Preparation and Evaluation of Sunscreen Containing Microspheres of Resveratrol

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Abstract: The objective of the current study was to formulate and evaluate the sunscreen loaded with microspheres of resveratrol. The method used for the preparation of microspheres was solvent evaporation method. Eudragit was used as a polymer for the coating of the drug and ethanol: dichloromethane were used as the solvent.The pure drug was highly compatible with the polymers in various ratios. The percent drug entrapment in the prepared ratios was found to be 58.07±1.02%, the percent drug release from in-vitro study was found to be 99.08% while the percent drug release from the ex-vivo study was found to be 98.47% in 12hrs. From the histopathological study it was seen that the prepared cream formulation was found to be safe for the use with good sun protective activity. The sun screen efficiency and in-vitro SPF study revealed that the prepared formulation was having a maximum absorbance of 2.432nm with the SPF value of 3.909257.The photostability study of the cream suggested the results that the prepared cream was stable and the unstable product started to degrade as soon as exposed to the sun while the batchA5 can provide a better UV protection with a stable sunscreen product. From the kinetics study it was revealed that the formulation shows a zero order drug release with a mechanism of Korsmeyers peppas modelfollowed by super case –II transport. The product was tested under the stability study and was found that the changes were not significant. The prepared microspheres were having a good sun protective action and is also safe on skin with higher stability.

Keywords:Resveratrol, Sunscreen, Microspheres, Antioxidants, UV Radiations,

Introduction: Ultraviolet (UV) radiation can cause wrinkles, premature aging, sunburns and cancer; there's a permanent need for cover from UV radiation and prevention from their side effects. Sunscreens are used to aid the body's natural defense mechanisms and to protect against harmful UV radiation from the sun. Its function is predicated on its ability to soak up, reflect or scatter the sun's rays[1, 2]. To measure the effectiveness of a topical formulation against UVB radiation, sun protection factor (SPF) is used. The SPF of a sunscreen is calculated by comparing the quantity of your time needed to supply sunburn on sunscreen protected skin to the quantity of your time needed to cause sunburn on unprotected skin. Higher SPF sunscreens offer greater protection from sunburn[3-5].

Resveratrol has been shown to prevent the damage to skin against UVB mediated skin by boosting anti-oxidant defenses, and it has been shown to help alleviate skin wounds. Resveratrol is a polyphenolic phytoalexin[6]. It is also classified as a stilbenoid, a derivate of stilbene, and is produced in plants with the assistance of the enzyme stilbene synthase. Resveratrol may be a very effective anti-oxidant with 95% efficiency in preventing lipid peroxidation[7]. It has very strong peroxyl radical scavenging abilities, quite gallic and ellagic acids and epicatechins. The antioxidants properties possessed by resveratrolcan protect cells against oxidative damage related to the consequences of free radicals and UV radiation on the skin by reducing the expression of AP-1 and NF-kB factors and it slows down the method of photoaging of the skin[8,9].

Methodology:

Materials: Resveratrol was procured from Yarrow Chem Pvt. Ltd, Mumbai, Eudragit RS100 and S100 were obtained as a gift sample from Evonik India Pvt. Ltd, White Soft Paraffin, Liquid Paraffin and Hard Paraffin were procured from S.D. Fine Chem, Pvt. Ltd. Mumbai, Poly vinyl alcohol was procured from Loba Chem Pvt.Ltd, Mumbai. All other reagents and chemicals used were of analytical grade and were used as obtained.

Standard Calibration Curve: Accurately weighed 10 mg of Resveratrolwas dissolved in small quantity of methanol and the volume was made up to 100 ml by methanol. This resulted in preparation of stock solution of concentration 100µg/mL. from this resultant stock solution aliquots of 0.8, 1.6, 2.4….up to 5.6 mL respectively was withdrawn using pipette into a series of 10 ml volumetric flasks of some specification and volume was made up to 10 mL with methanol resulting in solutions of concentration 8, 16, 24….56µg/mL respectively[10]. The solutions were then analyzed at wavelength maximaλ max 304 nm using UV-visible spectrophotometer

Drug –Excipient Interaction Study: Drug polymer interactions were studied by FT-IR spectroscopy. 1:1 ratio of drug and polymer was taken and the physical mixture of samples were weighed and mixed properly with potassium bromide to a uniform mixture. A small quantity of the powder was compressed into a small pellet by applying pressure[11]. The IR spectrum of the beads from 400-4000cm^{-1} was recorded and compared with standard to study any interference.

Thermal Study: Differential scanning calorimetry drug and excipient was performed by employing DSC. 5mg of pure drug and the physical mixture of drug with the polymers were scanned in aluminum pans over a temperature range between 50 and 400˚C at a scanning rate of 10˚C/min. Nitrogen was used for purging the sample holder at a flow rate of 20 Ml/min[12].

Preparation of Microspheres: Accurately weighed amount of polymers were taken in a separate beaker with the accurate amount of drug in it and the required amount of ethanol and dichloromethane in a ratio of 1:1 was added into it and this solution was known as the organic phase. Now in another beaker inorganic phase was prepared by preparing 2% solution of Polyvinyl Alcohol in distilled water and kept warm until preparation of microspheres. Now the organic solution was incorporated into the inorganic solvent drop by drop by using a hypodermic needle with continuous stirring until the whole organic phase has been added to the

inorganic phase. After this the whole assembly was allowed to stir for 3 hours while the inorganic phase was kept warm for the faster evaporation of the organic phase. After the stirring has been completed the microspheres were filtered and washed with distilled water and dried in a petri dish for 24 hours at room temperature[13, 14]. The dried microspheres were packed in ambered color glass vials with a rubber stopper and kept in a dark place for further evaluation[15].

Table 1: Formulation Chart of Resveratrol Microspheres

Batch	Resveratrol (mg)	Eudragit RS100 (mg)	Eudragit S100 (mg)	Ethanol: Dichloromethane
A1	250	100	100	1:1
A2	250	100	200	1:1
A3	250	100	300	1:1
A4	250	200	100	1:1
A5	250	200	200	1:1
A6	250	200	300	1:1
A7	250	300	100	1:1
A8	250	300	200	1:1
A9	250	300	300	1:1

Preparation of Cream Base: All the ingredients were accurately weighed separately in a beaker and were melt according to the decreasing order of melting range of the paraffin. Stir them homogeneously and add wool alcohol and stir until temperature falls to equilibrium. Add perfume when cooled to 35°C with stirring to obtain a homogeneous preparation[16].

Table 2: Formula for Preparation of Plain Cream Base

Sr. No.	Ingredients	Quantity prescribed (gm)
1	Wool alcohols	0.6
2	Hard paraffin	2.4
3	White soft paraffin	1.0
4	Liquid paraffin	6.0
5	Perfume	q.s
6	Antioxidant	q.s

Evaluation of Microspheres:

Micromeretics Study:

MicromereticsProperties: The prepared microspheres were evaluated for their micromeretics properties such as Tap density, Bulk density, Carr's index, Hauser's ratio, Angle of repose.

Tap Density: Tapped density of prepared microspheres was determined by the tapping method. Accurately weighed quantity of microspheres was transferred in to a 10 mL measuring cylinder. After observing the prevailing volume of microspheres, the tapping was continuing on a tough surface till no progressive modification in the volume was noted and therefore the tapped density was calculated[17].

Tap Density: Mass of Microspheres

Volume of Microspheres after tapping

Bulk Density: It is the quantitative relation between a given mass of a powder and its bulk volume. Bulk density of the formulated microspheres was calculated by pouring about 2 g of formulated Microspheres in a clean measuring cylinder, and initial volume was measured. The bulk density was calculated by the following equation[18]:

Bulk Density: Mass of Microspheres in gram

Volume of Microspheres in cm^3

Carr's Index: The Carr's index of the powder is a direct measure of the potential powder arch or bridge strength and stability. It is calculated by the following formula[19]:

Carr's Index: Tap Density – Bulk Density × 100

Tap Density

Hauser's Ratio: It is associate degree indirect index of easy powder flow. It is calculated by the following formula[20]:

Hauser's Ratio: Tap Density

Bulk Density

Angle of Repose: The flow property of floating microspheres is usually estimated by determining the angle of repose. The angle of repose of microspheres was determined by using a fixed funnel on a burette stand employing fixed funnel method. The microspheres were allowed to freely fall through the fixed funnel until apex of conical pile formed just touched the tip of the funnel. The angle of repose (θ) was determined according to the following formula[21]:

Tan θ = Height of Pile

Radius of Pile

Particle Size Measurement and Percent Drug Entrapment: Particle size and size distribution of different microsphere formulations were measured using an optical microscope and the mean particle size was calculated by measuring some calculated particles with the help of a calculated ocular micrometer. The average particle size was expressed because the volume mean diameter in micrometers[22]. For the entrapment efficiency 100mg of microspheres from all batches were accurately weighed and crushed. The prepared microspheres were powdered in mortar pestle and were dissolved in 10 mL methanol in 100 mLvolumetric flask and volume was made up with methanol. The resulting solution was then filtered with the help of buchner funnel by using whatman

filter paper, suitably diluted and the absorbance was measured at 304nm[23]. The percentage drug entrapment was calculated as follows:

$$\% \text{ Drug Entrapment} = \frac{\text{Actual amount of Drug Found}}{\text{Theoretical Amount of Drug}} \times 100$$

***In-Vitro* Drug Release:** USP dissolution apparatus Type I (basket type) was used to study the drug release behavior of pure drug and microspheres. Dissolution behavior of pure resveratrol and prepared microspheres was studied using phosphate buffer pH 6.8 as dissolution medium. Drug (250 mg) in a dialysis membrane which was tightly tied was placed in basket containing 900 ml of solution of pH 6.8 for 12 hours. After each regular interval of time 5ml of solution was withdrawn and diluted with buffer 6.8 solution. After each withdrawal of the samples the fresh dissolution medium was added into the apparatus in order to maintain the sink condition. The diluted samples were then analyzed at 304nm using UV-spectrophotometer [24, 25]. The cumulative drug release (% CDR) was then calculated for every batch of microspheres containing different ratio of polymers.

***Ex-vivo* Study:**Freshlyremoved skin of goat was obtained from the local slaughterhouse was then shaved carefully without damaging the skin tissues. Tissue samples were inserted in Franz diffusion cells displaying a permeation area of 3.14 cm^2. 25 ml of phosphate buffer of pH 6.6 was added to the acceptor chamber. The temperature was maintained at 34^0C. After a pre-incubation time of 20 minutes, formulation equivalent to 1gm of prepared cream was placed in the donor chamber. At predetermined time points, 1ml samples were withdrawn from the acceptor compartment, replacing the sampled volume with phosphate buffer pH 6.6 after each sampling, for a period of 12 hours[26, 27]. The withdrawn samples were filtered and used for analysis. Blank samples were run simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using a UV-visible spectrophotometer at 304 nm.

Histopathology Study: Freshly obtained skin tissue samples were subject for the use of histopathological study. The skin tissues were carefully removed from the skin of goat obtained from the local slaughterhouse. The skin was washed carefully and shavedwith the help of depilatories. The skin tissue of size $2cm^2$ were carefully removed and used for the testing. 2 samples of skin tissue were used on which the optimized cream was applied while the other sample was remained as it is i.e. tested without applying cream[28, 29]. Then both the samples were exposed in mid-day sunlight for the time period of 2hrs. After this both the samples were subject to microscopic testing to study the damaging effect of UV rays on the skin tissue samples.

Sunscreen Efficacy Testing: A 0.05% w/v solution of sodium nitroprusside in distilled water was prepared and 40 ml of this solution was placed in the various petridish. These petridish were covered with cellophane membrane to expose it directly to sunlight. Then 2 gm of the different preparations was spread uniformly over the membrane as a layer. The petridish were exposed to sunlight for 2 hr. during mid-day[30]. After exposure to sunlight, the samples were analyzed using UV spectrophotometer method for resveratrol content at 304 nm.

Determination of in vitro SPF: 1.0 g of cream from the optimized formulation was weighed to a 100 mLvolumetric flask, diluted to volume with ethanol and water (40:60) and ultrasonication for five minutes after filtering with Whatman filter paper and collecting filtrate by injectingthe first 10 mLof filtrate. 5.0 mL of aliquot was taken in a 50 mL volumetric bottle and diluted to volume with ethanol and water (40:60). After that 5.0 mL of aliquot was transferred to a 25 mL volumetric flask so the volume was filled with ethanol and water (40:60). The absorption values of all prepared aliquots were determined from 290 nm to 320 nm at aninterval of 5min, using ethanol and water solution (40:60) as incomplete. The study was taken three times so decisions were made in average. The detection values found are between 290 and 320 nm multiplied by the appropriate EE (λ) values[31, 32]. Their summary was taken and repeated with the corrective factor to obtain SPF values.

Photo Stability Determination:2mg / cm2 of 3 highly oiled sunscreen creams are weighed and spread evenly between two plates of quartz silica coated (5 mm thick and 25 mm wide). To avoid distortion of absorption, apply a thin layer. AUC for UVA, UVA1 (340-400 nm), UVA2 (320-340 nm) and UVB measured each spectrum before (AUC front) and rear (AUC rear) UV artificial (980 kJ / m2) UVA with 12 kJ / m^2 of UV radiation (including UVB) and before and after natural UV. If AUCI (AUCI = AUC in the background / AUC in front) was> 0.80, sunscreen was taken as a stable one[33]. The AUC is calculated by the following equation[34].

$$\sum_{\lambda min}^{\lambda max} A(\lambda)\Delta\lambda$$

Where, A is absorption and λ is wavelength. For UVA λmax = 400 nm and λmin = 320 nm. The same measurement was done for every UV range respectively, before and after UVartificial and before and after UV natural.

Kinetics Study: The drug release data were fitted to zero order (cumulative % drug release versus time), first order (log of cumulative % drug retained versus time), and Higuchi models (% cumulative drug released versus square root of time) and Korsmeyer-Peppas model (log % of cumulative drug release Vs log of time) to calculate the kinetics of drug release and determine the release mechanism of the drug from the prepared floating microspheresof resveratrol[35].

Surface Morphology: The surface morphology of the prepared microspheres was determined by the scanning electron microscopy (SEM). The samples for SEM were prepared by sprinkling the microspheres on a double adhesive tape which stuck to a stub. The stubs were then coated with platinum underneath an argon atmosphere employing a gold sputter module in an exceedingly high vacuum evaporator.

Stability Study: The need for rigorous testing to test the product and provide evidence of how the quality of a drug or drug product varies over time under the influence of various environmental factors such as temperature, light, humidity, and allows elevated storage conditions, re-test times and shelf lives will be established. Stability studies were performed at room temperature: 25^0C ± 2^0C / 60% RH ± 5% RH and accelerated testing: 40^0C ± 2^0C / 75% RH ± 5% RH for 3 months of prepared construction[36].The optimized formulation was analyzed for the percent drug content, percent cumulative drug release and sunscreen efficacy testing.

Results and Discussion:

Standard Calibration Curve: The results of standard calibration curve revealed that it follows the beers lamberts law as the equation obtained was linear with the values of y =0.014x + 0.005 and the regression value of R^2 = 0.999.

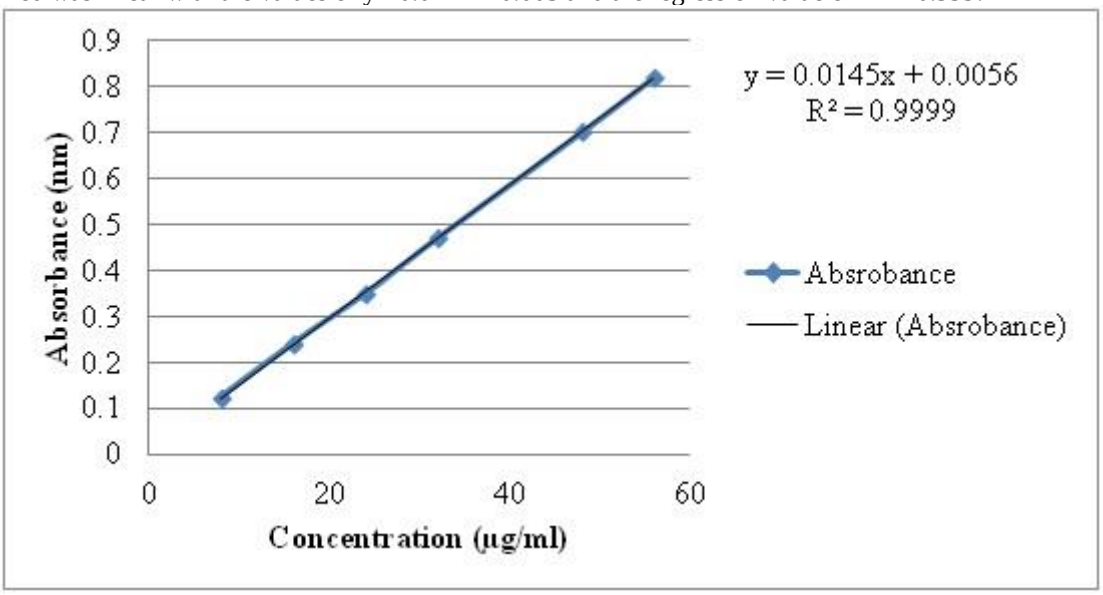


Figure 1: Standard Calibration Curve of Resveratrol

Drug –Excipient Interaction Study:

The
broad band at 3,192 cm
1
refers to O-H stretching of
phenolic hydroxyl bonds. The narrow and low-intensity
band at 3,017 cm
1
is related to the =C-H axial stretch-
ing of aromatic hydrogen, confirming the presence of
unsaturation. The stretching related to C=C bonds of the
aromatic rings is observed in bands at 1,611 cm
1
,
1,583 cm
1
, and 1,520 cm
1
. The presence of the band
at 1,155 cm
1
confirms C-O bond stretching for phenolic
compounds. The stretching at 1,377 cm
1
confirms the
O-H phenolic hydroxyl group. The =C-H band at
965 cm
1
is characteristic for the alkene in the trans-con-
figuration of the resveratrol. The stretching at 860-
770 cm
1
is characteristic of =C-H vibration bands of
arene conjugated to the olefinic group (831 cm
1
). The
deformation bands at 650-500 cm
1
correspond to =C-H
of the olefinic group (677 cm
1

).

The results from FTIR of pure resveratrolrepresented the following band characteristics at 3309cm^{-1}of Free O-H stretching vibration, 1458, 1504, 1597cm^{-1}exhibitedbenzene skeleton vibrations, and 995cm^{-1}represented bending vibration of C=C-H, the typical transolefinic band.The results of FTIR were compared with the standard and it was found that the pure drug was having the same peaks as that of the standard which confirmed that the drug was pure and the optimized formulation was then matched with the peaks of pure drug and it was seen that there was no new formation, disappearance, mismatching of peaks. From this it was confirmed that there was no physical or chemical interaction between the drug and excipients used and were compatible with each other.

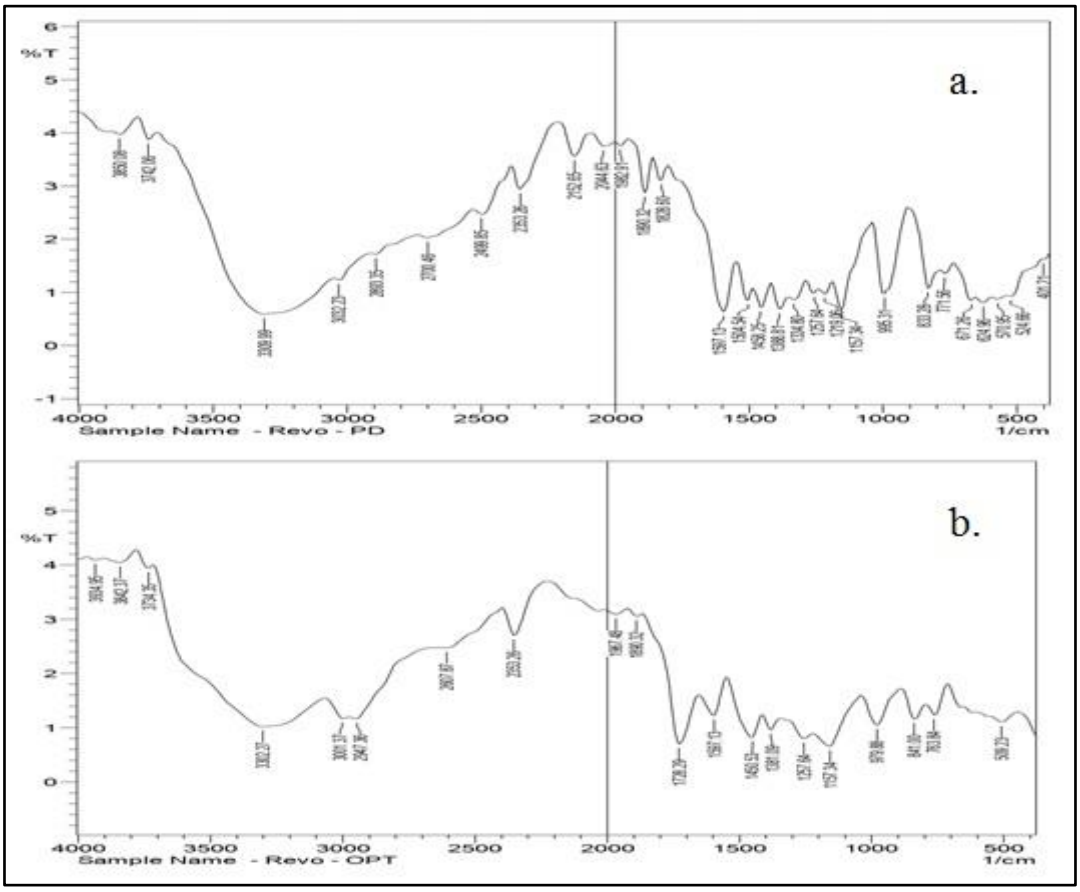
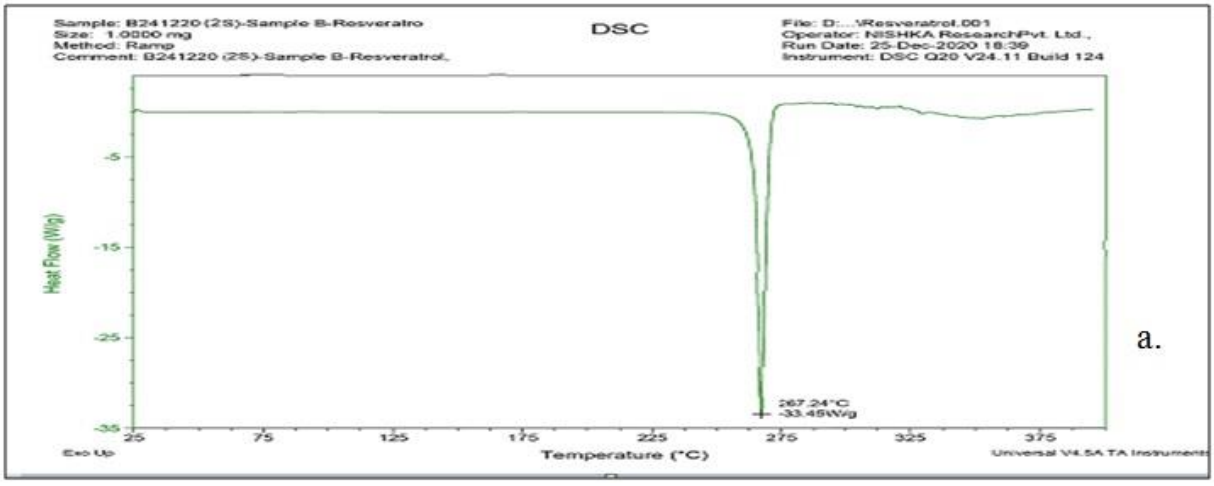


Figure 2: FTIR of a) Pure Drug b) Optimized Formulation

Thermal Study: The DSC study of pure drug shows the melting point of 267.24ºC which indicates its purity and the optimized formulation reveals the melting point of 281.56ºC. From thethermal study of the pure drug and the optimized formulation revealed that the drug was pure and when mixed with the excipients it shows a slight difference in the melting point which was not a significant difference in the temperature and therefore it was concluded that the drug was pure and it shows less physical or chemical interaction with the excipients.



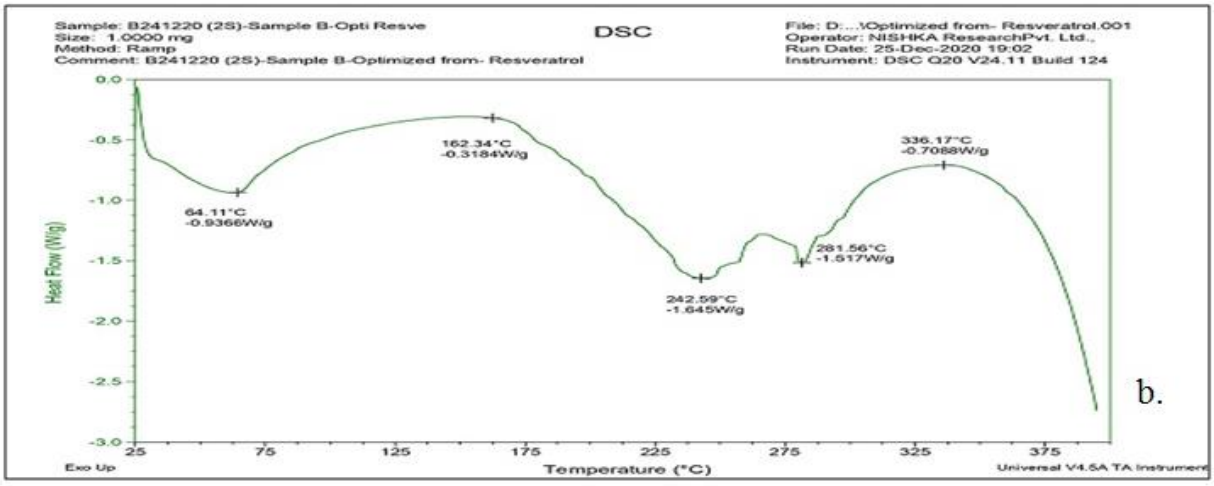


Figure 3: DSC of a) Pure Drug b) Optimized Formulation

Micromeretics Study: The micromeretics study of the prepared microspheres showed that the flow property of the prepared microspheres was good. The tap density was found to be in the range of 0.713±0.36-0.929±0.07gm/cm^3, the bulk density was found to be in the range of 0.798±0.29-0.878±0.17 gm/cm^3, the carr's index and hausner's ratio was found to be in the range of 4.8-7.2 and 1.03-1.08. The angle of repose was found to be in the range of 13.37^o±0.32-21.25^o±0.71.

Table 2: MicromeriticsEvaluation of Prepared Microspheres

Batch No.	Tap Density (gm/cm^3)	Bulk Density (gm/cm^3)	Carr's Index	Hauser's Ratio	Angle of Repose
Pure Drug	0.803±0.36	0.741±0.04	7.7	1.08	24.56 ±0.22
A1	0.713±0.36	0.752±0.04	6.7	1.07	19.60^0±0.25
A2	0.797±0.02	0.786±0.36	4.8	1.03	21.25^0±0.71
A3	0.833±0.06	0.878±0.17	7.2	1.04	16.87^0±0.06
A4	0.883±0.58	0.784±0.29	6.3	1.08	19.58^0±0.02
A5	0.922±0.12	0.862±0.07	4.3	1.03	18.84^0±0.08
A6	0.929±0.07	0.902±0.03	6.1	1.05	15.88^0±0.47
A7	0.843±0.58	0.798±0.29	5.3	1.05	19.98^0±0.18
A8	0.872±0.12	0.825±0.07	5.3	1.05	13.37^0±0.32
A9	0.909±0.07	0.862±0.03	5.1	1.05	20.18^0±0.22

Particle Size Measurement and Percent Drug Entrapment: The evaluation of percent drug content revealed that the percent entrapment was found to be in the range of 46.18 ± 1.55%- 58.07 ± 1.02% from which the batch A5 shows the maximum drug entrapment efficiency. The particle size of the prepared batches was found to be in the range of33.12± 2.50μm-43.78± 1.68 μm.

Table 3: Evaluation of Particle Size and Drug Entrapment Efficiency

Batch	Particle Size(μm)	Drug Entrapped (%)
A1	33.12± 2.50	46.18 ± 1.55
A2	36.44± 3.16	55.15 ± 2.17
A3	38.50± 2.09	55.72 ± 3.01
A4	40.56± 1.22	51.60 ± 3.61
A5	43.78± 1.68	58.07 ± 1.02
A6	41.07± 2.19	57.01 ± 1.26
A7	38.46± 2.38	55.70 ± 2.50
A8	35.61± 3.16	54.12 ± 2.40
A9	36.16± 2.01	55.70 ± 2.50

***In-Vitro* Drug Release:** The percent cumulative drug release of the prepared microspheres showed that the drug release was found to be in the range of 1.203%-99.08% in the time of 12hrs. It can be seen that the eudragit ratio of 1:1 (200:200) was having the maximum drug content entrapment and it also released the maximum amount of drug in the predicted time period. From the results it can be concluded that the batch A5 was the perfect combination for the microspheres and therefore from the results it was concluded that the batch A5 was optimum. This batch was further studied for the kinetics and stability study.

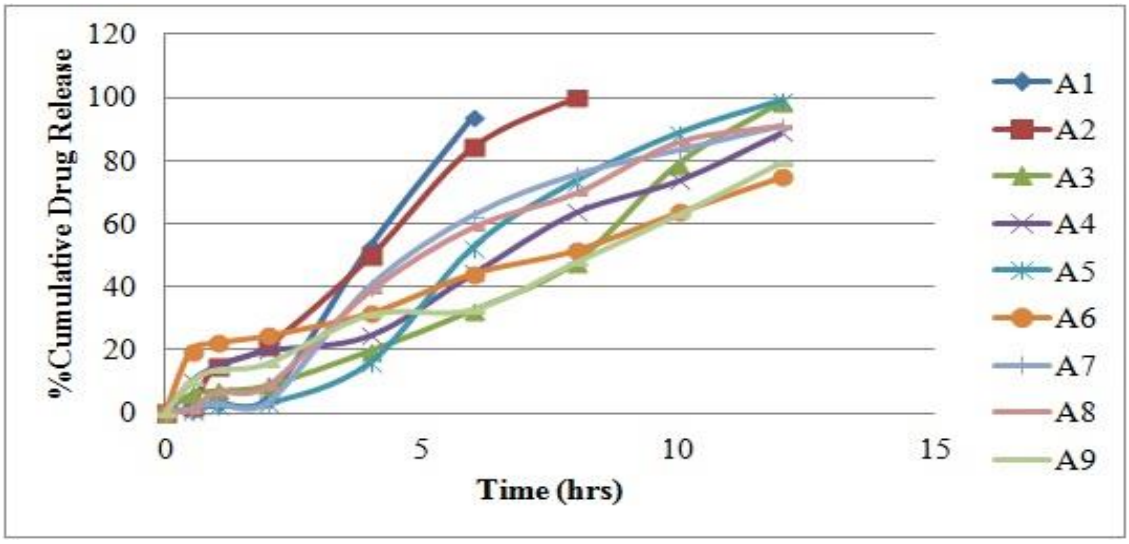


Figure 4: Percent Cumulative Drug Release of the Prepared Formulations

***Ex-vivo* Study:** The molecular permeability of the biological edge is a challenge of the multistep process. Many factors such as chemical composition, body composition, and biological interactions can affect molecular saturation. A study from the penetration of the drug from the skin revealed that drug withdrawal was between 1.134% to 99.98% over a 12-hour period. The highest drug release was seen in A2 formulation which is 99.98% but drug withdrawal in the A2 group was a 10-hour period. But batch A5 showed 98.47% drug withdrawal in the predicted 12-hour period. So the A5 collection was still an improved collection.

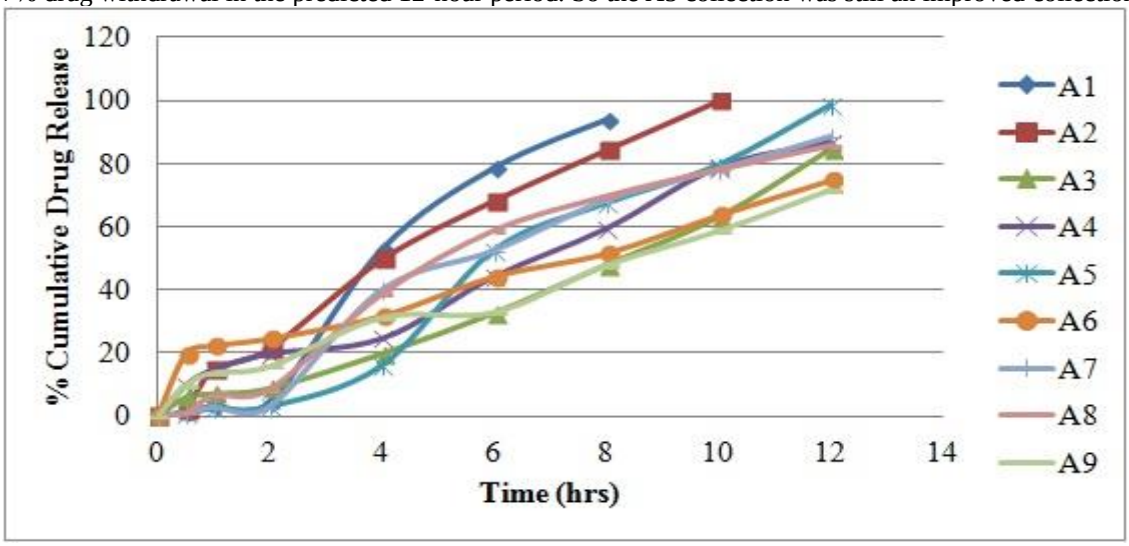


Figure 5: *Ex-vivo* Study of the Prepared Formulations

Histopathological Study:

The histopathological study was conducted to examine safety of the prepared sunscreen cream loaded with resveratrol microspheres. The safety was evaluated through determining any abnormalities like any kind of damage to the tissues. The first image (a) revealed that due to the exposure of tissue with UV rays the tissues were damaged while the second figure (b) revealed that the prepared sunscreen was protecting the skin from the harmful UV rays. These results suggest that sunscreen loaded with resveratrol microspheres might be regarded as a safe with respect to topical administration.

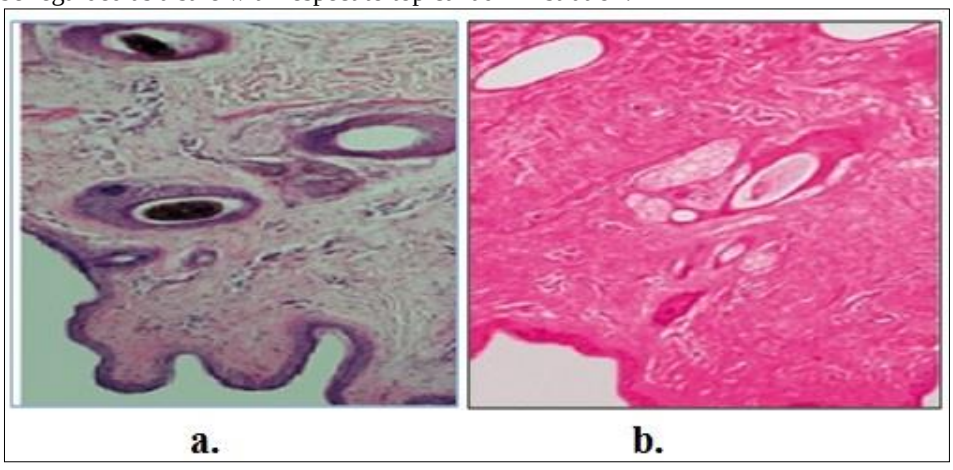


Figure 6: Histopathological Study of the Skin a) Untreated b) Treated

Sunscreen Efficacy Testing: The UV rays absorbance of all the formulations were taken in order to find out the batch which can absorb the maximum amount of UV radiations and from the study it can be seen that the batch of A5 is having the maximum absorbance of 2.432nm. Fromthese results it was concluded that the batch A5 was optimium.

Table 4: Absorbance ofsodium nitroprusside solution (0.05% w/v) at 304 nm after exposed to sunlight for 2 hrs

Sr. No.	Petri Plates Covered With Formulation	Absorbance of Sodium Nitroprusside Solution (nm)
1	A1	2.011
2	A2	1.112
3	A3	2.077
4	A4	1.107
5	A5	2.432
6	A6	0.252
7.	A7	1.487
8.	A8	1.987
9.	A9	0.872

Determination of *in vitro* SPF:The SPF value obtained from the optimized cream formulation with the values of 3.909257, it was evident that the prepared microspheres loaded cream will have the propertytoblockaround73%ofUVradiation,which will eventually reflectthe overallsunscreenactivityofthecream.

Table 5: *In-Vitro* SPF Value of Cream Formulation Measured Under DifferentWavelength

S. No.	Wavelength (λ nm)	EE×I (normalized)	Absorbance×CF×EE×I	SPF=∑EE (λ)×I (λ)×Absorbance (A)×10
1	290	0.0150	0.06405±0.002	3.909257
2	295	0.0817	0.339055±0.002	
3	300	0.2874	1.155348±0.001	
4	305	0.3278	1.27842±0.003	
5	310	0.1864	0.702728±0.001	
6	315	0.0839	0.305396±0.002	
7	320	0.018	0.062640±0.002	

Photo Stability Determination: All the ingredients in the sunscreen formulation showed good stability. Samples on the plate showed the same width before and after heating for 20 minutes at 50°C. Less stable sunscreensbegin to deteriorate as soon as they are exposed to the sun. After 120 minutes of UV exposure, AUCI found the A3 and A6 sunscreen which showed the instability. While the A5formulation exposure revealed a change in length to short range and was found to be stable with all UV activity once and for all and natural UV. It can therefore be said that the A5 can provide a better UV protection with a stable sunscreen product. All the cream has shown good firmness and can be considered an antioxidant environment.

Table 4: Results of Photo Stability Evaluation of Sunscreen Cream Batches

			After Natural UV Exposure		After Artificial UV Exposure	
Formulation	**Exposure Time (min)**	**UVA Radiation (kJ/m2)**	**UVA**	**UVB**	**UVA**	**UVB**
A3	30	55	0.65	0.68	0.72	0.72
	90	165	0.68	0.63	0.65	0.69
	120	235	0.59	0.61	0.71	0.77
A5	30	62	0.75	0.70	0.60	0.81
	90	155	0.79	0.72	0.65	0.80
	120	242	0.84	0.87	0.90	0.82
A6	30	58	0.45	0.59	0.65	0.73
	90	160	0.85	0.88	0.78	0.81
	120	230	0.65	0.69	0.82	0.87

Kinetics Study: All the release data was fitted into various kinetic models like, zero order, first order, Higuchi, and Korsmeyer-Peppas, in order to find out order and mechanism of drug release from microbeads. The value 'n' indicates the output method; where n = 1, the release rate is independent of time (zero-order) (case II transport), n = 0.5 Fickian release and when 0.5 <n <1.0, non-Fickian distribution and distribution are affected. Finally, when n> 1.0 major case II transactions appear.Regression coefficient and 'n'were calculated the value showed that, the prepared microspheres exhibited zero order kinetics followed by super case –II transport.

Table 5: Model fitting data for in-vitro releases kinetic parameters of prepared microspheres

Formulation	Zero order	Korsmeyer-Peppas	Higuchi Diffusion	Best Fit Model	Value of 'n'
A5	0.944	0.963	0.966	Korsmeyer Peppas	0.491

Surface Morphology: The surface morphology of the prepared microspheres was performed at 5K and 25K magnification to study the surface characteristics. From the results it can be seen that the prepared microspheres were spherical in shape having a rough surface with few cracks or fissures over the surface and presence of holes /hollow cavity due to the collapse of the wall of the

microspheres during in situ drying process. Thus the rate of solvent removal from the embryonic microspheres exerts an influence on the morphology of the end product. Porous structure was observed on the surface of microspheres shell thanks to the rapid diffusion of the solvent. It can also be seen that the surface might have a porous surface which will enhance the drug release from the prepared microspheres.

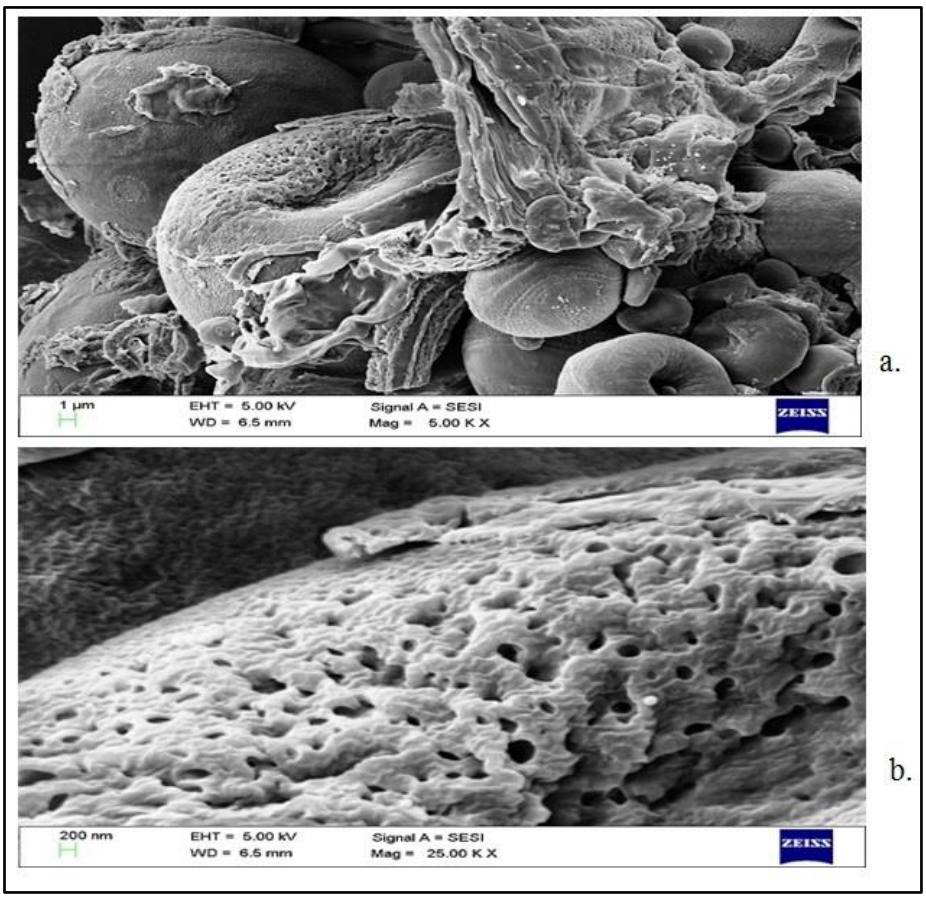


Figure 5: Scanning Electron Microscopy of the optimized formulation at 5K and 25K

Stability Study: The stability study of the prepared optimized cream loaded with resveratrol microspheres revealed the results of decrease in drug content by 0.27%, but there was no change in the drug release behavior, and the absorption of UV to test the sunscreen efficiency was changed and having a difference of 0.06nm in the stability study of room temperature. The stability testing during accelerated study has seen the difference of 0.51% in the drug content, while the percent drug release has seen a increment of 0.18%, and lastly the sunscreen efficiency saw a difference of 0.245nm. From the overall study it can be concluded that the obtained values were having a slight difference which were not significant and hence it can be concluded that the prepared formulation was stable during the study process.

Table 6: Stability Study at Room Temperature (25°C ± 2°C / 60 % RH ± 5% RH)

Day	Drug Content (%)	Drug Release (%)	Sunscreen Efficiency (nm)
0	58.07 ± 1.02	99.08	2.432
15	58.07 ± 1.02	99.08	2.432
30	58.07 ± 0.58	99.08	2.412
60	57.87 ± 0.15	99.08	2.380
90	57.80 ± 1.85	99.08	2.372

Table 7: Stability Study at Accelerated testing (40°C ± 2°C / 75 % RH ± 5% RH)

Day	Drug Content (%)	Drug Release (%)	Sunscreen Efficiency (nm)
0	58.07 ± 1.02	99.08	2.432
15	58.07 ± 1.02	99.08	2.432
30	57.97 ± 1.47	99.08	2.358
60	57.71 ± 1.85	99.18	2.241
90	57.56 ± 1.27	99.26	2.187

Conclusion: The microspheres loaded cream formulation containing resveratrol was prepared in order to test its efficiency against the UV rays for its sunscreen protection factor. From the prepared batches the A5 batch was found to be optimized as it was having the maximum SPF factor and so it can absorb maximum amount of UV rays when compared with other batches. The photosensitivity of the prepared batches showed that it can be highly stable and the same results can also be seen in the stability study of the prepared optimized batch. From the above study it can be concluded that resveratrol microspheres can be proved to a better and natural alternative to the synthetic sunscreens.

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