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FORMULATION AND EVALUATION OF ANTIOXIDANT AND ANTIMELANOGENIC ACTIVITY OF NANOEMULSION CONTAINING L-GLUTATHIONE

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ABSTRACT

This research aims to formulate and quantify nanoemulsion which contains L-glutathione reduced with antioxidant and antimelanogenic activity. The drug was obtained from RP chemical Pvt. Ltd. Mumbai India identified with certificate of analysis. Total 9 batches preformulation were prepared by taking the proportion of the excipients and variables as factors. The nanoemulsions were prepared using the Phase Inversion Emulsion (EPI) technique. Nanoemulsion formulations were prepared simultaneously by heating both phases, dissolving the reduced L-glutathione in aqueous form. It used orange oil as an oily stage, and PEG-400.

Parameters such as organoleptic properties, appearance tests, dye solubilization, pH, viscosity, dispensability tests, refractive index, product consistency, particle size analysis, zeta potential, thermodynamic stability tests, stability tests were performed for all batches.

This work offers a stronger release profile of nanoemulsion. Droplet size in nanoformulation is a crucial parameter. Formulation would be suitable for having the smallest particle size. The smaller the larger particle size will be drug absorption in systemic circulation at the lowest possible dosage and optimum therapeutic activity. Reducing dosages of prescribed drugs will further decrease toxicity and side effects.

Keywords: Formulation, Antioxidant, Antimelanogenic and L-glutathione

1. INTRODUCTION

L-Glutathione is a drug that is considered the body's principal antioxidant. This is produced in our livers, and has a very important role to play for us. In light of a reduction in the synthesis of L-Glutathione, we will suffer from a weakened immune system that threatens our wellbeing from disease and infection **[4]**. Doctors advise patients with cancer, asthma, heart disease, glaucoma, etc. in oral glutathione. Reduced L-Glutathione can also be injected into a muscle to help prevent harmful chemotherapy side effects, or to help overcome male fertility issues. Though used primarily for medicinal purposes, due to its many advantages, it is also one of the core components in many skins lightening products **[3]**. In light of a reduction in the synthesis of L-Glutathione, we will suffer from a weakened immune system that threatens our wellbeing from disease and infection **[2]**. Doctors advise patients with cancer, asthma, heart disease, glaucoma, etc. in oral glutathione. Glutathione has recently become the most common "systemic skin lightening agent" with additional anti-melanogens, a potent antioxidant **[1]**.

It is assumed that L-glutathione was a more complex form of glutathione saturated with an accompanying sulphur molecule. This provides a very effective antioxidant in the body which helps to grow healthy cells because of the regeneration the L-glutathione molecule can perform on the cell walls. The form of solution that limits its use is highly vulnerable to oxidation. L-glutathione provides a possible solution to this issue with nano-oil droplets. The proposed work will therefore be conducted to formulate nanoemulsion loaded with L-glutathione and to test its studies of stability together with its antioxidant capacity and protective function against Sun **[1]**.

2. MATERIALS

Reduced L- Glutathione was purchased from PC Chemical Pvt. Ltd. Mumbai India. Orange oil, Tween 80, Polyethylene Glycol 400, Methyl paraben RL Fine Chemical Pvt. Ltd. Mumbai India.

3. METHODS

3.1 Preformulation studies- Preformulation is the initial phase in basis improvement of any pharmaceutical dose structure in another medication, since preformulation study centers around those new exacerbate that can partner impact on medication execution and advancement of a viable inconclusive amount type. Just these preformulation examinations affirm that there are no noteworthy obstructions to the mixes improvement. The details are given in **Table 1 [18]**.

3.2 Organoleptic properties- This includes testing of properties like color, taste, odour, etc.

Melting point determination- Melting purpose of reduced L- glutathione was controlled by open narrow technique utilizing Thiele's cylinder **[9]**.

3.3 Spectroscopic analysis- Stock arrangement of 100 µg/ml was set up by including 10mg of unadulterated reduced L- glutathione in 10 mL of dissolvable distilled water. At that point, 1mL of stock arrangement had taken and appropriately weakened with arrangement of water to make 10 µg/ml of Reduced L-glutathione arrangement. The arrangement was then sifted and its UV range was recorded in the wavelength go 200 - 400 nm **[26]**.

3.4 Preparation of calibration curve for reduced L- glutathione- 100µg / ml of stock solution was dissolved in distilled water with a solvent and further diluted with a distilled water solution. As a solvent for solutions entering concentration range of 5-25 µg / mL. The solutions were eventually filtered and analysed at 249 nm spectrophotometrically **[24]**.

3.5 Fourier transform infrared spectrometry (FTIR)- Using Jasco FTIR 4100, Japan, an infrared spectrum of pure drug, drug mixture with every excipient, and physical mixture was registered. The scanning range was 650–4000 cm−1, and the sample IR spectra was collected using ATR. Any alteration in drug spectrum pattern due to excipient involvement was analyzed to identify any chemical interactions **[20]**.

3.6 Interpretation of IR spectrum of reduced L- glutathione- The spectrum of pure reduced L-glutathione powder in infrared was reported and its spectral analysis was performed **[27]**.

3.7 Compatibility studies between drug and excipients- Incompatibility is the result of at least two substances being mixed, and is defined by physical, concoction and useful properties. It can affect the fitness, performance and existence of the measuring device. Subsequently, comprehensive researchers are of prime importance in determining the potential incongruity between complex fixations and excipients used to construct the last measuring structure. We examined infrared testing during this investigation to determine any association (compound or physical) or bond forming between the medication and the excipient **[26]**.

3.8 Excipients screening- Excipients such as oil, surfactant, and co-surfactant were chosen based on the solubility of L-glutathione reduced in various oils and surfactants. To assess the solubility the Eucalyptus oil and Castor oil were screened. To obtain stable formulation,

tween-20, Tween-60, Tween-80, PEG-400, PEG-200, propylene glycol and ethanol were tested for solubility. Just 1.0 ml of oil or surfactant was added with a maximum amount of L-glutathione reduced. Using the cyclomixer Remi CM-101 the tube was then vortexed (72.0 hours, 25.0 ^{0}C ±1^0 C). A cumulative reduced volume of L-glutathione was extracted from the mixture by centrifugation (Remi centrifuge) for (10.0 minutes rcf) followed by 72.0 hours. 10.0 Supernant microliters were transferred to fresh tube and length, and ethanol concentrations were up to 1.0 ml. The mixture was first filtered with a syringe filter (0.22 micrometer), then vortexed. The right absorbance dilution at 249 nm to show a calibration curve, followed by an unspecified quantity of reduced L-glutathione dissolved in a definite amount of oil or surfactant and co-surfactant **[28]**.

3.9 Preparation of Nanoemulsion- They prepared emulsions using the Inversion of the Emulsion Phase (EPI) method. The primary formulation of the base nanoemulsion was prepared using a magnetic stirrer to separately heat up the oil and water system in the water bath in two different 60 ° c beakers with continuous stirring at 920 rpm for 30 minutes. The oil phase consists of Orange oil, propyl paraben (0.05 per cent) and PEG-400 while the water phase containing Tween 80, L-glutathione and methyl paraben (0.1 per cent) was reduced by distilled water dissolving. Through the water phase the oil component was then continuously mixed with a magnetic stirrer at 920 rpm. After a while the mixture was stirred for 15 minutes at 1500 revaluation per minute and homogenized with T25 Ultra-Turrax (IKA, USA) **[25]**.

3.10 Evalution of nanoemulsion

A. **Appearance Test-** The appearance check was visually performed, and would involve observation of the physical character of the nanoemulsion such as color, texture and homogeneity of the nanoemulsion **[23]**.

B. **Dye Solubilization- A** water soluble dye is solubilized in the aqueous process of the w/o globule, but is dispersible in the o / w globule. In the o / w globule oil phase the oil-soluble dye is solubilized, but is dispersible inside the w/o globule **[24]**.

C. **pH-** The pH of their nanoemulsion loaded with reduced L-glutathione was measured using a digital pH meter. For nanoemulsion another important parameter is pH. The formulations used in formulations decide the pH of its final deployment and, therefore, the

exposure process. The pH shift can affect the formulation's surface charge which will in turn affect the preparation's stability. The formulations were calculated by means of a automated pH meter. The tests were taken in triplicate and consideration was given to the mean **[22]**.

D. **Viscosity-** Viscositiy was measured for determining the rheological properties of formulations. The Brookfield Rheometer viscometer fulfills this function at 30°C with a CPE 64 spindle at 30 rpm. The measurements were carried out in triplicate, and attention was given to the mean **[26]**.

E. **Dispersibility tests-** Dispersibility tests were performed using a 2.1 mL dissolution apparatus of each form, applied to 500 mL of water at 37±0.5 ^{o}C. A standard, 50 rpm rotating paddle for dissolution of stainless steel provided gentle agitation. The consistency of the formulation was visually evaluated in vitro using the grading system such as from Grade A to Grade E **[23]**.

F. **Refractive index-** The nanoemulsion refractive index determined at 25±0.5 ° by Abbes style refractometer, by putting a decrease in nanoemulsion mainly on the slide and comparing it to the water refractive index (1.333). The refractive index for reduced Abbe refractometer L-gluatathione nanoemulsion was reported to be close to that of water and the formulations' refractive index value **[25]**.

G. **Drug Content -** Using a UV spectroscopic system the drug content of reduced L-gluatathione nano-emulsion formulation was determined. Aliquot 10 µg / mL was prepared using nano-emulsion formulated using water as a solvent. The samples were measured as 249 nm using UV-spectroscopic method **[20]**.

H. **Particle size measurement-** The formulation droplet sizes were determined using Zeta Sizer 1000 HSA (Malvern Instrument, UK), based on the principle of spectroscopy photons correlations. The solution was diluted with the buffer before reading the particle scale to obtain the K count from 50-200, as required by machine consistency **[19]**.

I. **Zeta Potential Determination-** Zeta potential is measured for

variety of factors, such as particle surface charge density, solution counter ion concentration, solvent polarity, and temperature. To determine zeta potential, use the Malvern Zeta sizer or the Nicomp particle sizer **[18]**.

J. **Thermodynamic stability tests-** Selected formulations have had to undergo various thermodynamic stability checks. Heating cycle cooling **[27]**.

K. **Heating cooling cycle-** It was analyzed from six cycles with storage at no less than 48h at each temperature between refrigerator temperature 4^{o}C and 45^{o}C. These formulations, which were stable at these temperatures, underwent centrifugation **[26]**.

L. **Centrifugation-** Those formulations that went through were centrifuged with centrifuge at 3500 revaluation per minute for 30 minutes. The formulations which showed no separate steps have been taken for further testing **[20]**.

M. **Freeze thaw cycle-** Between – 21 ^{o}C and +25 ^{o}C, for the formulations that passed these thermodynamic stress tests were further taken for dispersibility testing, three freeze thaw cycles were performed with storage at each temperature of about 48 h **[18]**.

N. ***In-vitro* release studies-** Works on the release of drugs in vitro were performed using a Franz diffusion. That formulation was applied to the surface of the egg membrane which was located between the donor and receptor compartment of the Franz diffusion. Sample (5 ml) was removed at reasonable time intervals and diluted with the same solvent up to 10 ml, and replaced with equal quantities of fresh dissolution paper **[23]**.

O. **Accelerated stability studies-** Temperature stability investigation was applied by holding the sample at two different temperatures (40 °C± 2 °C, Room Temperature) for 90 days, and visual observation was obtained by drawing samples at intervals for the next days. The formulations were centrifuged for 15 minutes at 0°C at 1000 revolution per minute and checked for any improvement in the homogeneity of formulations with nanoemulsion **[20]**.

P. **Determination of *in vitro SPF* –** The reported procedure for summation was taken and

multiplied with the correction factor (10) to obtain the SPF values **[21]**.

3.11 Determination antioxidant activity using Hydrogen peroxide scavenging (H_2O_2) assay- The ability of nanoemlsion to scavenge hydrogen peroxide can be estimated according to the reported method of Ruch **[24]**.

4. RESULTS

4.1 Organoleptic properties- Physical appearance of medication was analyzed for following organoleptic properties appears good for B4 batch as compared to others.

4.2 Melting point- Temperature was noted at which solid drug changes into liquid. It was found to be 193°C.

4.3 Preparation of standard curve of reduced L-glutathione- The decreased arrangement of standard reduced L-glutathione concentration of 100 µg / ml showed the most extreme absorbance at 249 nm wavelength. Later, the reduced L-glutathione was found to be 249 nm.

4.4 Interpretation of IR spectrum of reduced L-gluatathione- They found that significant peaks present at Stretching, which defined the purity of reduced L-glutathione. Drug and excipient analysis say FTIR spectroscopic drug and excipients.

4.5 Excipients screening- Excipients such as oil, surfactant, and co-surfactant were selected based on L-glutathione solubility reduced in different oils and surfactants. To determine the highest solubility of the Orange oil obtained, different oils, coconut oil and orange oil, eucalyptus oil and castor oil were screened. Solubility studies on Tween-20, Tween-60, Tween-80, PEG-400, PEG-200, propylene glycol, and ethanol were carried out. Surfactant from Tween-80 and PEG-400 was obtained, and co-surfactant was selected for stable formulation.

4.6 Evalution of nanoemulsion-

A. Appearance Test: The appearance test was conducted visually. This is an examination of the physical character of a nanoemulsion. It includes colour, texture and homogeneity of the nanoemulsion.

B. **Dye Solubilization -** Dye solubilization test was performed with a water soluble dye (amaranth) and oil soluble dye (scarlet red) solution appearance in under microscope. Showing water-soluble dye (amaranth): Disperse phase is colored and continuous phase is colorless. Oil-soluble dye (scarlet red) continuous phase is colored and disperse phase is colorless.

C. pH- The pH levels of all ready-made information ranged from 5.5 to 5.7, which, when absorbed through the skin, was considered

adequate to hold away from the danger of disruption as the adult skin pH is 5.5.

D. **Viscosity -** The pivoting at 10 (min.) and 100 (max.) revolution at any moment with an axle 64. At each point, the comparative dial perusing was noted, and the consistency of different plans was observed at 558-564 centipoise.

E. **Dispersibility tests-** As grade A and grade B formulations will remain as nanoemulsions when dispersed in GIT, formulations which passed dispersibility tests in grades A and B were taken for further study from based on the results.

F. **Refractive index-** In B4 formulation, an increase in water content may be due to the lowest RI values, because water has a relatively different refractive index (the water refractive index is 1.333).

G. **Drug Content -** The percentage of drug content of all formulations ranged from 89% to 93%.

H. **Particle size measurement-** The droplet size of formulations batch number B4 is 225±2.9nm. It was clear that formulations batch number B4 had smallest particle size may due to its unique composition of oil, surfactant and water.

I. **Zeta Potential Determination-** The values of Z potential of the drug loaded nanoformulation batch number B4 were -60.8mV.

J. **Thermodynamic Stability Studies-** Through centrifugation testing, B4 formulations have been found to be stable and are submitted for further characterization and evaluation.

K. ***In-vitro* release studies-** The *in-vitro* release of reduced L-gluatathione nanoemulsion was varied in amount according to concentration of emulsifying agents used on formulations. The result of *in vitro* percentage amount of drug released at different time intervals is plotted against time to get the discharge profiles, as appeared in Table 2.The medication discharge was recorded between 91-96%.

L. **Accelerated stability studies-** The temperature stability check was performed by holding the nanoemulsion sample for 90 days at temperatures (40±2^{o}C, room temperature, and 75±5 percent RH) and performing a visual evaluation.

M. **Determination of *in vitro SPF-*** Simple mathematical equation

which substitutes the *in vitro* method proposes by utilizing UV spectrophotometry and the following equation:

$$SPF_{spectrophotometric}=CF\times\sum EE(\lambda)\times I(\lambda)\times Abs.(\lambda)$$

The invitro SPF nanoemulsion containing L-glutathione was found to be 6 determined by putting values in the above mentioned formula.

N. Determination antioxidant activity

The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\%\ \text{scavenged}\ (H_2O_2)= [(A_i-A_t)/A_i] \times 100$$

Where Ai is the absorbance of control and At is the absorbance of test. The hydrogen peroxide scavenging was found 65 %, calculated by putting values in the above formula.

Table 1: Formulation table of reduced L-glutathione nanoemulsion

Batches	Orange oil %	Surfactant %	Co-Surfactant %	Aqueous phase %
B1	5	44.6	45.4	4
B2	9	40.5	49.5	9
B3	14	35.3	34.7	14
B4	10	45.6	18.4	26
B5	20	29.2	30.8	19
B6	25	25.4	24.6	24
B7	10	34.7	35.3	18
B8	18	34.5	35.5	10
B9	30	30.3	19.7	19

Table 2: *In-vitro* release studies of various formulations

Time (min)	B1	B2	B3	B4	B5	B6	B7	B8	B9
0	1.82±0.3	1.75±0.2	1.63±0.3	1.88±0.4	1.47±0.2	1.45±0.2	1.52±0.4	1.65±0.4	1.72±0.6
5	4.27±0.2	4.17±0.3	4.77±0.4	4.85±0.3	4.36±0.4	4.18±0.4	4.23±0.2	4.76±0.2	4.22±0.4
10	8.15±0.6	6.37±0.2	7.63±0.6	8.85±0.4	6.29±0.4	7.35±0.2	7.32±0.3	7.27±0.3	6.46±0.4
15	11.23±0.2	9.42±0.4	10.47±0.4	11.77±0.6	10.67±0.6	11.58±0.2	9.44±0.3	9.31±0.4	9.58±0.2
20	16.37±0.3	14.68±0.4	14.27±0.4	16.82±0.6	14.31±0.4	15.23±0.4	15.19±0.2	14.35±0.6	14.78±0.2
25	25.46±0.4	24.32±0.5	24.08±0.6	25.79±0.2	24.16±0.4	24.43±0.4	25.29±0.4	24.45±0.6	24.66±0.4
30	42.39±0.5	41.32±0.2	40.47±0.6	42.27±0.3	40.58±0.6	40.63±0.2	41.82±0.4	41.54±0.4	41.44±0.4
60	75.17±0.4	74.28±0.6	74.38±0.2	75.27±0.4	74.70±0.2	74.47±0.2	74.38±0.2	74.29±0.4	74.83±0.6
90	83.45±0.2	82.38±0.2	82.40±0.4	83.87±0.4	82.34±0.4	82.14±0.4	82.68±0.2	83.76±0.4	82.29±0.6
120	94.28±0.2	92.77±0.4	92.33±0.4	95.26±0.7	93.17±0.2	93.88±0.4	92.64±0.6	92.41±0.6	93.58±0.6

5. DISCUSSION

Nanoemulsion containing L-Glutathione formulated by the use of surface reaction method has been evaluated and developed in the present research model. The most effective way to blanch your skin tone is to do so directly to your skin by applying decreased L-Glutathione directly to your skin, which is absorbed deeper into your bloodstream without having to go through your body's digestive system. If you are considering applying reduced L-glutathione topically, you should be mindful that it is available as soaps, creams and lotions fade in the skin. The most effective way to blanch your skin tone is by adding reduced L-Glutathione directly to your skin, which is absorbed more deeply into your

bloodstream without having to go through the digestive system of your body. If you are considering topically applying reduced L-glutathione, you should be aware it is available in the skin as soaps, creams and lotions disappear. Human skin is a special organ that allows for terrestrial life by controlling the loss of heat and water in the body while preventing noxious chemicals or micro-organisms from entering the body. People have used different molecules mostly on body for medicinal or esthetic purposes for centuries, and in the modern times a number of topical formulations have been developed.

6. CONCLUSION

In the most recent research review, L-glutathione reduced the take-up of topical pathways. Marketed topical emulsions containing creams do not increase the rate of absorption. This work offers a stronger release profile of nanoemulsion.

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