: 302.28 g/mol

As interest is growing in the use of dietary flavonoids to combat the oxidative stress-mediated neurodegeneration in CNS-associated pathophysiological processes, including Alzheimer's disease and Parkinson's disease, there is a growing concern regarding its entry into the CNS and penetration through the blood–brain barrier. Before absorption from the gut, flavonoids must be released from plant sources by chewing or by the action of the digestive enzymes in the gastrointestinal tract [7][8]. After ingestion, the absorption of flavonoids depends on its physicochemical properties such as molecular size, lipophilicity, solubility, and pKa values [9]. Several studies have analysed the penetration of flavonoids through the BBB, which has added value to the studies conducted so far. Here, in the case of hesperetin, some studies have been conducted in animals and brain endothelial cells, which showed that citrus flavonoids like hesperetin, naringenin, dietary anthocyanin and polyphenols are taken up by brain cells [10][11].

2. MATERIALS AND METHODS

.1 Experimental requirements

The hesperitin was obtained from the Sigma-Aldrich, India. Stearic acid, Span 20, Tween 80 (Sigma Aldrich, India), distilled water, ethanol were procured from the local chemical shop in Haridwar, UK.

Table 1. List of chemicals and their manufacturers

Ingredients	Use	Source
Hesperitin	Active ingredient	Sigma-Aldrich
Cholesterol	Polymer	SD Fine Chemicals
Stearic acid	Polymer	SD Fine Chemicals
Span 20	Plasticizer	SD Fine Chemicals
Tween 80	Plasticizer	SD Fine Chemicals
Methanol	Solvent	SD Fine Chemicals

Table 2. List of instruments and their manufacturers

Ingredients	Model	Manufacturer
Melting point apparatus	-	Scientech
Digital weight balance	CX 220	Citizen scale

Magnetic stirrer	-	Scientech
DSC	Jade DSC	Perkin Elmer, USA
Ultrasonicator	DP120	PCI
SEM	LEO 435 VP	SEM, Cambridge
FTIR	FTIR-8400SCE	Shimadzu Corp.
Zetasizer	-	Malvern, Ltd
UV Spectrophotometer	UV-1800	Shimadzu

2.1 Preformulation studies

Preformulation studies conducted prior to the initiation of formulation development aim primarily to create stable, safe, and therapeutically effective dosage forms, focusing on the characterization of the drug substance [159][160][161].

2.2.1 Organoleptic properties

The obtained hesperitin was observed for its physical characteristics like colour, odour, texture of drug and compared with as reported in official monograph.

2.2.2 Melting point

Capillary tuble equipment was used to determine the melting point of hesperitin. The capillary tube was filled with a small amount of hesperitin by tapping it on the drug bed, connecting it to the graduated thermometer, turning on the device, and checking for the melting point of hesperitin.

2.2.3 Solubility

Hesperitin was tested for solubility in Surfactant (Tween 80, Span 20). Hesperitin was added in excess to vials designed to hold 2 ml of surfactant. Following vial capping, the surfactant was agitated on a vortex shaker for 24 hours, occasionally being interrupted by the shaking process. Thus, solubility was estimated in different solvent systems at room temperature.

2.2.4 Drug excipients compatibility studies

to determine whether the drug's chemical makeup has changed after being combined with the excipients or polymers. A mixture of hesperitin and potassium bromide was applied, then compressed into a disc form. The Shimadzu FTIR spectroscopy (4000-400cm-1) was used to evaluate the disk.

2.2.5 Preparation of Std. Calibration Curve and λ_{max} Identification

The standard stock solution was prepared by dissolving 10mg of hesperitin with 10ml of methanol to give a 1000µg/ml concentration. To develop stock II, which has a concentration of 100µg/ml, take 1ml of this stock solution and diluted it with methanol (solvent) up to 10ml. A 10ml volumetric flask was filled with 1ml of the stock solution (100µg/ml), and the line was then filled with ethanol to a volume equal to 10µg/ml. The sample was then scanned with a UV-Visible spectrophotometer in the 200-400nm wavelength range using methanol as a blank. Further dilutions of 0µg/ml - 12µg/ml were made from the stock solution (100µg/ml). The absorbance of the dilutions was measured at absorption maxima. The calibration curve was then constructed [162].

2.3 Preparation of SLN by spray-drying method

Hesperitin was added and swirled until completely dissolved after the lipid components- stearic acid & cholesterol melted at 60ºC. Tween 80 and Span 20 were dissolved in distilled water to develop aqueous phase. Water that had been deionized was heated to the same temperature with constant stirring. Using homogenizer, the aqueous phase was mixed with the lipid phase for 5 minutes at a speed of 10,000 rpm. After that, a 25 Kw ultrasonicator was used to sonicate the suspension for 5 minutes. The nanosuspension was finally cooled to room temperature.

Table 3. List of composition for SLN formulation

Formulation	Hesperitin (mg)	Stearic acid (mg)	Cholesterol (mg)	Tween 80 (%)	Span 20 (%)	Deionized water (ml)
F1	50	200	0.2	1	2	100
F2	50	300	0.2	1	2	100
F3	50	400	0.2	1	2	100
F4	50	500	0.2	1	2	100
F5	50	600	0.2	1	2	100
F6	50	700	0.2	1	2	100

2.4 Characterization parameters [163][164][165]

2.4.1 Analysis of particle size & PDI

The average globule size of solid lipid nanoparticles of hesperitin (Malvern Instrument, UK) was measured using the Zetasizer Nano ZS. The intensity of the scattered light was verified to be within the sensitivity range of the device by taking measurements at a 90 degree angle at a temperature of 25 degrees Celsius. At 25°C, all measurements were taken. The PDI of the formulation was determined using the same tool. The polydispersity index showed the width of the size distribution.

2.4.2 Entrapment efficiency

The centrifugation technique was used to calculate the entrapment efficiency of formulation of hesperitin. Methanol was used to make suspension of SLN, which was subsequently centrifuged using a high-speed cooling centrifuge for 30 min at -4ºC. The amount of free SLN was determined through UV visible spectrophotometers at respective wavelengths, respectively.

2.4.3 Drug content

To guarantee that the medication was released from its entrapment in the medium, a predetermined amount of SLN dispersion (200 mg) was added to a volumetric flask, suspended in methanol, and continuously shaken for 30 min. This solution was put into a centrifuge tube, spun for 15 minutes at 20°C at 12000 rpm. After being diluted with enough methanol, the supernatant filtered-off through a 0.45um membrane filter. UV-VIS Spectrophotometer, Shimadzu Japan 1601, was used to measure the concentration of the hesperitin in the supernatant at 299.5 nm and 238 nm, respectively.

2.4.4 pH estimation

The procedure of evaluating SLN must include calculating pH. The excipients used in the formulation control the final preparation's pH, which has an impact on the route of administration. The formulation's pH was measured using a digital pH monitor. The findings were taken in three copies to reduce error.

2.4.5 Swelling index

SLNs were weighed (W), placed on an agar gel plate containing 2% water-by-volume, and left to sit for an hour at 37°C. Every hour (3 hours), it was taken out of the petri dish and the surface water was wiped off with a dry piece of tissue. The swelling index was determined by reweighing the formulation after it had expanded.

% Swelling index = W_1-W/W × 100

2.4.6 Differential Scanning Calorimetry

Additionally, it provides information on the creation of new substances and medicine excipient compatibility. As a purge gas, dried nitrogen was employed. As per usual practise, the apparatus was calibrated for heat flow and heat capacity using indium. Every sample was heated to a temperature between 25 and 300ºC at a rate of 5ºC per minute. Flow of N_2 was kept at 5ml per minute.

2.4.7 SEM analysis

It was employed in the analysis of film structure. Samples were placed on round brass stubs (12mm in width) using double-sided adhesive tape before being inspected by a scanning electron microscope. After 8 minutes of sputter coating at 1.1 LV in an argon environment, the samples were gold-palladium coated. Black and white Ilford PANF 50 film was used for the photography.

2.4.8 In vitro drug release

A modified Franz diffusion cell was used for the in vitro diffusion testing. The diffusion cell was a 10-centimeter-tall glass cylinder with a 3.7-centimeter-outside diameter and a 3.1-centimeter-inside diameter. To create a diffusion cell, a shep mucosa was attached to the cylinder at one end. One milliliter of the nano emulsion was applied to a cell, which was subsequently put in the receptor part of a beaker filled with 100 milliliters of pH 6.8 phosphate buffer. The receptor compartment, which was magnetically agitated and maintained at 37 degrees Celsius, was in contact with the whole cell surface. Ten milliliters of the receptor compartment's samples were taken out and refilled with an equivalent volume to maintain the sink condition.

2.4.9 Stability

For six months, stability tests on the final optimised SLN dispersion were conducted in sealed aluminium collapsible tubes at three distinct temperatures- 5°C, 25°C, & 40°C while being periodically assessed for percent drug content, pH, texture, and other physical parameters.

3. RESULTS AND DISCUSSION

3.1 Pre-formulation studies

3.1.1 Melting point

The melting point was observed as 260°C which was found in the range of Hesperitin (reference) as 250-262°C. Below table refers the melting point observed:

Table 4. Melting point of Hesperitin

Drug	Reference	Sample
Hesperitin	250-262°C	260°C

3.1.2 Solubility

Hesperitin showed very poor soluble in distilled water and ethanol. Both stearic acid and cholesterol dissolved it freely. It was found soluble in Tween 80 and Span 20. Hesperitin also reported good solubility in DMSO. Thus, it suggests that hesperitin is more soluble in a lipid/organic environment than in aqueous.

Table 5. Solubility of Hesperitin

Solvent	Hesperitin
Distilled water	Very poor soluble
Stearic acid	Freely soluble
Cholesterol	Freely soluble
Tween 80	Soluble
Span 20	Soluble
Ethanol	Poor soluble
DMSO	Soluble

3.1.2 Drug-excipients compatibility study

Hesperitin was also the subject of drug-excipient compatibility investigations using FT-IR spectroscopy, both alone and in combination. A log and example of this compatibility may be seen below:

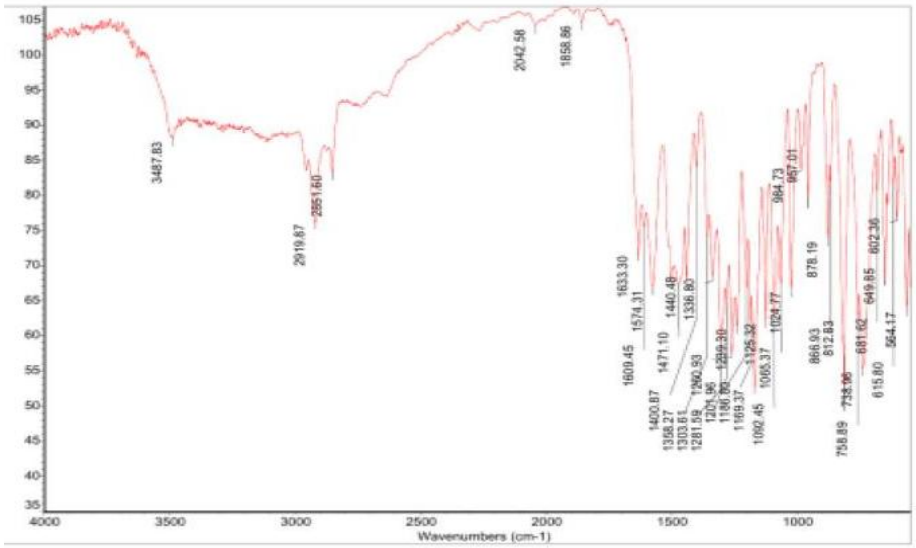


Fig 2. FTIR spectrum of Hesperitin

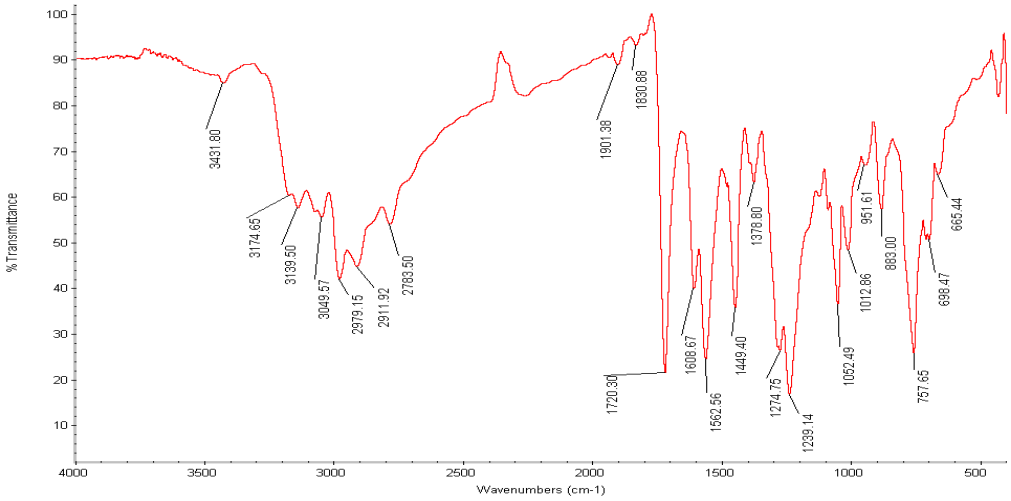


Fig 3. FTIR Spectrum of Hesperitin + Stearic acid

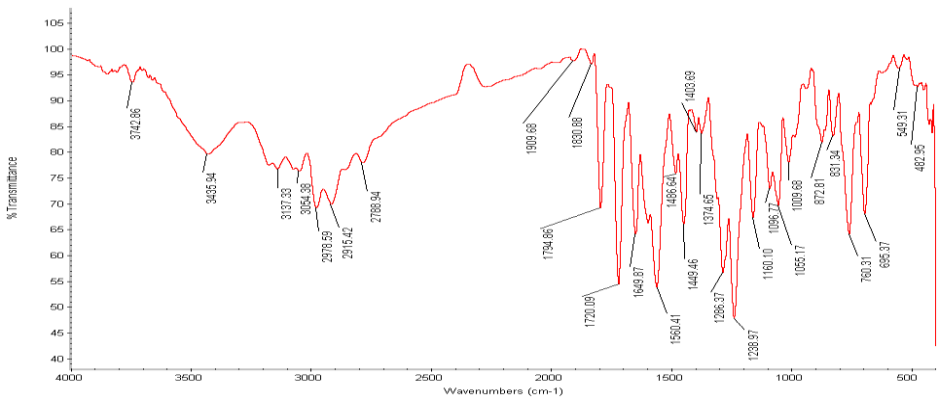


Fig 4. FTIR Spectrum of Hesperitin + Tween 80

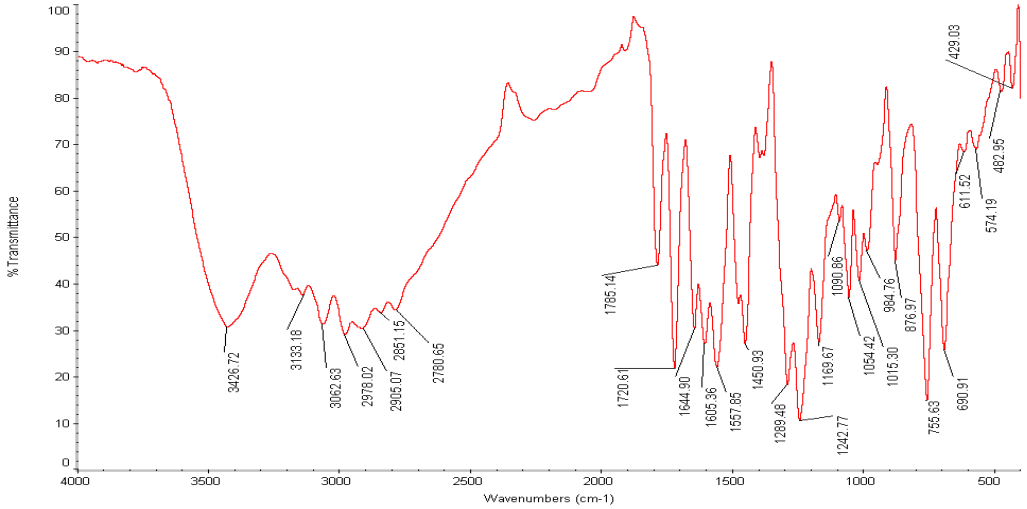


Fig 5. FTIR Spectrum of Hesperitin + Span 20

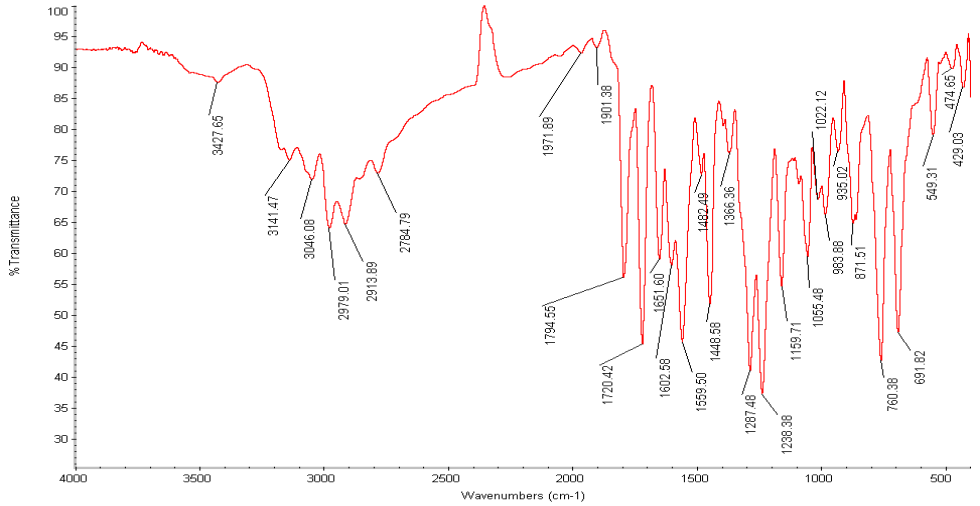


Fig 6. FTIR Spectrum of Hesperitin + Cholesterol

3.1.3 Standard Calibration Curve

Hesperitin was analysed using UV spectrophotometry. At 322nm, in PBS (pH 7.4) containing a small amount of methanol, the absorbance of the drug was determined. Between 0 and 12µg/ml, the griseofulvin standard curve in PBS at pH 7.4 was linear. Beer-Lambert's law can be seen in action here.

Table 6. Std. calibration curve of Hesperitin

Conc. (µg/ml)	Optical density
0	0.00
2	0.19
4	0.35
6	0.53
8	0.66
10	0.81
12	0.93

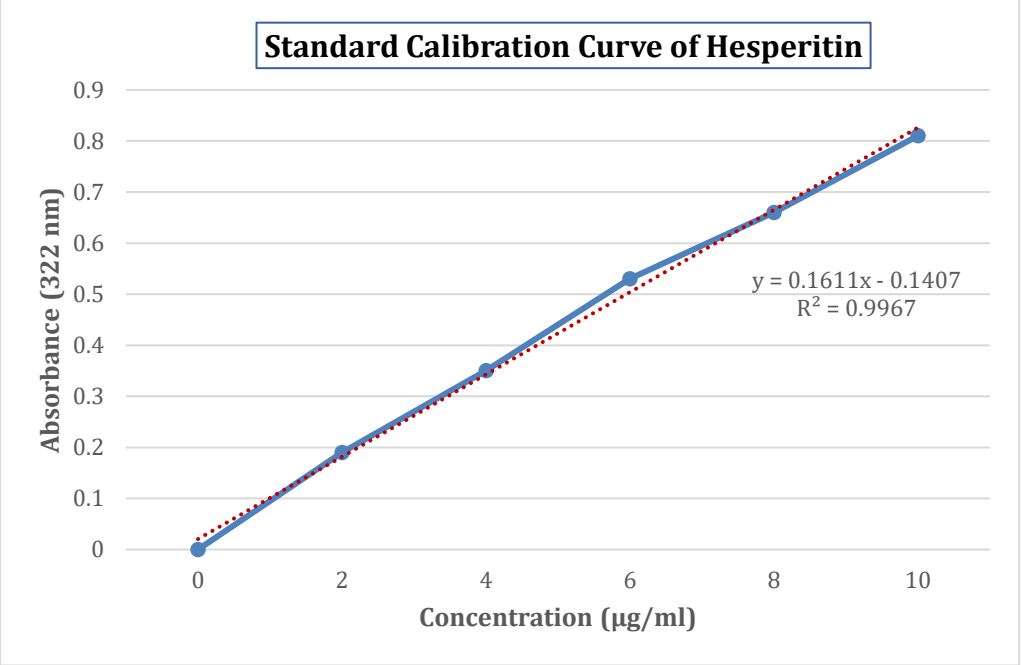


Fig 7. Standard calibration curve of hesperitin

3.2 Characterizations of formulated SLN

3.2.1 Physical appearance

6 different types of SLNs were examined based on their outward appearances of transparency, homogeneity/heterogeneity, whiteness, and turbidity.

SLNs 1-4 were observed to be clear and uniform in appearance, while SLNs 5 and 6 were observed to be colourless and uniform in appearance. This is indicative of the substantial method in which most SLN subtypes were produced, with a transparent and homogeneous appearance.

Table 7. Physical appearance of hesperitin-based SLN

Formulation	Physical appearance
F1	White
F2	White
F3	White
F4	White
F5	White
F6	White

3.2.2 Entrapment efficiency

Entrapment efficiency was determined as 86.19±0.27 %, 84.10±0.56 %, and 87.23±0.16% in the F3, F4 and F6, respectively. Moreover, the highest entrapment efficiency was found in F5 as 91.17±0.33 %. It is a special feature of solid lipid nanoparticles to characterize them.

Below table demonstrates the entrapment efficiency of hesperitin-based SLN:

Table 8. Entrapment efficiency (%) of hesperitin-based SLN

Formulation	Entrapment efficiency (%)
F1	83.45±0.11
F2	81.20±0.34
F3	86.19±0.27
F4	84.10±0.56
F5	91.17±0.33
F6	87.23±0.16

3.2.3 Drug content

In hesperitin-based solid lipid nanoparticles, the drug content was estimated as 74.23± 0.23 %, 77.12± 0.43 %, 73.45± 0.23 and 76.10± 0.31 % in F2, F3, F4 and F6, respectively. The highest drug content was reported in F6 (82.23± 0.12 %). All the formulations showed remarkable and almost identical drug content. Its effective in-vitro drug release is indicative of improved solubility, co-adhesion between the drug and the excipient, and homogeneity of drug content.

Below table represents the drug content of hesperitin-based SLN:

Table 9. Drug content of hesperitin-based SLN

Formulation	Drug content (%)
F1	71.43± 0.30
F2	74.23± 0.23
F3	77.12± 0.43
F4	73.45± 0.23
F5	82.23± 0.12
F6	76.10± 0.31

Droplet size analysis

Nanodroplet analyser was utilized for the droplet size analysis of hesperitin-based SLN. It was lowest in F5 (19.23±0.54 nm. While it was observed moderated increased in the case of F1, F3, and F4 as 26.21±0.23 nm, 28.43±0.27 nm, and 25.34±0.13 nm, respectively.

Table 10. Droplet size analysis hesperitin-based SLN

Formulation	Droplet size (nm)
F1	26.21±0.23
F2	22.19±0.43
F3	28.43±0.27
F4	25.34±0.13
F5	19.23±0.54
F6	24.11±0.19

3.2.5 Analysis of PDI

PDI is a useful measure of particle homogeneity in topical dose formulations. The PDI values found as 0.49±0.04, 0.46±0.02, 0.51±0.04, 0.44±0.01, and 0.41±0.03 in F1, F2, F3, F4 and F6, respectively. Moreover, lowest PDI was estimated in the F5 (0.41±0.03).

Table 11. Analysis of PDI of hesperitin-based SLN

Formulation	PDI
F1	0.49± 0.04
F2	0.46± 0.02
F3	0.51± 0.04
F4	0.44± 0.01
F5	0.41± 0.03
F6	0.47± 0.02

3.2.6 Surface pH estimation

The produced formulation's surface pH was measured and found to be slightly basic/acidic. The pH values for the formulations F1, F2, F3, F4, F5 and F6 were as 6.9± 0.28, 6.4± 0.19, 6.6± 0.24, 7.2± 0.16, 6.8± 0.27 and 6.5± 0.21, respectively. The surface pH was estimated, 1 hour post formulation of SLN. Therefore, it might confirm that the pH range used to manufacture the solid nanoparticles was ideal for their tolerance and solubility in saliva.

Table 12. Surface pH of hesperitin-based SLN

Formulation	pH
F1	6.9± 0.28
F2	6.4± 0.19
F3	6.6± 0.24
F4	7.2± 0.16
F5	6.8± 0.27
F6	6.5± 0.21

3.2.7 Swelling index estimation

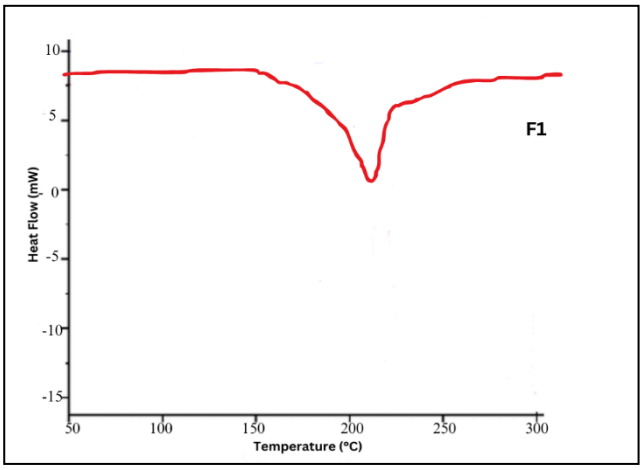
Minimum swelling index was seen in SLN 2 (0.82± 0.23) whereas maximum swelling index was calculated in SLN 1 (0.86± 0.26), SLN 4 (0.85± 0.22) and SLN 5 (0.87± 0.48). The concentration of polymers utilized to create solid lipid nanoparticles is shown by this power.

Table 13. Swelling index of Hesperitin-based SLN

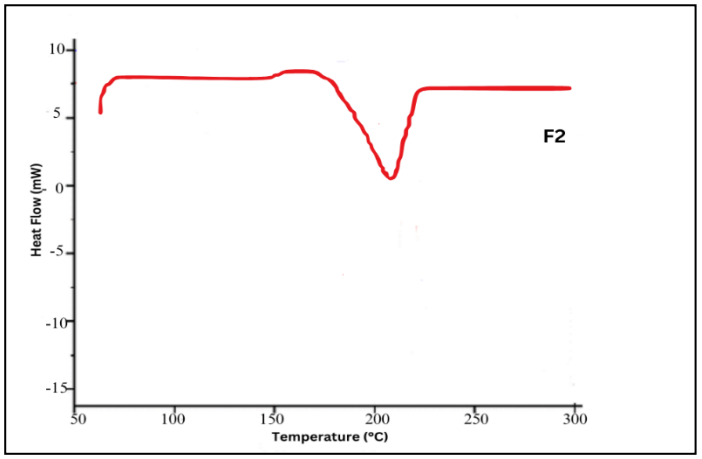
Formulation	Weight (g)	Weight after swelling (g)± S.D.
F1	0.76	0.79±0.23
F2	0.81	0.84±0.21
F3	0.79	0.83±0.34
F4	0.72	0.76±0.23
F5	0.83	0.87±0.17
F6	0.74	0.79±0.29

3.2.8 DSC estimation

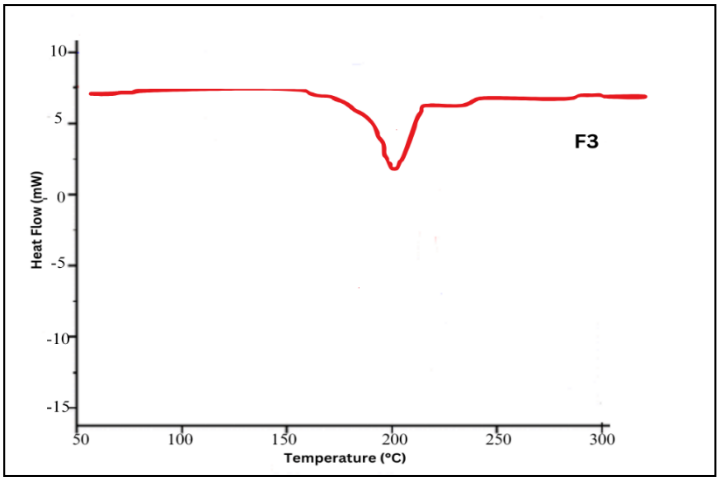
DSC analysis was performed to check that how the formulations are resistant to the moisture and increasing temperature. Formulations F1-F6 were shown almost identical response with the temperature in range of 205ºC to 246ºC.



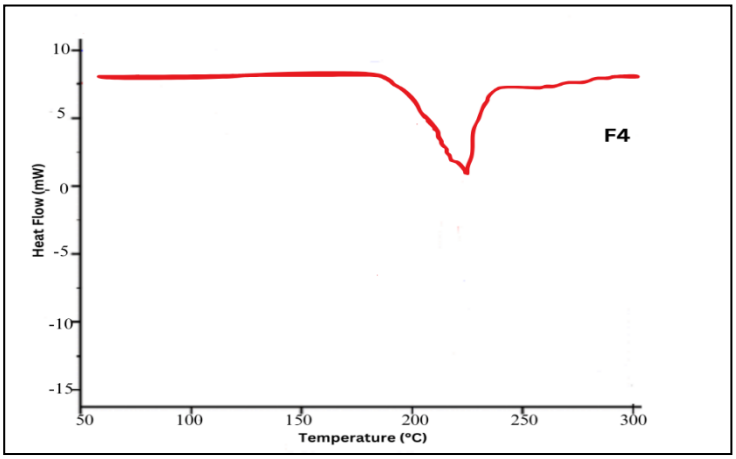
DSC thermographs of F1



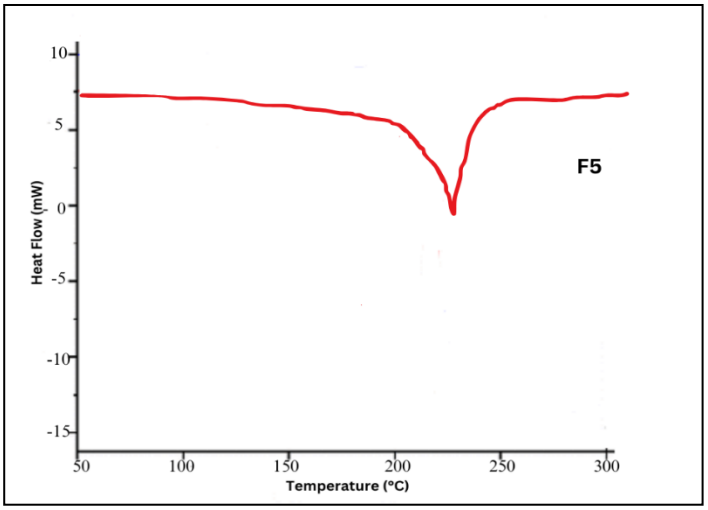
DSC thermographs of F2



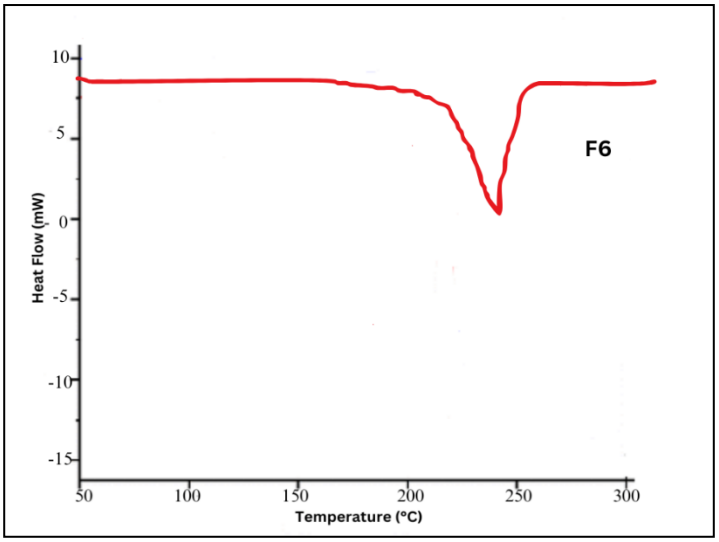
DSC thermographs of F3



DSC thermographs of F4



DSC thermographs of F5

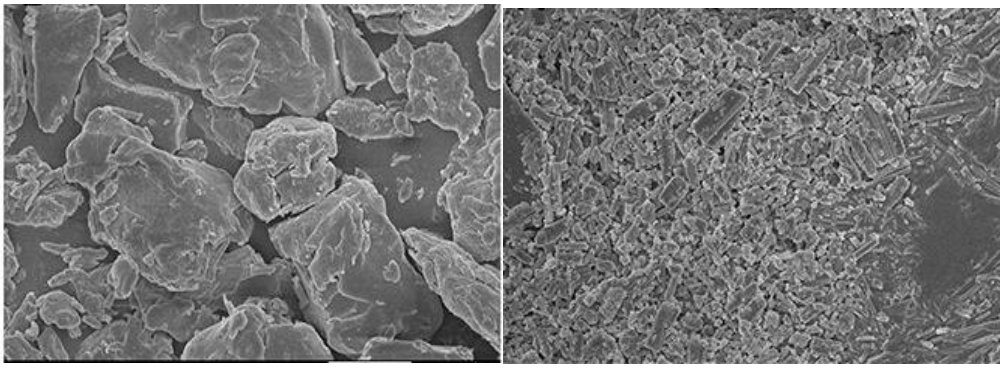


DSC thermographs of F6

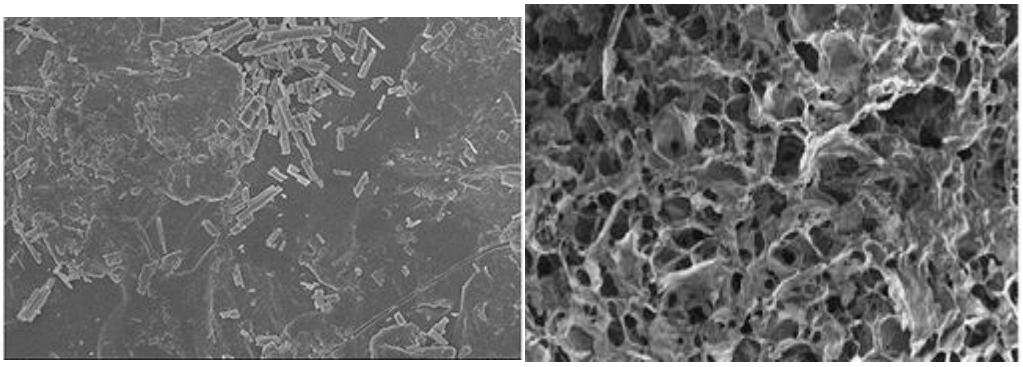
Fig 8. DSC thermographs of hesperitin-based SLN

5.2.9 SEM determination

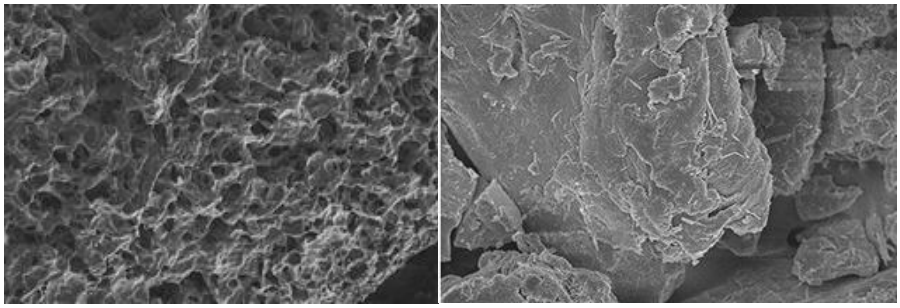
All the formulations of solid lipid nanoparticles were analysed for SEM. In this parameter, F2, F4 and F5 demonstrated almost near analysis of pictures when observed in SEM but F1, F3 and F6 were observed in crystalline form.



F1 **F2**



F3 **F4**



F5 F6

Fig 9. Illustration of SEM determination of hesperitin-based SLN

2.2.10 In-vitro drug release

The generated SLN formulations were used to estimate the in vitro drug release. It was determined to be optimistic and close to 100%, indicating exceptional stability for the API and various excipients utilized.

Six hours later, the percentage of drug release in SLN 1, SLN 2, and SLN 3 was 89.3±0.4, 92.6±0.3, and 91.4±0.2, respectively. In contrast, SLN 1 had a minimum drug release percentage of 89.3±0.4. Of the six preparations, SLN 4 exhibited the highest percentage of drug release (92.9±0.2), whereas SLN 5 and SLN 6 showed moderate percentages of drug release (90.2±0.3 and 91.8±0.4, respectively).

Table 14. In-vitro drug release of hesperitin-based SLN

Time (hr)	% Drug release± S D					
	F1	F2	F3	F4	F5	F6
1	32.4±0.1	33.1±0.4	31.4±0.2	35.2±0.1	34.1±0.6	32.1±0.6
2	44.1±0.6	46.2±0.1	43.1±0.4	45.4±0.6	43.1±0.3	42.4±0.2
3	56.2±0.1	58.2±0.3	53.4±0.3	57.1±0.2	59.2±0.4	60.4±0.2
4	65.6±0.2	67.3±0.1	63.2±0.1	64.2±0.1	68.2±0.1	72.3±0.1
5	82.6±0.3	83.5±0.2	77.3±0.2	81.4±0.2	84.4±0.1	79.2±0.6
6	91.3±0.4	92.1±0.4	88.4±0.1	90.6±0.1	94.2±0.2	89.6±0.2

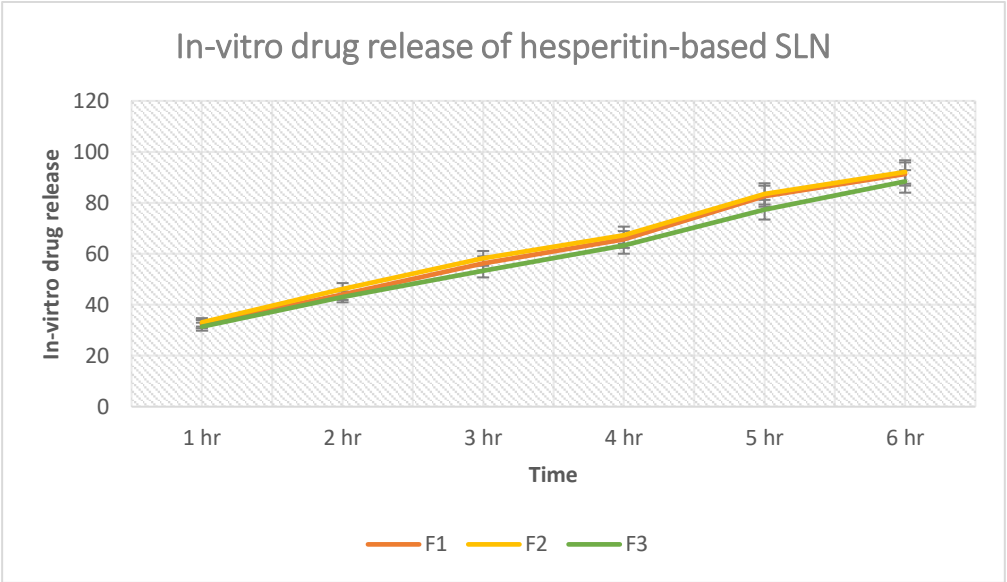


Fig 10. % drug release of SLN 1- SLN 3

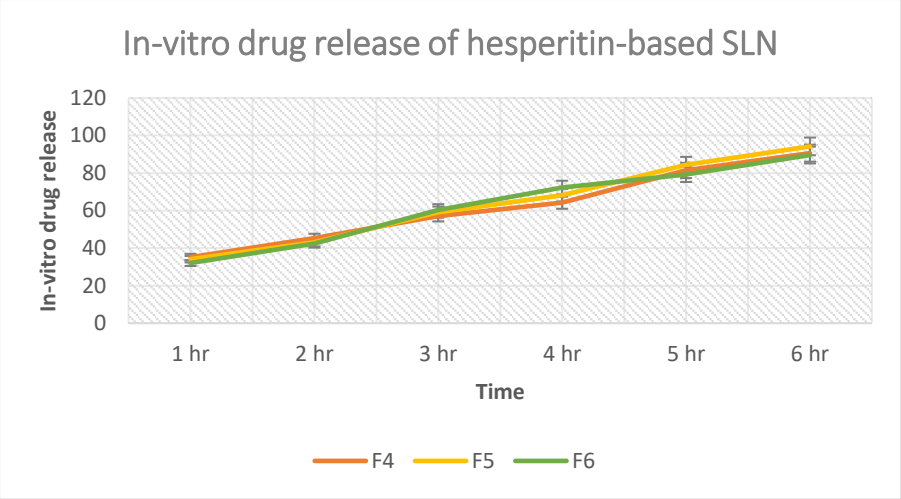


Fig 11. % drug release of SLN 4- SLN 5

3.2.11 Stability

The physical appearance of solid liquid nanoparticles was investigated to establish their stability profile. The physical appearance of several solid liquid nanoparticles was found to be consistent after 30 days of storage. Thus, it is possible to draw the conclusion that hesperitin's solid lipid nanoparticles prepared with these excipients were highly effective and stable.

The promise of solid lipid nanoparticles drug delivery technology to advance medical therapies has not been fully realised. The composition of SLN, the production process's speed and efficacy, including its capacity for large-scale production, and the potential to produce carriers with improved encapsulation efficiency are all evident benefits. Partitioning effects during manufacturing reduce SLNs' loading capacity, making it difficult to administer hydrophilic medicines.

Thus, it can be concluded that SLNs developed using hisperitin demonstrated as a stable formulation when observed for pH, in-vitro drug release and physical appearance after 1 month of storage.

4. CONCLUSION

As drug carriers for enhancing the delivery of pharmaceutical active ingredients, solid lipid nanoparticles are gaining popularity because they provide a few benefits for the delivery of pharmaceuticals. They are adaptable to practically all distribution methods and consequently show potential in a variety of industries, including biotechnology, cosmetics, and medicines. This novel technology might be created to overcome some phytopharmaceuticals' low absorption and poor miscibility with the lipids found in cell membrane linings.

In conclusion, among the several forms of solid lipid nanoparticles of hesperitin, F5 was found to be the most significant formulation in terms of in-vitro drug release, droplet size, and drug content. It also showed improved stability, with no significant change in pH, % drug release and physical appearances after being stored for a month.

Hesperetin is promising natural compounds with a wide range of potential health benefits. Further research, including clinical trials, is needed to fully realize their therapeutic potential and optimize their use in various health conditions.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Tyagi P, J.A. Subramony. Guidance for Industry: Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology, Food and Drug Administration (Accessed 02 Fig. 4. Schematic showing flexibility for developing immunotherapy combinations based on patient parameters such as tumor phenotype and tumor biomarker information. Journal of Controlled Release, 2018; 272:159-168.

[2] Liu L, Q. Ye, M. Lu, Y.C. Lo, Y.H. Hsu, M.C. Wei, Y.H. Chen, S.C. Lo, S.J. Wang, D.J. Bain, C. Ho, A new approach to reduce toxicities and to improve bioavail- abilities of platinum-containing anti-cancer nanodrugs, Sci. Rep. 5 (2015) 10881.

[3] Merisko-Liversidge E, G.G. Liversidge. Nanosizing for oral and parenteral drug delivery: a perspective on formulating poorly-water soluble compounds using wet media milling technology, Adv. Drug Deliv. Rev. 63

(2011) 427-440.

[4] Khadka P R, H. Kim, I. Kim, J. Kim, H. Kim, J. Cho, G. Yun, J. Lee, Pharmaceutical particle technologies: an approach to improve drug solubility, dissolution and bioavailability, Asian J. Pharm. Sci. 9 (2014) 304–316.

[5] Maeda H, H. Nakamura, J. Fang, The EPR effect for macromolecular drug delivery to solid tumors: improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo, Adv. Drug Deliv. Rev. 65 (2013) 71–79.

[6] Amjad Khan, Muhammad Ikram, Jong Ryeal Hahm and Myeong Ok Kim. Antioxidant and Anti-Inflammatory Effects of *Citrus* Flavonoid Hesperetin: Special Focus on Neurological Disorders. *Antioxidants* 2020, *9*(7), 609.

[7] Hostetler, G.L.; Ralston, R.A.; Schwartz, S.J. Flavones: Food Sources, Bioavailability, Metabolism, and Bioactivity. *Adv. Nutr.* 2017, *8*, 423–435.

[8] Youdim, K.A.; Dobbie, M.S.; Kuhnle, G.; Proteggente, A.R.; Abbott, N.J.; Rice-Evans, C. Interaction between flavonoids and the blood-brain barrier: In vitro studies. *J. Neurochem.* 2003, *85*, 180–192.

[9] Hollman, P.C. Absorption, Bioavailability, and Metabolism of Flavonoids. *Pharm. Biol.* 2004, *42*, 74–83.

[10] Makarova, N.M. [Bioavailability and metabolism of flavonoids]. *Eksp. Klin. Farmakol.* 2011, *74*, 33–40.

[11] Figueira, I.; Garcia, G.; Pimpão, R.C.; Terrasso, A.; Costa, I.; Almeida, A.F.; Tavares, L.; Pais, T.F.; Pinto, P.; Ventura, M.R.; et al. Polyphenols journey through blood-brain barrier towards neuronal protection. *Sci. Rep.* 2017, *7*, 11456.

[12] Samiullah, Jan SU, Gul R, Jalaludin S, Asmathullah, "formulation and evaluation of transdermal patches of pseudoephedrine HCL", International Journal of Applied Pharmaceutics, 2020,12(3), 121-127.

[13] Chauhan SB, Saini S, "formulation and evaluation of transdermal patches of metoprolol tartrate using permeation enhancers of natural and synthetic origin", Int J App Pharm, 2019,11(5), 293-298.

[14] Kulkarni S. Formulation and Evaluation of Transdermal Patch for Atomoxetine hydrochloride. JDDT, 2019;9(2-A):32-5.

[15] Jirapornchai Suksaeree, Chomnapas Chuchote. Accelerated Stability Testing of a Polyherbal Transdermal Patches Using Polyvinyl Alcohol and Hydroxypropyl Methylcelluloseas a Controlling Polymer Layer. Journal of Polymers and the Environment (2018) 26:4056–4062.

[16] Kavitha K and More Mangesh Rajendra, Design and Evaluation of Transdermal Films of Lornoxicam, IJPBS, 2011, 2(2), 54-62.

[17] Shalu Rani, Kamal Saroha, Navneet Syan, Pooja Mathur, Transdermal Patches A Successful Tool In Transdermal Drug Delivery System: An overview, Der Pharmacia Sinica, 2011, 2 (5), 17-29.

[18] D NV, Shrestha N, Sharma J. Transdermal drug delivery system: An overview. Int J Res Pharm Sci. 2012;3(2):234–41.