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DEVELOPMENT AND EVALUATION OF TOPICAL ANTI-AGEING FORMULATION OF RESVERATROL

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ABSTRACT

Topical application gel formulations are designed to be spread on to the skin or specific membranes of mucous for lubricating or restorative properties, or for the local or transdermal entry of medication. By integrating the medication inside the matrix of hydrogel, topical distribution of medications can be accomplished, preventing first-pass metabolism and promoting localized response in the treatment of soreness and skin related conditions. The purpose of this work was to characterise and assess the anti-aging properties of hydrogel formulations loaded with resveratrol. An effort was made to create topical hydrogel formulations of resveratrol using hydrophilic polymers such as Carbopol 934 at different doses. Physical appearance, viscosity, pH, drug content, spreadability, *in-vitro* release of drug, and analysis of anti-ageing property utilising the DPPH scavenging assay technique were all used in this study to characterise hydrogel resveratrol. According to the results of the organoleptic test, every formula was homogenous in consistent behaviour soft, and white in colour. Tests for spreadability, pH, and viscosity revealed that every formula was within the typical range for topical treatments. Tests of anti-aging activity have also shown that raising the dose increases the formula's IC50 value and percentage of inhibition. Following exposure to increased temperatures (40 ± 2^{o}C) and humidity levels (75 ± 5%RH), no significant alterations in the formulation's physicochemical characteristics were noted. Based on in vitro testing, the gel formulation (F4) was determined to be appropriate for topical use. These findings imply that resveratrol topical gel formation as an anti-aging hydrogel is feasible.

KEYWORDS: Resveratrol. Hydrogel. Anti-ageing, DPPH, Topical Delivery.

INTRODUCTION

Pharmaceutical technology advancements have prompted formulation scientists to investigate methods other than oral or parenteral for the proficient and effective delivery of drugs to the intended place. The appropriate distribution of medicines at the site of action within the allotted time frame is a component of effective medication administration.[1] Topical administration of medicines uses the skin, vaginal cavity, the rectal, and ocular channels to administer drugs locally throughout the body.[2] Topical delivery is the direct treatment of dermatological problems by applying a drug-containing product to the outer layer of the skin.[3] When a medication is given directly on the skin, it eliminates variations in the concentration of plasma, hepatic metabolism during the first pass, and stomach pH changes that are often encountered when a medication is taken in oral form.[4] The primary channel of topical medication delivery is the skin, which is also one of the most vulnerable organs on the body of an individual for application on the skin. Research and development of hydrogel systems for drug delivery has advanced scientifically and technologically in the past few decades by conquering physiologic obstacles such first pass metabolism and for better targeted action.[5]

Hydrogels are three-dimensional networks of natural or synthetic polymers with a great degree of elasticity because of their high water content. They are the perfect material for a number of uses because, under physiological circumstances, they can hold a lot of water or bodily fluids and have a soft, rubbery consistency that is comparable to that of live tissues.[6,7] One of the emerging classes of polymer-based technologies with a wide range of commercial and biological uses is hydrogels. Because they are soft, rubbery, and hydrophilic, they cause less tissue distress and have a low propensity for proteins and cells to stick to the hydrogel surface. Drug delivery technique has benefited greatly from the use of hydrogels.[8]

A polyphenolic molecule belonging to the stilbens family, resveratrol is mostly present in peanuts, skin of grapes, and Polygonum cuspidatum roots.[9] Resveratrol has recently been linked to several possible benefits for health, including anti-inflammatory properties, anti-carcinogenic, and cardio/neuroprotective actions, according to plenty of in vitro and in vivo research investigations.[10] Trans epithelial diffusion is assumed to be the primary mechanism for the approximately 75% oral absorption of resveratrol in humans. The oral bioavailability is significantly less than 1% due to extensive processing in the liver and intestine.[11] It has

been demonstrated that this molecule has remarkable biological, medicinal, and therapeutic qualities, including relaxation of vessels, anti-inflammatory, antioxidant properties, and peroxidation of fatty acids and platelet association inhibition.[12]

The goal of the current study was to develop and assess topical hydrogel drug delivery devices. By regulating the rate of medication release from dosage forms, efforts were made to improve therapy by increasing drug exposure and absorption. Using thickening, gelling, or cross-linking agents, the rate of medication release was altered. By combining hydrophilic polymers, the principal objective was to increase the drug's bioavailability and enhance its market formulation. In this study, resveratrol loaded topical hydrogels were formulated at definite dose of resveratrol, propylene glycol and benzalkonium chloride with variable concentrations of Carbopol-934. All the formulations were characterized for physical appearance, viscosity, pH, spreadability, drug content and *in vitro* drug release to find out the best optimized formulation.

MATERIALS AND METHODS

MATERIALS

Resveratrol was bought from Cell Fusion Biotechnology. Carbopol 934, Propylene glycol, Ethanol and Sodium hydroxide was bought from Rankem, Benzalkonium chloride was procured from Sisco Research Laboratories and utilised in this present research.

Development of Resveratrol Topical Hydrogel

A hydrophilic polymer such as Carbopol 934 was chosen, and 0.1N solution of sodium hydroxide was utilised as a crosslinking agent. In water, carbopol 934 dissolves. Separate polymeric dispersions with concentrations ranging from 0.1 to 1% w/v were produced, and their mechanical qualities were determined to be good. As indicated in Table 1, the topical hydrogels were made with varying amounts of Carbopol 934.

Hydrogels were made with varying amounts of polymeric dispersions. Distilled water was used to produce Carbopol 934 colloidal dispersions at concentrations of 0.25, 0.375, 0.5, and 1%. The polymer solutions were allowed to fully swell for 24 hours in the dark following full dispersion. Polymer dispersions were manufactured employing a 500 rpm magnetic stirrer. 0.0025% w/v benzalkonium chloride and 1% v/v propylene glycol were introduced. Following the addition of sodium hydroxide solution, ethanolic drug solution was incorporated to the polymeric solution. To achieve a uniform gel dispersion with magnetic

stirring, the remaining distilled water was then added.

Table 1: Formulation Composition of Hydrogels.

Ingredients (mg)	F 1	F 2	F 3	F 4
Resveratrol	200	200	200	200
Carbopol 934	0.25	0.375	0.5	1
Propylene Glycol	1	1	1	1
Benzalkonium Chloride	0.25ml	0.25ml	0.25ml	0.25ml
Purified Water (q.s.)	100ml	100ml	100ml	100ml

Characterization of Resveratrol

Fourier transform infrared spectroscopy (FTIR) analysis

The potassium bromide (KBr) pellet method was used to perform FTIR analysis of the medication, physical combination, and optimised batch. One milligramme of the medication was weighed, triturated with around ten milligrammes of dry potassium bromide, formed into a pellet using a hydraulic press, and then examined using an FTIR spectrophotometer (Perkin Elmer Spectrum, BX II) in the 4000-400 cm-1 range.[13]

Differential scanning calorimetry (DSC) analysis

A DSC analyser (Q-10, TA Instruments Waters) was used to obtain the drug's DSC thermogram and the optimised batch. DSC was used to determine the drug's and formulation's melting point and thermal stability. Five milligrammes of the drug sample were placed in a DSC pan and heated under nitrogen conditions at a steady rate of 10 degrees Celsius per minute over a temperature range of 20 to 300 degrees Celsius for this study.[14]

Powder X-Ray diffraction (XRD) analysis

Utilising a Cu radiation source at a voltage of 30 kV and a current of 15 mA, powder X-ray diffraction (XRD) patterns were examined using an X-Ray diffractometer (Miniflex-II), located at Rigaku, Japan. Prior to analysis, the powder samples were placed in the X-ray sample holder from the top. Over a range of 5 to 70°, these samples were continuously rotated and scanned at a rate of 1°/min.[15]

Evaluation of Resveratrol Topical Gel

Physical appearance: Through visual inspection, the produced gels' homogeneity and physical characteristics were evaluated. The commercially available formulation served as a standard for comparison.[16]

PH determination: A digital pH meter was used to measure the pH. After precisely weighing 2.5 g of resveratrol-loaded hydrogel, it was solubilized in 25 millilitres of pure water. Prior to every use, solutions of buffer with a pH reading of 4.0, 7.0, and 9.0 were used to calibrate the pH meter. Three separate measurements of the formulation's pH were made, and the mean results were computed.[17]

Determination of viscosity: The Brookfields viscometer LVDV II was utilized for the assessment of the viscosity of the developed formulation. Using a Brookfield viscometer with spindle number seven, the viscosity of gel compositions was assessed at 250°C and 100 rpm.[5]

Spreadability Test

One millilitre of the produced hydrogel was injected onto the glass plate employing a sterile syringe. A calibrated plate has been placed over the formulation, and weights ranging in mass from 20 to 50 to 200 to 300 to 400 to 500 to 600 g were placed on its surface. Twenty seconds after the subsequent weight was placed, the gels' radii were measured. The following formula can be used to determine the area occupied by the developed hydrogels based on the findings obtained:

$P=\pi r^2$

where P—surface area occupied by the hydrogel (m^2); r—radius of the hydrogel (cm).

The equation S = (m × l)/t was used to determine the spreadability, where S is the spreadability, m is the amount of weight on the higher slides, and l is the length of the slide of glass, and t for duration in seconds.

At room temperature, triplicate spreadability experiments were performed on the produced hydrogel.[18]

Drug Content Determination

One gramme of hydrogel was precisely weighed and mixed with one hundred millilitres of phosphate buffer (pH 7.4). To ensure appropriate mixing, the volumetric flask was shaken vigorously in a shaker for four hours. The solution was filtered after being run through the filter paper. A 10 ml volumetric flask was filled with 1 ml of the solution, and 7.4 phosphate buffer was used to get the final volume. Following the proper dilution, the absorbance was estimated spectrophotometrically at 306 nm using a blank of matching phosphate buffer pH 7.4.[19]

***In-Vitro* Drug Release Study**

Franz diffusion cells with cellophane sheets were used for the *in-vitro* drug release experiments. The 30-milliliter water jacketed recipient compartment featured a sample arm on one side and a water inlet and outlet on the other. The inside dimension of the donor compartment was 2.8 cm^2. The donor compartment was situated so that it only touched the diffusion medium in the receptor compartment. The pH 7.4 phosphate buffer solution was present in the receptor compartment. The temperature was kept at 37°C ± 1°C. The hydrogel containing resveratrol was applied to the donor side after the membrane had been equilibrated. 1ml of samples were regularly removed from the receptor compartment and replaced with the same quantity of fresh PBS solution before being analysed at 306nm using a spectrophotometer.[19]

Anti-oxidant activity (in vitro)

Preparations

a) DPPH solution at 0.3 mM After weighing and dissolving 0.012 grammes of DPPH in 90 millilitres of methanol, the volume was increased to 100 millilitres.

b) Standard preparation: In a volumetric flask with an amber colour, after being weighed, 10 mg of ascorbic acid was immersed in 100 millilitres of distilled water. On a cyclomixer, the solution was vortexed before being utilised as a standard.

c) Sample preparation: One gramme of the sample was dissolved in ten millilitres of methanol to create a stock solution. After ten minutes of sonication, the solution was vortexed. The resulting clear methanolic solution was employed in the test.

PROCEDURE

Test samples at varying concentrations were made in methanol. One millilitre of 0.3 mM DPPH solution was combined with 2.5 millilitres of each test sample. For 30 minutes, these samples were incubated at room temperature in the dark. ii) At 516 nm, absorbance was measured with a Jasco V-630 UV-visible spectrophotometer. iii) 2.5 millilitres of the test sample and 1 millilitres of methanol have been mixed to create the blank. One millilitre of a 0.3 mM DPPH solution was mixed with 2.5 millilitres of methanol to create the control sample. The reference standard was ascorbic acid. Every measurement was made three times. iv) The percentage of inhibition of the DPPH radical caused by the tests and standard samples was used to express the results. IC50 values were computed and contrasted with the standard value. v) The following equation was utilized to examine how much the samples inhibited DPPH radicals: Activity of DPPH-

scavenging (%): [1-(A1-A2) / A0] x 100, where A0 is the control absorbance. A1 represents the sample's absorbance. A2 = blank absorbance Plotting graphs and calculating IC50 values using the point-to-point curve method were done using a one-month trial edition of the Graph Pad Prim 6.5 program.[20]

Accelerated stability studies

In accordance with the standards of the International Conference on Harmonisation (ICH), stability experiments were conducted on the optimised formulation. According to ICH guidelines, the formulation in an aluminium tube underwent three months of accelerated stability testing at 40 ± 2oC and 75 ± 5% relative humidity. The samples have been collected at one-month intervals throughout the course of three months, and the previously described process was used to analyse the changes in pH, drug content, spread-ability and in-vitro drug release. Each change to the assessed variables that were noticed were noted. The mean as well as the standard deviation of the observed data were noted, and the experiments were carried out twice.[21]

RESULTS AND DISCUSSION

Resveratrol hydrogel formulation was made using different amounts of Carbopol 934. The results of the visual appearance, drug content, viscosity, pH, spreadability are shown in Table 2 and Table 3 displays the formulations' in vitro release of drug. The goal of the current study was to develop and assess topical hydrogel drug delivery devices. Efforts have been taken to modify medication exposure by controlling the rate of release of medicine from dosage forms and absorption in order to enhance pharmacokinetics and pharmacodynamics. The use of thickening, gelling, or cross-linking agents will regulate the rate of medication release. Different amounts of Carbopol 934 were used to create topical resveratrol hydrogels. Better physicochemical hydrogels were chosen from the produced hydrogel. Table 1 lists the various ratios of polymers employed in the creation of hydrogel. Figure 1 shows the calibration graph of resveratrol.

Table 2 displayed the physicochemical characteristics of the gel compositions. It is clear from the outcomes that every hydrogel formulation exhibited consistent uniformity and spreadability. The hydrogel compositions had a white, translucent texture. The hydrogel formulations had pH values between 6.7 ± 0.07 and 7 ± 0.1, which is within the skin's natural pH range and won't irritate it. For every composition, there was no discernible variation in pH levels over time. In general, the consistency of gel compositions is reflected in their viscosity. When compared to other developed gels, the F4 formulation has a higher viscosity (5841 ± 09.83 cps) and is comparable to the marketed formulation (5861 ± 07.93 cps). It is observed that viscosity varies when the

concentration of polymers changes. A test of spreadability was performed on each formulation. With increases in the formulation's viscosity and polymer concentration, the hydrogel's spreadability increased. Spreadability is crucial because it demonstrates how the hydrogel behaves when it exits the tube. The gel formulations' medication content demonstrated consistency, falling between 98.7 ± 0.2 and 99.7 ± 0.1. The physicochemical parameters of developed gel compositions were similar to those of a commercial medication. To choose the right concentration of polymer for a hydrogel development with the right uniformity for topical administration, *in vitro* release of drug tests were conducted. The Franz Diffusion apparatus have been employed to conduct *in vitro* drug dissolving investigations in order to evaluate in vitro drug release. The release of resveratrol from the hydrogel was varied according to the viscosity of different polymer. The release of resveratrol from the hydrogel formulation can be ranked in the following descending order: F4 > F3 > F2> F1, Where the amounts of the drug released after 8 hr were 63.36%, 60.23%, 57.28%, 54.8 % respectively as shown in figure 2.

According to Table 3's cumulative in vitro drug release data, F4 releases 63.36% while the marketed product releases 62.89%. When compared to the commercial formulation, the F4 formulation exhibits similar in vitro release of medication, according to the data (Fig. 3). It was determined that formulation F4 was appropriate for topical usage by considering its physicochemical characteristics and *in vitro* release of the drug.

Anti-oxidant activity: Resveratrol loaded hydrogel formulations showed significant and promising anti-oxidant activity. F4 formulations showed highest anti-oxidant activity.

Accelerated stability studies: No notable alterations were seen. After three months of exposure to elevated temperatures and humidity levels, the formulation F4 was determined to be stable. The physical evaluation parameters, which are listed in Table 5, did not show any notable alterations. Table 6 provided the in-vitro drug release data. Tables 5 and 6 demonstrate that, even after being exposed to accelerated temperature (40^{o}C) and humidity conditions (75 ± 5%RH), the optimised formulation's physicochemical characteristics and in-vitro drug release profile did not significantly alter. Therefore, after being put through accelerated stability tests, the new formulation was determined to be stable.

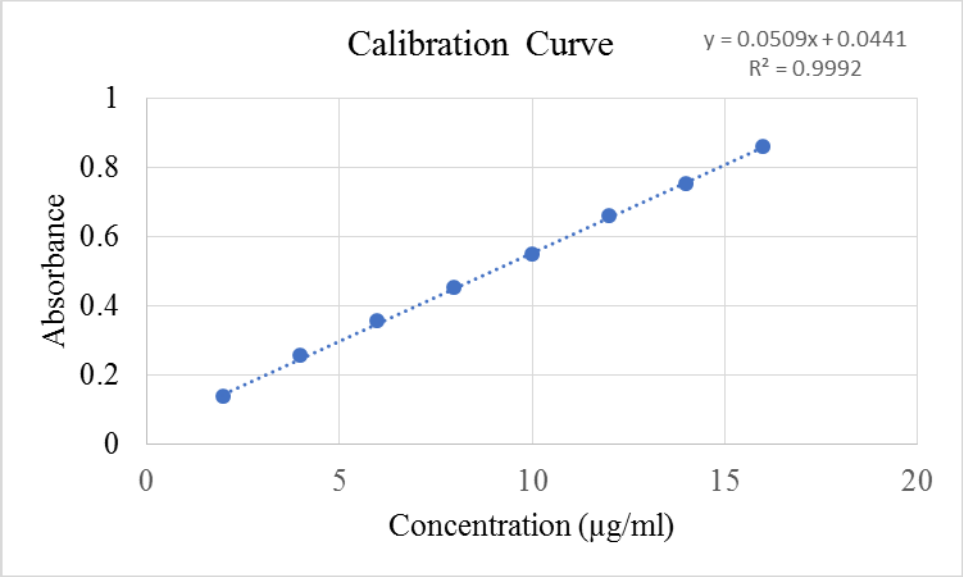

Figure 1: Calibration Graph of Resveratrol.

Table 2: Assessment of Topical Hydrogels Loaded with Resveratrol.

Formulation coding	Physical Appearance	Drug Content	Viscosity	PH	Spreadbility
F-1	Thick & Translucent	98.5	4756 ± 10.27	6.8	5.18 ± 0.28
F-2	Thick & Translucent	98.7	4812 ± 08.56	6.7	5.25 ± 0.15
F-3	Thick & Translucent	99.2	5291 ± 11.02	6.8	5.45 ± 0.41
F-4	Thick & Translucent	99.6	5841 ± 09.83	6.9	5.89 ± 0.13
Marketed	Thick & Opaque	99.89	5861 ± 07.93	7.0	5.94 ± 0.11

Table 3: *In Vitro* Dissolution Analysis of Resveratrol Topical Hydrogel Preparations F-1 To F-4, Pure Drug and Marketed Preparation.

Time (in hrs)	F1	F2	F3	F4	Pure Drug	Marketed Formulation
0.5	9.93	8.45	6.05	8.98	93.23	9.23
1	13.64	10.42	20.42	12.01	93.6	15.01
2	28.12	26.53	29.98	22.54	99	24.56
4	39.19	35.71	37.94	36.25	99.8	38.09
6	47.01	42.83	44.12	49.09	99.8	50.23
8	54.8	57.28	60.23	63.36	99.9	62.89

Table 4: Anti-oxidant activity data of ascorbic acid.

Concentration (µg/ml)	% Inhibition of DPPH radical
0	0
10	14.472
20	27.133
30	40.83
40	49.23
50	68.45

60	83.29
70	89.68
80	95.14
90	96.09

Table 5: Physical Variables Following Accelerated Stability Analysis of Formulation F-4.

Physical Variables	Initial	Preceding 1 month	Preceding 2 months	Preceding 3 months
pH	6.9 ± 0.07	6.9 ± 0.07	7 ±0.04	7 ±0.07
Drug Content	99.6 ± 0.1	99.5 ± 0.1	99.4 ± 0.1	99.3 ± 0.1
Viscosity	5841 ± 09.83	5841 ± 09.83	5851 ± 09.83	5854 ± 09.83

Table 6: *In-Vitro* Drug Release Findings Following Accelerated Stability Analysis of Formulation F-4.

Time (in hrs)	Initial	After 1 month	After 2 months	After 3 months
0.5	8.98	8.45	8.01	7.99
1	12.01	11.98	11.78	11.23
2	22.54	22.45	22.03	21.79
4	36.25	36.15	35.88	35.69
6	49.09	49.09	49.01	48.93
8	63.36	63.12	62.89	62.01

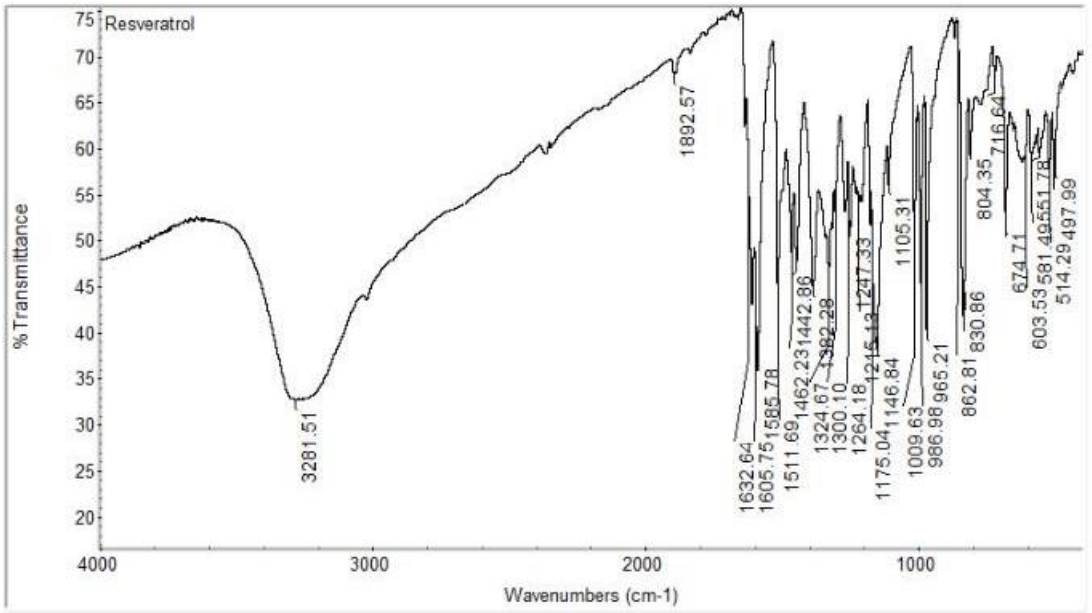

Figure 2: FTIR Spectra of Resveratrol.

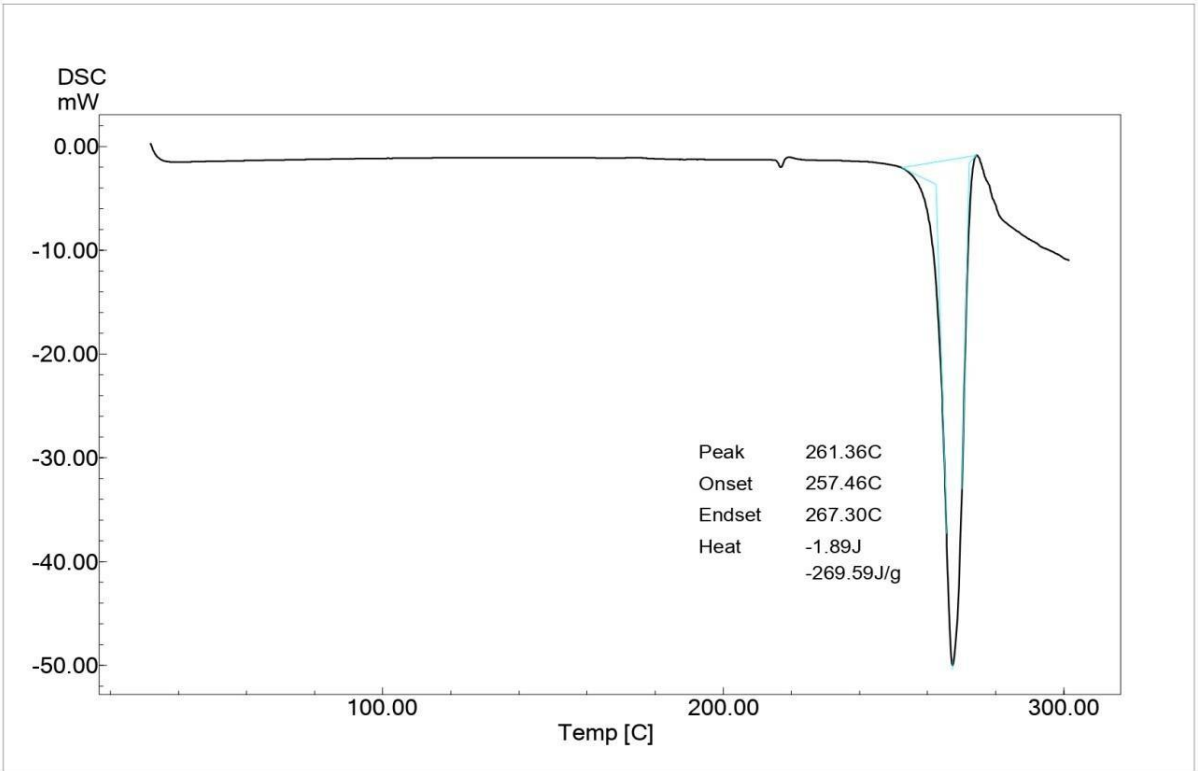

Figure 3: DSC Thermogram of Resveratrol.

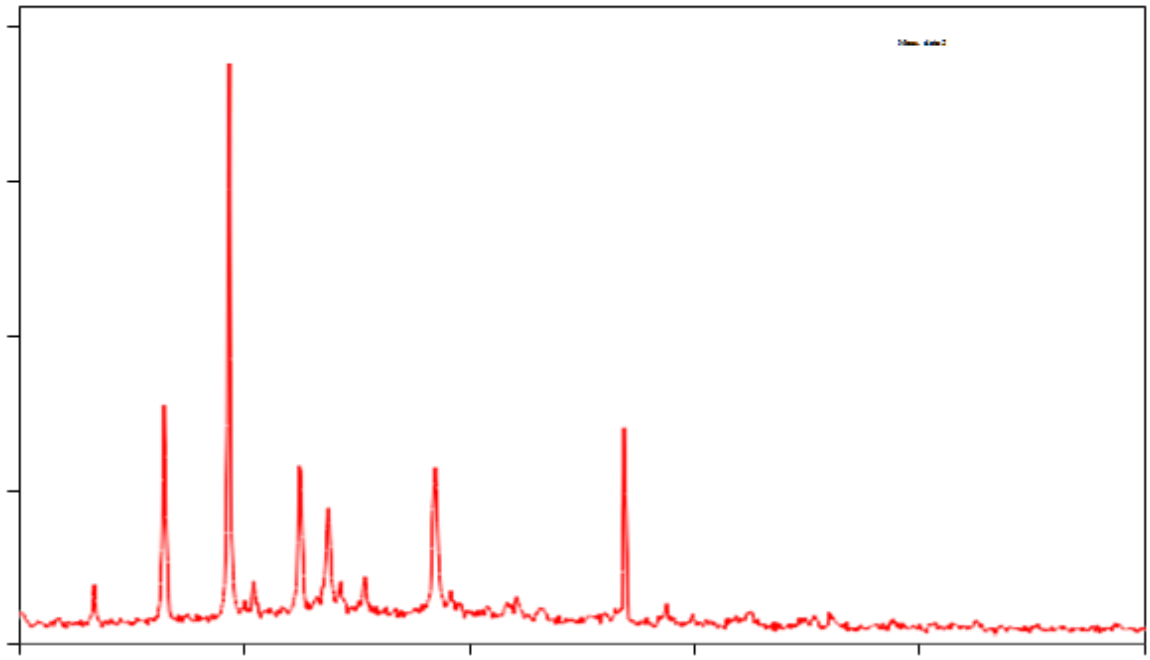

Figure 4: XRD Graph of Resveratrol.

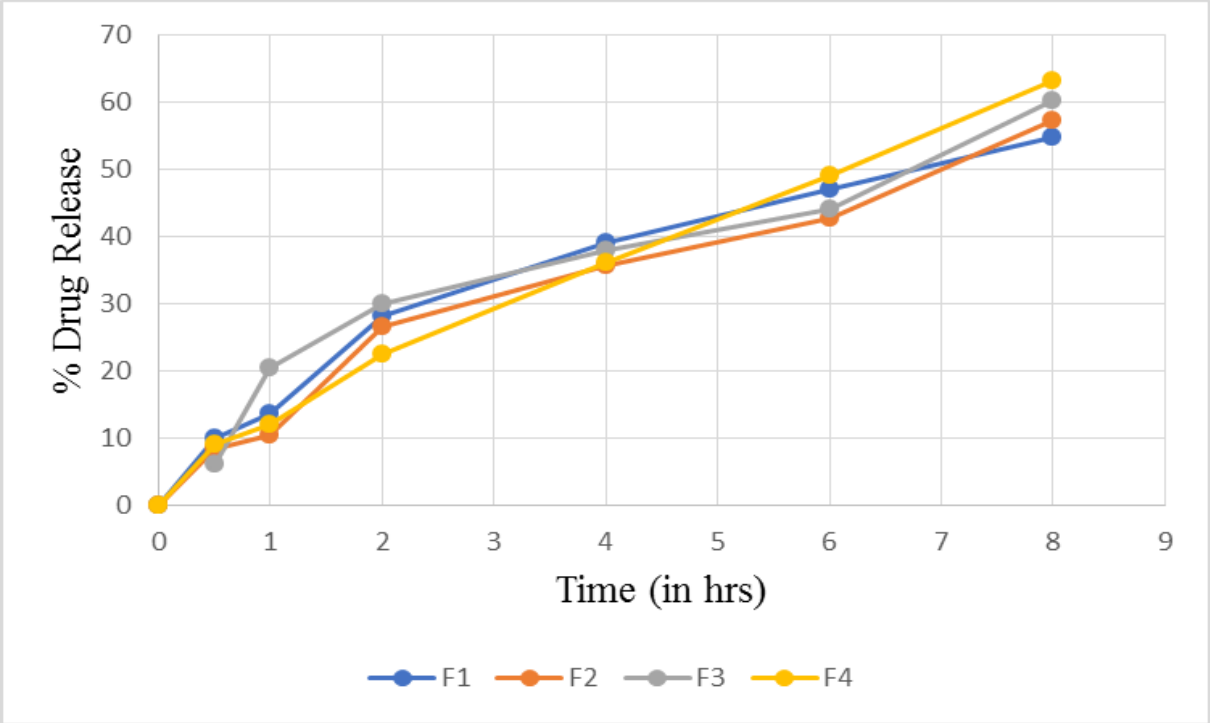

Figure 5: *In- Vitro* Release of Drug from Prepared Hydrogel Preparations (F1 – F4).

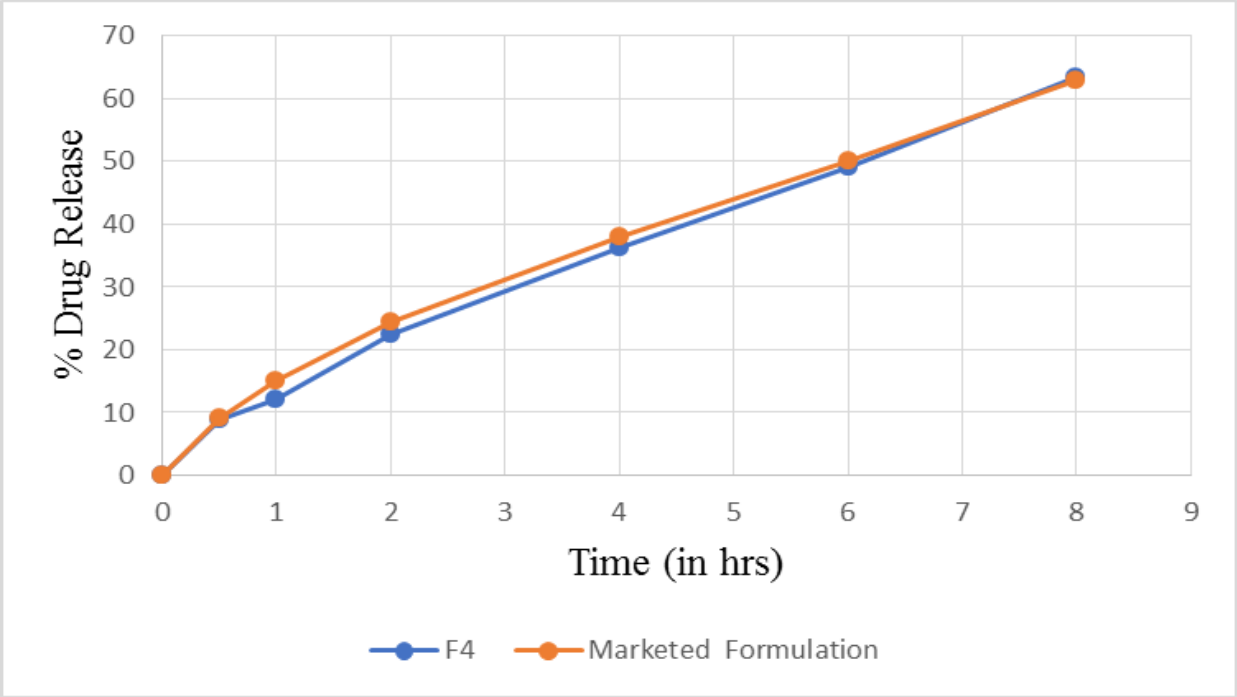

Figure 6: *In-Vitro* Drug Release of F4 and Marketed Product.

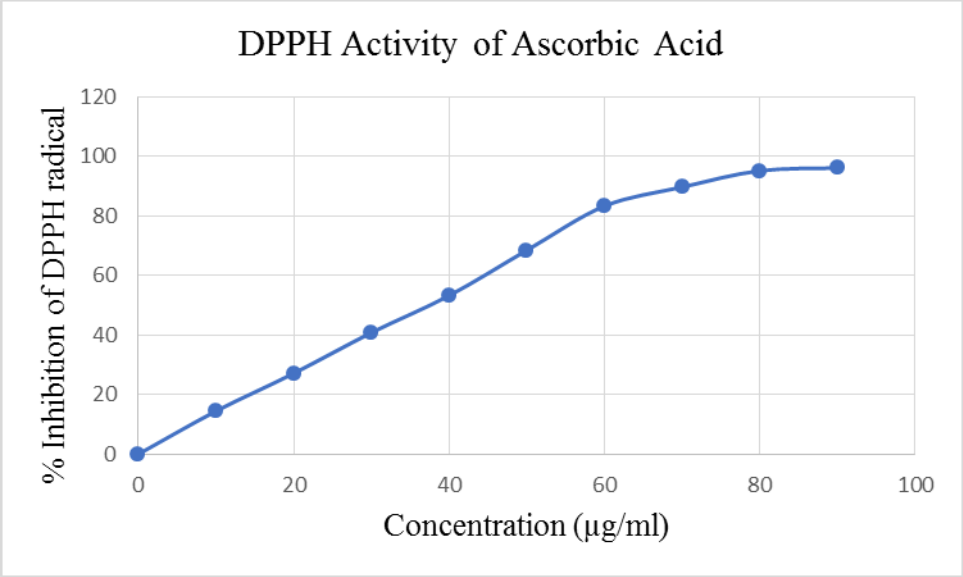

Figure 7: Plot of % inhibition of DPPH v/s concentration of ascorbic acid.

CONCLUSION

In conclusion, a number of hydrogels loaded with resveratrol were created and their potential as topical delivery methods assessed in this study. After oral treatment, resveratrol has a short elimination half-life and substantial first-pass metabolism due to its hydrophobic nature. According to the results, the hydrogel loaded with resveratrol was white, translucent, and homogeneous, with no lumps. Spread-ability, viscosity, pH, content of drug, and *in-vitro* release of drug were all good for formulation F4, and during the accelerated stability tests, the appearance was unambiguous and there was no discernible change in any of these parameters.

When compared to the commercial formulation, the F4 formulation exhibits similar in vitro drug release. It was determined that formulation F4 was appropriate for topical application considering its physicochemical characteristics and *in vitro* release of drug. Hydrogel's antioxidant activity is evaluated using the DPPH assay. Resveratrol hydrogel can therefore be applied topically as an anti-aging agent.

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