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Development and performance evaluation of γ-Oryzanol loaded albumin nanoparticle gel.

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Abstract

Transdermal drug delivery offers an attractive alternative to the conventional drug-delivery methods of oral administration and injection. Nanocarriers have been developed for efficient drug delivery and diagnostic tools. Nanocarrier enables an effective, targeted biomolecular interaction in order to lower side effects caused during the treatment.Cancer therapies are currently limited to surgery, radiation, and chemotherapy. Nanotechnology offers the means to target chemotherapies directly and selectively to cancerous cells and neoplasms, guide in surgical resection of tumors, and enhance the therapeutic efficacy of radiation-based and other current treatment modalities.Nanocarriers as drug delivery systems are promising and becoming popular, especially for cancer treatment. In addition to improving the pharmacokinetics of poorly soluble hydrophobic drugs by solubilizing them in a hydrophobic core, nanocarriers allow cancer-specific combination drug deliveries by inherent passive targeting phenomena and adoption of active targeting strategies.γ-Oryzanol and its components have been evaluated for their anticancer propertiesγ -Oryzanol has a property to treat cancer and hyperlipidemia. It is not soluble in water due to this property γ -Oryzanol give very poor bioavailability but in the nano drug delivery with albumin it has good solubility and permeation ability.The present investigation attempted to find out the anticancer properties of γ-Oryzanol Loaded Albumin Nanoparticles.

Keywords : Transdermal drug delivery, Nanocarrier, Cancer, Albumin, γ-Oryzanol

Introduction

Drug

Drugs are obtained from many sources. Many inorganic materials such as metals are chemotherapeutic; hormones, alkaloids, vaccines, and antibiotics come from living organisms; and other drugs are synthetic or semi-synthetic. Synthetics are often more effective and less toxic than the naturally obtained substances and are easier to prepare in

standardized units. The techniques of genetic engineering are being applied to the production of drugs and genetically engineered livestock that incorporate human genes are being developed for the production of scarce human enzymes and other proteins. The advancement of medicine and chemistry complemented and were complemented by pharmacology during the eighteenth and nineteenth centuries.[1]

Transdermal drug delivery system

Transdermal drug delivery systems are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug, through the skin, at a controlled rate to the systemic circulation.Transdermal drug delivery systems are topically administered medicaments in the form of patches that deliver drugs for systemic effects at a predetermined and controlled rate.[2] Now a day many drugs are administered orally, but they are observed not more effective as desired so to upgrade such character TDDS was created. Drug delivery administered by the skin and attain a systemic effect of drug is called as transdermal drug delivery system.[3] These are kind of dosage form which includes drug transport to reasonable epidermis and potentially dermal tissue of the skin locally therapeutic effect.[4]

Skin

Skin is the layer of usually soft, flexible outer tissue covering the body of a vertebrate animal, with three main functions: protection, regulation, and sensation.[5] Other animal coverings, such as the arthropod exoskeleton, have different developmental origin, structure and chemical composition. The adjective cutaneous means "of the skin" (from Latin cutis 'skin'). In mammals, the skin is an organ of the integumentary system made up of multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments, and internal organs. Skin of a different nature exists in amphibians, reptiles, and birds.[6]

Epidermis

The epidermis is a stratified, squamous, keratinizing epithelium. The complex layer of epidermis varies incontingent upon cell size, thickness and number of cell layers of epidermis which are going from 0.8 mm on palms and soles down to 0.06 mm on the eyelids. About 90% epidermal cells are keratinocytes or chest rated in five layers and creates keratin protein and 8% melanocytes are available.[7]

Dermis

The dermis is the layer of skin beneath the epidermis that consists of connective tissue and cushions the body from stress and strain. The dermis provides tensile strength and elasticity to the skin through an extracellular matrix composed of collagen fibrils, microfibrils, and elastic fibers, embedded in hyaluronan and proteoglycans. Skin proteoglycans are varied and have very specific locations.[8]

Nanoparticles

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.[9]

Nanoparticles For transdermal drug delivery

Topical or transdermal drug delivery is a challenge as the skin acts as a natural and protective barrier [10]. The protective function of the skin is attributed to the epidermal stratum corneum layer. This layer also regulates the transport of compounds into the skin [11]. The nanoparticles act as a reservoir of lipophilic drugs and deliver them to the stratum corneum. Polymeric nanoparticles increase the drug adhesivity and duration for skin permeation [12]. The gelatinpilocarpine hydrochloride (HCl) or hydrocortisone nanoparticles produced using a desolations method showed sustained drug release compared to the aqueous solution of the drugs [13].

Cancer

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.[14] These contrast with benign tumors, which do not spread.Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements. While these symptoms may indicate cancer, they can also have other causes.[15] Over 100 types of cancers affect humans.[16]

Applications of Nanoparticles in Cancer Therapy

One of the most important impacts of nanoparticle-based drug delivery systems appears to be the localized treatment of solid tumors. Novel nanoparticle delivery technologies provide the opportunity for passive accumulation of intravenously injected nanoparticles (20- 150 nm) from leaky vasculature.Macrophages-evading nanoparticles. Folate-based targeting offers distinct advantages over approaches such as monoclonal antibodies. Folate is nonimmunogenic and folate nanoparticles are rapidly internalized by activated cells in a manner that bypasses cancer cell multi-drug-efflux pumps. Furthermore, the idea of targeting drugs to blood vessels of tumors is intuitively appealing and nanotechnology can contribute to the early detection and treatment of metastatic cancers or in the diagnosis and therapy of resistant tumors.[17]

Material and method

Pre formulation study of γ- Oryzanol

Physical appearance

Sense organ examined the physical appearance of γ-Oryzanol. Organoleptic characteristics including colour, odour, and taste were used to describe it physically. Comparing all physical parameters to stated parameters.

Identification of drugγ- Oryzanol

Melting point method

Melting point is the method used to assess the purity of drugs. Below is a picture of a Mel-Temp device with a digital thermometer.To determine the melting range, the sample was dried. Samples are packed in capillaries by the device. Put the capillary into the channels on the front of the thermometer tube and the thermometer into the thermometer well (there are slots for three capillaries).In order to select the power level to achieve the required heating rate, flip the green LED power switch to the "1" position and crank the black heating control knob. The eye should be around 15 cm away from the lens on the front of the equipment where the sample may be seen.

Keep in mind that for an accurate measurement, a modest heating rate at the melting point is required. First, we take a capillary tube and place it close to the burner's flame before sealing one end.To collect a little lump of powder, the capillary tube's opening end was pressed against a small mound of medication. The capillary tube was then gently tapped to encourage the medication to settle down. After the sample begins to melt, note the temperature on the thermometer. Later, when the sample has completely melted, note the temperature once more (this gives you the melting point range).Turn the power to off, remove the capillary, and dispose of it in the designated container after the sample has melted.The cooling process can be sped up by attaching a hose to the on-site compressed air and blowing air across the block. The block will need some time to cool. Using a digital capillary melting point instrument and the capillary fusion method, the melting point was determined. This process was repeated several times. Following that, a capillary tube was inserted in an apparatus with a melting point and a temperature at which samples start to transition from a solid to a liquid condition. There were three runs of this experiment. By using a thermometer, the temperature at which the medicine (γ-Oryzanol) begins to melt was observed and compared to a previously published value.

Figure 1 Melting-Temp apparatus equipped with a digital thermometer

Solubility

The solubility of γ-oryzanol was examined in a variety of solvents with varying pH values (1.2–8.0), including methanol, water, ethanol, acetone, and ether. As per the previously described procedure, buffer solutions (standard) were created. The solubility of oryzanol was determined by adding a significant amount of the medication (γ-oryzanol) to vials with distinct systems of solvent and keeping them at 250°C in a shaker (water bath) for 24 hours.The amount of medication dissolved was measured after the dispersion was filtered using a 0.45 micron pore size filter.

The amount of drug was determined using the absorbance of solutions with known drug concentrations (API) and standard curves. The solubility of the medication (API) was plotted versus the medium's PH and the specific solvent system.

UV Spectrum

γ-Oryzanol (10 mg) was dissolved in 1 ml of 1 M HCl (hydrochloric acid) and 9 ml of methanol before being diluted with solvents to 100 ml (Same mixture). Following this, a volume of 10 ml of the solution was withdrawn, made up to 10 ml with 1 ml of 1M hydrochloric acid and 9 ml of methanol R, and then scanned between 300 nm and 500 nm using a UV-visible spectrophotometer (company name). The UV (drug) spectrum was observed, and the greatest reported absorption was correlated with it (320nm).

Partition coefficient

A drug's capacity to penetrate a cell membrane and its lipophilicity are both determined by the partition coefficient.The value is computed when the drug content in one of the layers has been established. A ratio unionised (γ-Oryzanol) diffuse between an equilibrium organic and aqueous water system may be used to define the partition coefficient.The material will still be spread along(two) layers in a ratio of varied concentration if it is added to the immiscible(not miscible) solvents in an amount insufficient to submerge the solution. If C1 and C2 are in equilibrium, then C1/C2=K. Here, K is referred to as the partition coefficient or distribution ratio. The partition coefficients of γ-oryzanol in ethanol: water were determined. A rubber stopper containing each 20ml of the water and ethanol combination was filled with precisely weighed 20mg, and it was then agitated continuously for two hours. After two hours, the two phases were separated using a funnel, and the water was then examined using a Shimadzu UV-1700.

Loss on Drying

Loss on Drying is the weight loss, given as a percentage of weight, caused by the evaporation of water and other volatile substances under certain circumstances. A carefully prepared, well-mixed sample of the substance is used for these tests.Initial dose of the medication, γ-oryzanol, was put on a Petri disc and heated to 105°C in a hot air oven. After a while, the medication was removed from the hot air oven, and weight was collected. Formula was used to determine weight loss:-

Loss on Drying =[(Initial weight of material –final weight of material)/Initial weight of material] X 100

Analytical methods

Preparation of standard curve of γ- Oryzanol

Preparation of Phosphates buffer pH7.4γ- Oryzanol

When a tiny quantity of acid or alkali is added to a solution, a buffer solution is a unique kind of solution that helps to maintain a steady pH level. One of the buffer solutions that is frequently used in biological labs and in haematology labs for dilution of stains is phosphate buffer solution. 10 litres of water (purified and sodium hydroxide pellets) were mixed with 68 grammes of potassium dehydrogenate orthophosphate (KH2PO4). The PH was changed to 7.4 with the

addition of 3.24 grammes. According to the Indian Pharmacopoeia's recommended formula, phosphate buffered saline with a pH of 7.4 was created (volume 1).NaCl, Na2HPO4, and KH2PO4 were added to a 100 ml volumetric flask and mixed with double-distilled water in accordance with the aforementioned formula. A bath sonicator was used to sonicate the mixture for a while, and the pH was then set to 7.4. Finally, the volume met the required level. After that, 0.5 g of sodium lauryl sulphate was added to the previously prepared PBS and thoroughly dissolved using an ultrasonic bath sonicator for 5 min.

Preparation of standard curve of γ- Oryzanol in 7.4 phosphate buffer

In biological investigations, simple phosphate buffer is frequently employed because it may be adjusted to a range of pH values, including isotonic. Due to phosphoric acid's three dissociation constants, or "triprotic acid" in chemistry, it is possible to create buffers with a pH close to each of the following values: 2.15, 6.86, or 12.32. Although phosphate buffer has a high buffering capacity and is extremely water soluble, it inhibits enzyme activity and precipitates in ethanol.One of the most widely utilised tools today, the buffer is frequently used in fields including molecular and cell biology, chemistry, and material science, among many others.Methanol and 100 mg of γ-oryzanol were combined (100ml). Now, 5 ml of this product was collected and diluted to 100 ml of phosphate buffer (7.4pH). Once more, dilution (serial) from stock solution was made to get solution concentrations between 5 and 35 µg/ml. Then, these were analysed spectrophotometrically with the use of 319 nm absorbance calculations.

Preparation of standard curve for drug loading study

A volumetric flask made of borosilicate glass was used to combine 10 mg of γ-oryzanol with 100 ml of wateracetonitrile (HPLC grade) in a 40:60 ratio. The solvent was then completely dissolved by ultrasonically agitating the mixture for around 2 minutes.From this stock solution, various amounts of 0.2 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml were taken out and diluted to a volume of 10.0 ml using a water-acetonitrile combination to produce five distinct solutions with varying concentrations of γ-oryzanol, including 2 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg Utilizing a UV-visible spectrophotometer and a water-acetonitrile combination as a blank, each solution's absorbance was measured at 218 nm.

Determination of flow properties of pure drug

Bulk density

By measuring the volume of a known-weight powder sample that may have been put through a sieve and placed in a graduated cylinder, one may calculate the bulk density of a powder. The mass of the material's many particles divided by the entire volume they inhabit is how bulk density is calculated. Particle volume, inter-particle void volume, and internal pore volume are all included in the overall volume. The process of disposing of the sample—its preparation, handling, and storage—affects the measured value.. By measuring in graduated cylinders (USP type), bulk density was estimated. The needed amount of the material was precisely weighed, put through Sieve No. 22 to remove any agglomerates (clogging), and then transferred

into a 100ml measuring cylinder without compacting. The volume was carefully documented. In gm/ml, the bulk density was determined.

Bulk density= Mass of powder/volume of powder

Tapped density

The tapped value can be measured more accurately than the bulk value.The ratio between these two measured densities is used to determine a powder's "flowability."Following the determination of the bulk density, measuring cylinders were mechanically tapped on a holder in the tapped density tester, which produces a fixed drop at a nominal rate of 300 drops per minute (rate).The volume (tapped) Vt was determined to the closest graded unit after tapping the measuring cylinder about 500 times at first. Tapped volumes were computed after 100 additional taps were made. The following formula was used to determine the final tapped (volume) and measure the tapped densities:-

Tapped density= Mass of powder/Tapped volume

Compressibility index and Hausner ratio

The Compressibility Index is a measurement of a powder's propensity to be compressed as previously mentioned. As a result, it serves as a gauge for how well a powder settles and enables the evaluation of the relative significance of interparticulate interactions.A Carr index above 25 is seen as a sign of poor flowability, and one below 15 as a sign of high flowability.

The Hausner ratio, or simply p,/pb, or the tapped density divided by bulk density, is a more often used expression.The flow is poorer the larger the Hausner ratio. For a free-flowing powder, the Hausner ratio is around 1.2; for a cohesive powder, it is 1.6.Many different sectors utilise the Hausner ratio as a measure of a powder's flowability. A Hausner ratio above 1.25 to 1.4 is seen as a sign of poor flowability. The Carr index (C), another sign of flowability, and the Hausner ratio (H) are connected.

Angle of repose

For a powder to have high flowability, it is vital to consider its angle of repose. The angle of repose of a medication can be measured using a variety of techniques. The angle produced between the horizontal plane and a sloping line running down the face of a heap created by dumping material onto the horizontal surface is known as the angle of repose. The flowability of various powders may be measured quickly, accurately, and simply using the angle of repose. The cohesiveness of the powder increases with increasing angle of repose (poor flow), but the bulk material flows more freely with decreasing angle of repose.

Method of preparation of Nanoparticles

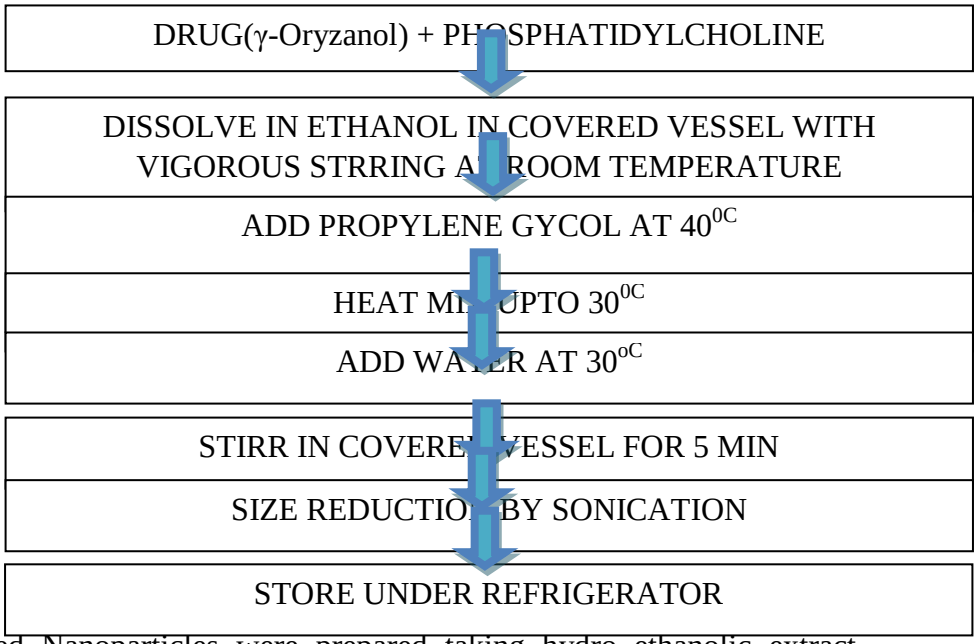
Emulsification

An oil-in-water (o/w) emulsion is ultimately produced by emulsifying an organic phase made up of an aqueous phase containing the stabilising agent and an aqueous phase constituted of a water-immiscible solvent in which the preformed polymer is dissolved. Under the right circumstances, the organic solvent's subsequent evaporation causes the polymer to aggregate into nanoparticles of a few hundred nm

diameter. The organic phase is then added after incorporating lipophilic properties into the drugs. Emulsion solvent evaporation was used to create γ- oryzanol-loaded nanoparticles. 1.5% (w/v) and 2.5% (w/v) aqueous solutions of polyvinyl alcohol were produced independently in the first stage. The following step involved creating an organic drug and albumin solution in dichloromethane (2 ml). the ratios of medication to polymer that are utilised in different formulations. A primary emulsion was created after adding dropwise 0.5 ml of previously produced 2.5% PVA solution to the drug-polymer combination and homogenising the mixture at 20,000 rpm for 5 minutes at room temperature.The 1.5% PVA solution was then mixed with 75 ml of this main emulsion drop by drop while being homogenised continuously for 8 minutes at 20,000 rpm. A common hydrophilic polymer that serves as a stabiliser is PVA. PVA possesses a hydrophilic -OH group, but it also contains a nonpolar vinyl component. As a result, the surface tension between the aqueous and nonpolar non-watery portions of the mixture decreases, with the vinyl group facing the non-polar portion and the OH-group facing the aqueous portion. It stabilises the main emulsion in this way.

Preparation of albumin loaded Nanoparticles

Nanoparticle could increase its percutaneous capability and promote the curative efficacy through gradual release of the drugs. Nanoparticle drug delivery system opens up doors for the development of new and novel therapies, as it is an easier way to prepare in addition to its safety and efficacy.



Albumin loaded Nanoparticles were prepared taking hydro ethanolic extract (10:90) γ-Oryzanol. All required necessities were weighed in distinct concentrations for the preparation of Nanoparticle formulation. Nanoparticles were prepared by slight modification of cold method (Dayan and Touitou, 2000). Initially (1-3%) of Phosphatidylcholine was taken and dissolved in (10-40%) of 90% ethanol by use of magnetic stirrer (Remi Motors Mumbai) in completely closed flask at 30 ºC. To this solution 10 mg hydro alcoholic extract dissolved in

hot distilled water (having temperature 30 ºC) (to make volume up to 100%) was added as fine stream by the use of syringe very slowly and then whole system was stirred for 15 minutes at 900 rpm. Further they were sonicated for 5-15 min. Finally, the formulations were stored under refrigeration (Dayan and Touitou, 2000).

The optimization was done on the basis of vesicular size and percent entrapment efficiency. After optimization of ratio of components, the albumin loaded Nanoparticles were prepared taking optimized ratio of extract individually and further evaluation parameters were performed.Based on that, curcumin Nanoparticle was produced using Nanoparticle as a drug delivery system of γ-Oryzanol.

Amongst all the formulations, NS-8 formulation was optimized based on % entrapment efficiency. The average size of Nanoparticles was determined by microscopy.Entrapment efficiency gives you an idea about the % drug that is successfully entrapped/adsorbed into nanoparticles.There are several particle characteristics that are of critical importance when considering pharmaceutical properties. Zeta potential was determined using Zetasizer (HORIBA SZ-100). Measurements were performed on the same samples prepared for size analysis.

Table 1 Optimization Of Components Of Albumin Loaded Nanoparticle Formulation

S.NO	Formulation Code (Nanoparticle Suspension-NS)	Tween 80	Phosphatidyl choline	Ethanol:Distilled Water	γ-Oryzanol (mg)
1.	NS-1	2%	1	25:75	10
2.	NS-2	4%	3	40:60	10
3.	NS-3	6%	2	20:80	10
4.	NS-4	2%	1	15:85	10
5.	NS-5	4%	2	10:90	10
6.	NS-6	6%	3	30:70	10
7.	NS-7	2%	1	35:65	10
8.	NS-8	4%	3	45:55	10
9.	NS-9	6%	2	40:60	10
10.	NS-10	2%	1	10:90	10

Physicochemical characterization of nanoparticles

Drug loading and loading efficiency

The ratio of the amount of drug in the nanoparticle to the overall amount of drug used in their formation is known as drug loading efficiency. The ratio of the quantity of drug in a nanoparticle to the overall amount of drug used in its formulation is known as drug loading efficiency. To determine the quantity of drug entrapped in the experimental formulations, drug loading was done. 2 ml of a water-acetonitrile solution (40:60 v/v) were used to suspend the necessary amount of nanoparticles (2 mg). The mixture was vortexed for five minutes, then shaken for three to four hours at 37°C in an incubator shaker. After 5 minutes of 10,000 rpm centrifugation, the supernatant was collected. The absorbance was measured spectrophotometrically at 218 nm following the proper dilution. The absorbance was measured using the same process for the blank formulation. The difference between the absorbance of the nanoparticle formulation and the blank formulation was used to quantify the actual quantity of drug contained in nanoparticles. The following formulae were used to determine the proportion of real medication loading and loading effectiveness:

$$\text{Percentage of loading} = \frac{Amount\ of\ in\ present\ drug\ nanoparticles}{Weight\ of\ nanopartic\ le\ analyzed} \text{ x } 100$$

$$\text{Percentage of efficiency} = \frac{Actual\ loading\ drug}{Theoretical\ \ drug\ loading} \text{ x } 100$$

Yield Percentage

Regarding the total amount of raw materials utilised for the formulation, the quantity of nanoparticles produced was calculated. The lyophilized nanoparticles were weighed, and the formulations' % yield was determined using the formula below:

$$\text{Percentage of loading} = \frac{Amount\ of\ nanopartic\ obtained}{Total\ of\ polymer\ and\ drug\ used} \text{ x } 100$$

Particle size, size distribution and zeta potential

The stability of therapeutic goods made possible by nanoparticles in complicated biological settings and the particle size distribution are crucial factors in determining the efficacy, safety, and quality of those products.

The electrical potential at the sliding plane is known as zeta potential. The interface that divides fluid that is mobile from fluid that is still affixed to the surface is this plane. Zeta potential, then, is the difference in potential between the dispersion medium and the stationary fluid layer affixed to the dispersed particle. The stability of colloidal dispersions is significantly and easily measured by the zeta potential. The size of the zeta potential represents the strength of electrostatic attraction between nearby particles with identical charges in a dispersion. For sufficiently tiny molecules and particles, a high zeta potential will impart stability, which means that the solution or dispersion will fend off aggregation.

Understanding the size range of the nanoparticles requires knowledge about their particle size distribution. The stability of colloidal dispersion is implied by the zeta potential, which is a measurement of the surface charges of nanoparticles. By using a dynamic light scattering approach, the average particle size, size distribution, and zeta potential of loaded nanoparticles were investigated. At a temperature of 25°C, a

scattering angle of 90° was used for the analysis. Before observation, samples were diluted with Milli-Q water.

Preparation of nanoparticle Gel

The gels were prepared by dispersion method using Polymer. Accurately weighed Propylene Glycol was taken in a beaker and dispersed in 50 ml of distilled water. Kept the beaker aside to swell it for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. Themixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. The Nanoparticle concentrate (pellet) with drug equivalent to 1%w/w was incorporated into the priorly formed gel base. Triethanolamine was added to maintain the pH and for the spontaneous gel formation.

To this gel solution optimized Nanoparticle dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. Paraben was added as a preservative. The prepared gels were filled in glass vials and stored at 4-8^{0}C.

Table 2 Preparation Of Nanoparticle Gel

S.NO	Formulation Code (Nanoparticle Gel -NG)	Tween 80	Purified Water	Coconut oil	Triethanolamine	Nanoparticle Suspension
1.	NG-1	10	20	As much as suffices	2	Nanoparticle Suspension 1
2.	NG-2	15	20	As much as suffices	4	Nanoparticle Suspension 2
3.	NG-3	18	20	As much as suffices	2	Nanoparticle Suspension 3
4.	NG-4	15	20	As much as suffices	4	Nanoparticle Suspension 4
5.	NG-5	11	20	As much as suffices	2	Nanoparticle Suspension 5
6.	NG-6	17	20	As much as suffices	4	Nanoparticle Suspension 6
7.	NG-7	19	20	As much as suffices	2	Nanoparticle Suspension 7
8.	NG-8	20	20	As much as suffices	4	Nanoparticle Suspension 8
9.	NG-9	15	20	As much as suffices	2	Nanoparticle Suspension 9
10.	NG-10	10	20	As much as suffices	4	Nanoparticle Suspension 10

Characterization & Evaluation of Formulation

Evaluation of Gel Formulation

All prepared formulations of gel were characterized for:

Physical Evaluation

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

Measurement of pH

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

Spreadibility

Spreadibility was determined by the apparatus which consists of a wooden block, provided with pulley at one end. By this method spreadibility was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped of from the edges. The top plate was then subjected to pull of 80 gms weight with the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicates better spreadibility. Spreadibility was calculated using the formula given below:

$S = M \times L / T$

Where,

S = Spreadibility,

M = Weight in the pan (tied to the upper slide),

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the slide completely each other.

Consistency

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

Homogeneity

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

Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle No. 7 at 50 rpm at room temperature. The gels were rotated at 0.3, 0.6 and 1.5 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brookefield Viscometer manual.

Results

Organoleptic properties

The organoleptic properties include drug substance appearance, colour and odour. They are sensory experiences of the distinctive attributes or qualities of a thing. It is a qualitative evaluation type in which the morphological and sensory characters of drugs are studied. Study of a drug's macroscopy involves its visual appearance to the naked eye. The properties are shown in table 3

Table 3 The organoleptic properties of γ- Oryzanol

S.NO	PROPERTIES	OUTCOME
1.	Colour	White to off white
2.	Shape	Crystalline
3.	Odour	Little or no odor,
4.	Texture	Powder
5.	Taste	Tasteless

Figure 3 γ- Oryzanol powder

Identification of γ- Oryzanol

Melting point

The melting point (or, rarely, liquefaction point) of a substance is the temperature at which it changes state from solid to liquid. At the melting point the solid and liquid phase exist in equilibrium. The melting point of the medicine(γ- Oryzanol) was measured using Melting-Temp apparatus equipped with a digital thermometer and was found 136.5^{0}C as shown in table 4

Table 4Melting point of γ- Oryzanol

Crude drug	Melting point
γ- **Oryzanol**	136.5^{0}C

Solubility ofγ- Oryzanol

A drug's ability to dissolve in solvents is a crucial characteristic that affects how well it is absorbed when taken orally. The solubility of γ- Oryzanol was done using the USP-designated "shake flask Method. The solubility was found to be 0.28 in ethanol, 2.68 in ether, 0.13 in water, 0.37 in methanol and 3.86 in acetone respectively and shown in table 5

Table 5 Solubility of γ- Oryzanol in different solvents

S.No	Parameters % w/w	Ethanol	Pet. Ether	Water	Methanol	Acetone
1.	Solubility	0.28	2.68	0.13	0.37	3.86

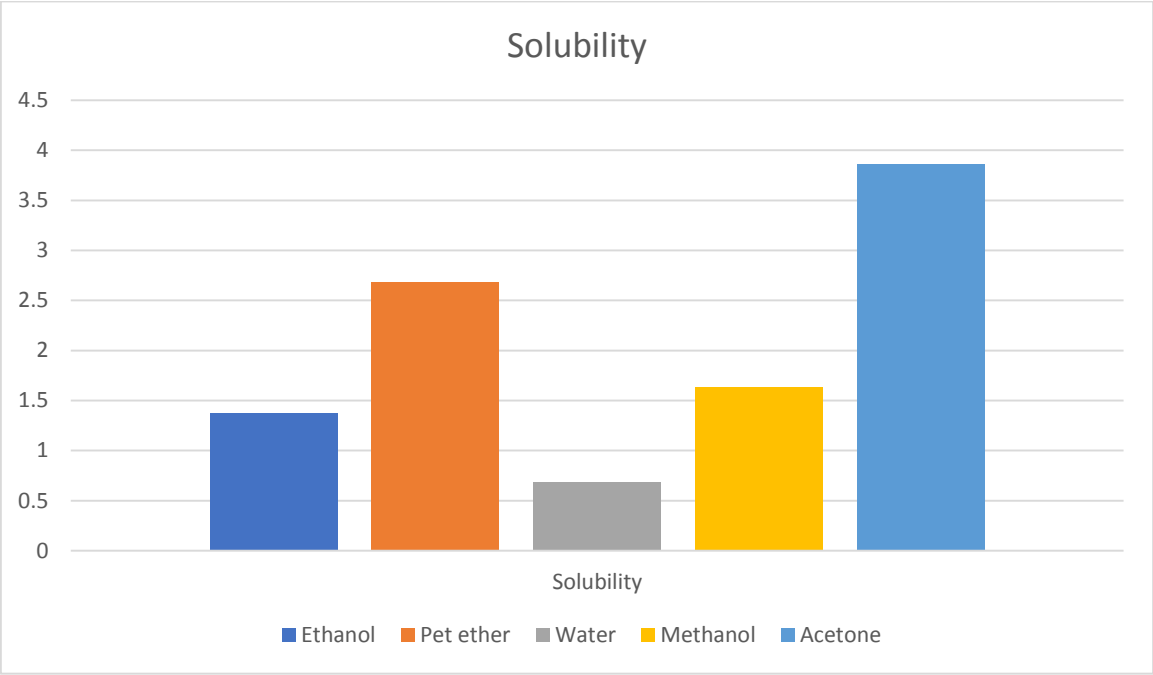


Figure 4 Solubility of γ- Oryzanol in different solvents

Figure 5 Pet ether solution

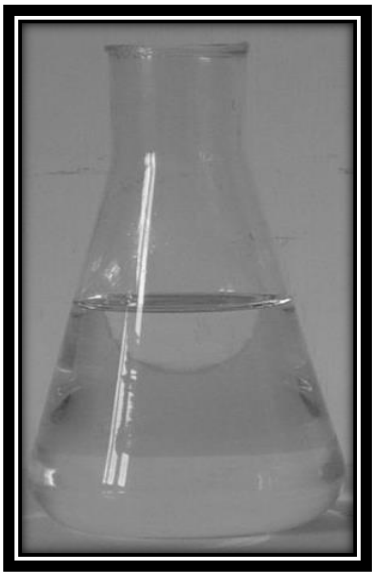


Figure 6 Ethanol solution

UV Spectrum

UV- spectroscopy has been applied in numerous ways to facilitate qualitative and quantitative analysis as well as structure determination and online reaction monitoring in a wide range of environments. The most commonly used techniques for the determination of γ-oryzanol in rice bran oil are UV Spectrophotometry. The sprectrum shows a complex sprectrum. γ - Oryzanol is composed of multiple molecular species. Depending on the separation conditions, the peak of γ-oryzanol may be a single peak or may be divided into a plurality of peaks. This separation condition is not particularly limited, but a condition in which a peak is separated for each molecular species is preferable in terms of easy identification. The UV spectrum of γ- Oryzanol is shown in figure 7

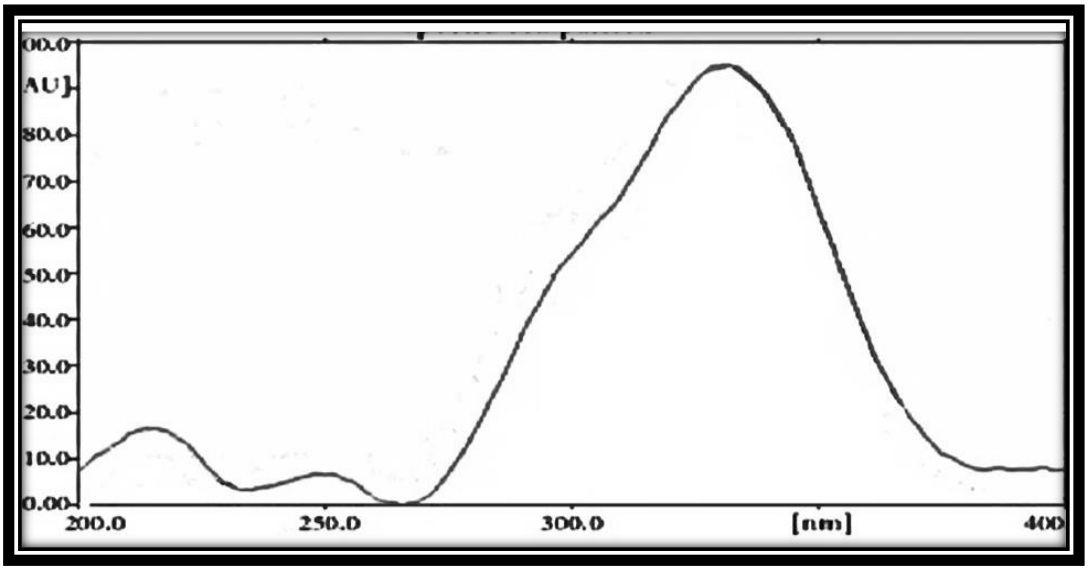


Figure 7 UV spectrum of γ- Oryzanol

Partition coefficient

Partition coefficient (P) is a ratio of a compound's concentrations in the mix of two immiscible solvents at the equilibrium. The ratio is the comparison of the solute's solubilities

in the two liquids. Partition coefficient (P) was determined to be 0.28 as shown in table 6Therefore, experimental data and standard data agree most closely.

Table 6Partition coefficient of γ- Oryzanol

Crude drug	Partition coefficient(P)
γ- Oryzanol	0.28

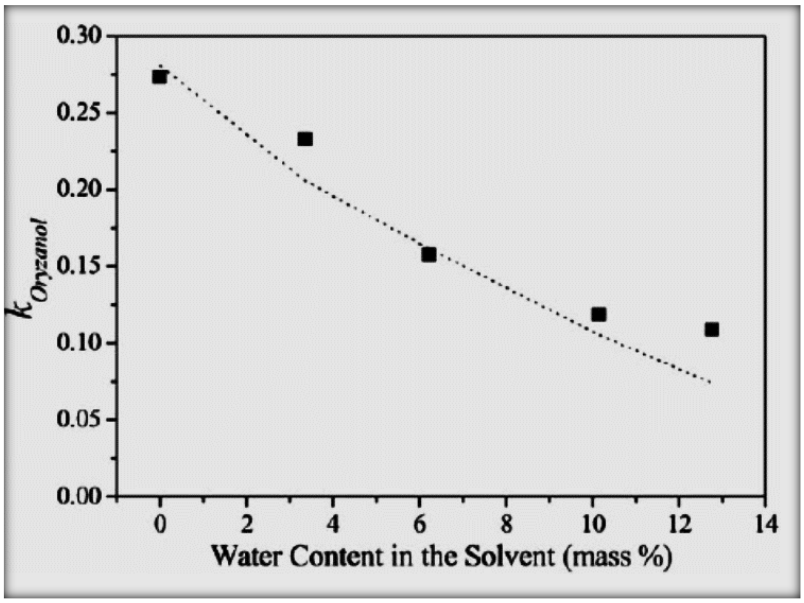


Figure 8Partition coefficien γ- Oryzanol

Loss on drying

The loss of drying test is a technique for calculating the sample's weight loss during drying under the circumstances listed in each monograph. The value was found to be 11.23 and shown in table 7

Table 7 Loss on drying

Crude drug	Loss on drying (% w/w)*
γ- Oryzanol	11.23

Preparation of standard curve of γ- Oryzanol

Preparation of Phosphates buffer pH7.4γ- Oryzanol

A simple phosphate buffer is used ubiquitously in biological experiments, as it can be adapted to a variety of pH levels, including isotonic. This wide range is due to phosphoric acid having 3 dissociation constants, (known in chemistry as a triprotic acid) allowing for formulation of buffers near each of the pH levels of 2.15, 6.86, or 12.32. Phosphate buffer is highly water soluble and has a high buffering capacity, but will inhibit enzymatic activity and precipitates in ethanol. The buffer is one of

the most popular currently used, and is commonly employed in molecular and cell biology, chemistry, and material science, among many others. The UV absorbance of OZ standard solutions in the range of 1-25µg/ml of drug in phosphate buffer pH 7.4 showed linearity at λmax 319 nm.

Table 8 Absorbance of working standard solution

S.no	Concentration(µg/ml)	Absorbance
1.	**0**	**0**
2.	**2**	**0.095**
3.	**5**	**0.18**
4.	**10**	**0.28**
5.	**12**	**0.4**
6.	**15**	**0.55**
7.	**25**	**0.7**

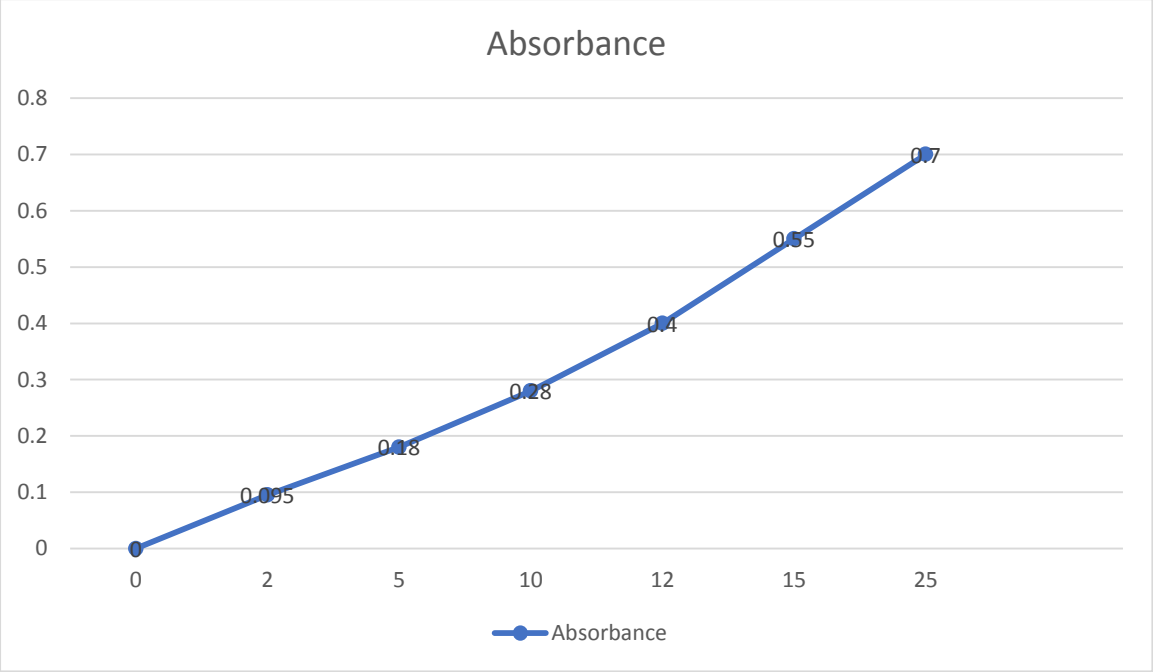


Figure 9 Graph of Phosphates buffer pH7.4γ- Oryzanol

Preparation of standard curve of γ- Oryzanol in 7.4 phosphate buffer

The UV absorbance of OZ standard solutions in the range of 1-25µg/ml of drug in phosphate buffer pH 7.4 showed linearity at λmax 319 nm.

Table 9 Absorbance of working standard solution

S.no	Concentration(µg/ml)	Absorbance
1.	**0**	**0**
2.	**2**	**0.098**
3.	**5**	**0.20**
4.	**10**	**0.31**
5.	**12**	**0.98**

6.	**15**	**0.45**
7.	**25**	**0.7**

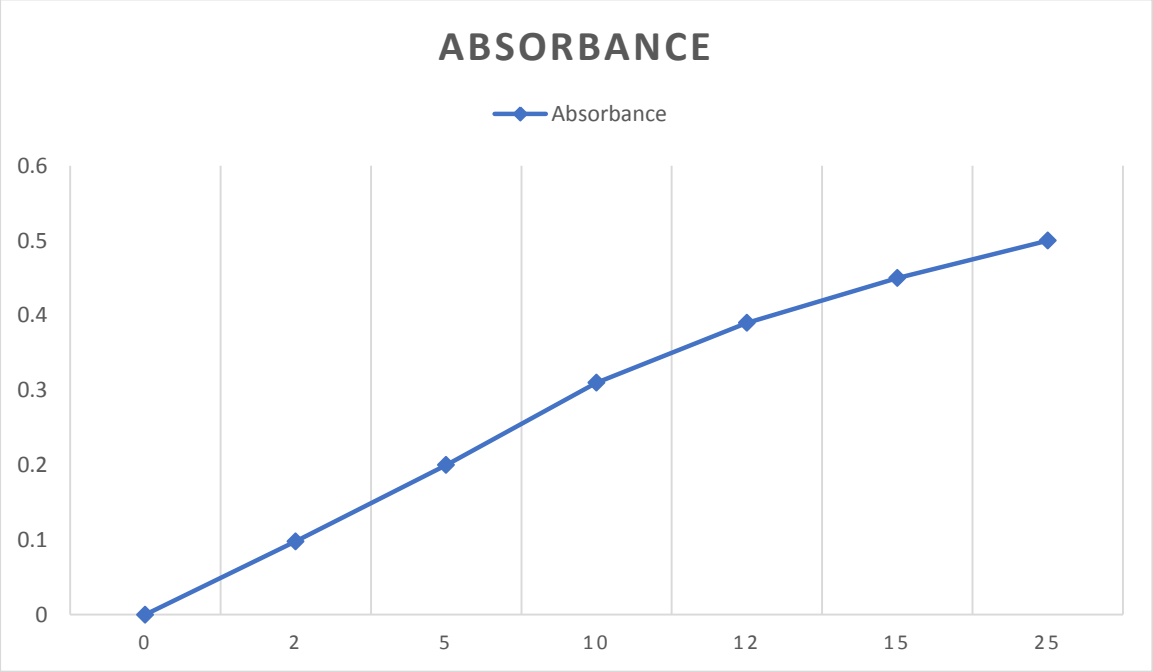


Figure 10 Graph of standard curve of γ- Oryzanol in 7.4 phosphate buffer

Preparation of standard curve for drug loading study

Table 10 Absorbance of working standard solution

S.no	Concentration(µg/ml)	Absorbance
1.	**0**	**0**
2.	**2**	**0.1**
3.	**5**	**0.190**
4.	**10**	**0.289**
5.	**15**	**0.40**

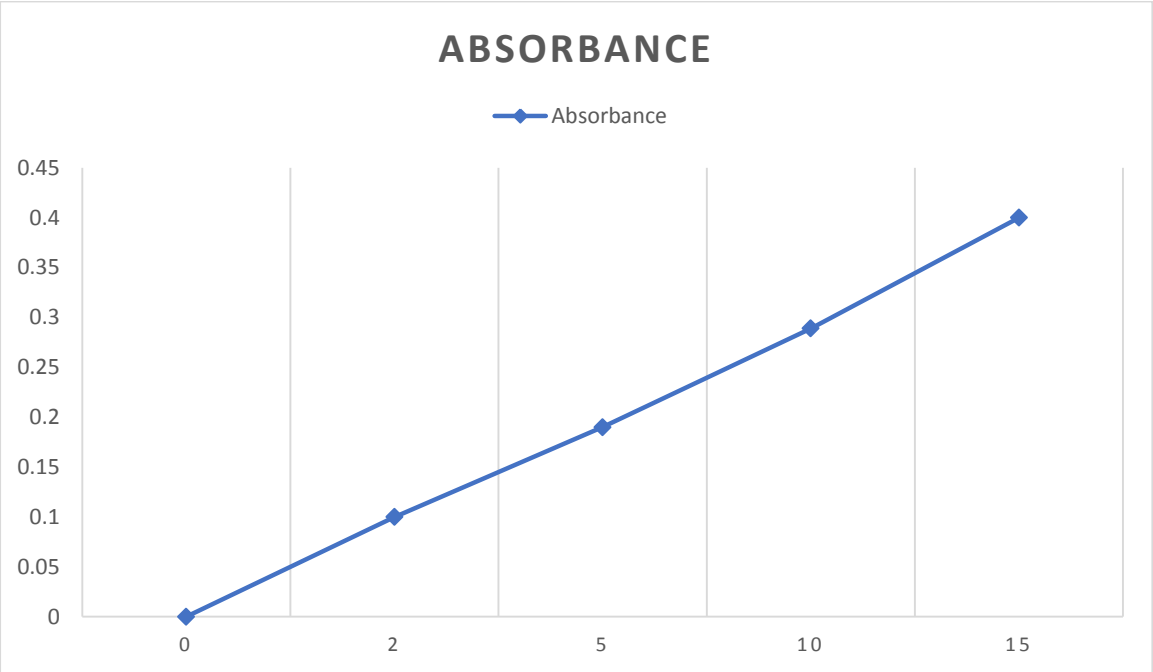


Figure 11 Graph of standard curve for drug loading

Determination of flow properties of pure drug

Bulk density and tapped density

Bulk density is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder. Tapped density is achieved by mechanically tapping a measuring cylinder containing a powder sample. After observing the initial volume, the cylinder is mechanically tapped, and volume readings are taken until little further volume change is observed. The values are shown in table 11

Table 11 Determination of flow properties of pure drug

Parameters	Values
Bulk density	0.68
Tapped density	0.56

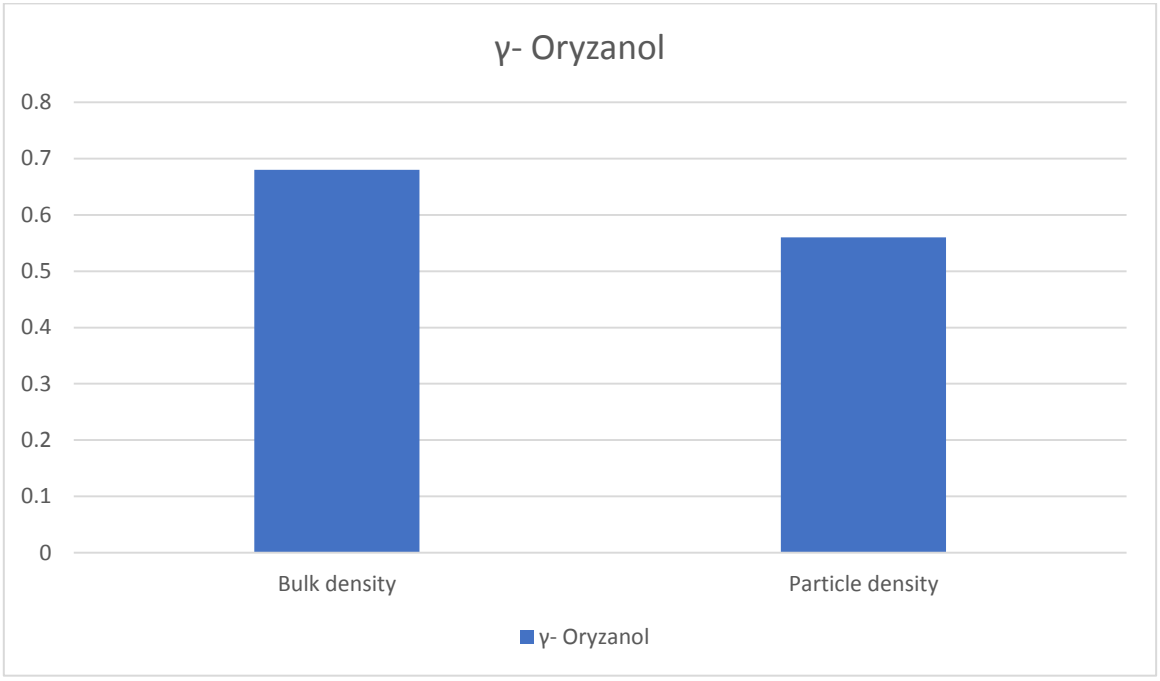


Figure 12 Graph of flow properties of γ- Oryzanol

Compressibility index, hausner ration and angle of repose

The compressibility index, hausner ration and angle of repose were found to be 6.68, 1.44 and $27.38^0$and are shown in table 12

Table 12 Determination of flow properties of pure drug

Parameters	Values
Compressibility index	6.68
Hausner ratio	1.44
Angle of repose	27.38^0

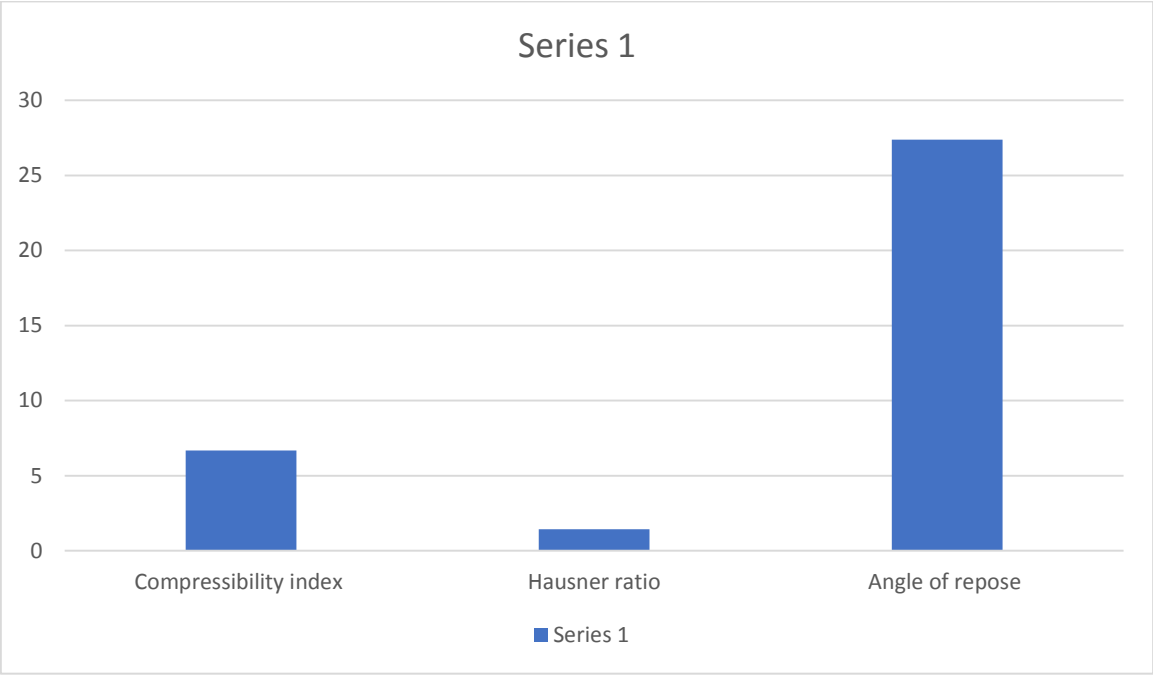


Figure 13 Graph of flow properties of γ- Oryzanol

Physicochemical characterization of nanoparticles

Understanding the physicochemical properties of nanomaterials and how functionalizations modify their surface, altering their properties, is fundamental to defining better strategies of use. Therefore, a good understanding of nanoparticle requires the use of several physicochemical and morphological techniques to adequately determine its drug loading Percentage, yield percentage, drug loading efficiency, particle size and zeta potential. Following characters were observed while preparation of nanoparticle suspension. Drug loading %, drug loading efficiency, yield percentage,particle size and zeta potential arde shown in table 13 and table 14

Table 13 Physicochemical characterization of nanoparticles

Formulation (Nanoparticle Suspension-NS)	Drug loading (%)	Loading efficiency (%)	Yield Percentage(%)
NS-1	**46.23**	61.46	65.16
NS-2	**44.21**	59.03	63.23
NS-3	**43.43**	58.33	74.21
NS-4	**42.22**	56.12	53.11
NS-5	**47.63**	60.23	58.47
NS-6	**47.87**	62.45	79.33
NS-7	**44.23**	57.56	80.45
NS-8	**43.73**	59.12	84.43
NS-9	**42.12**	55.43	71.12
NS-10	**44.43**	58.63	63.56

Table 14 Physicochemical characterization of nanoparticles

Formulation	Particle size(nm)	Zeta potential(mv)
NS-1	435	21.23
NS-2	213	23.56
NS-3	380	24.11
NS-4	355	25.63
NS-5	250	24.56
NS-6	330	25.93
NS-7	370	26.12
NS-8	400	25.62
NS-9	412	23.12
NS-10	250	22.36

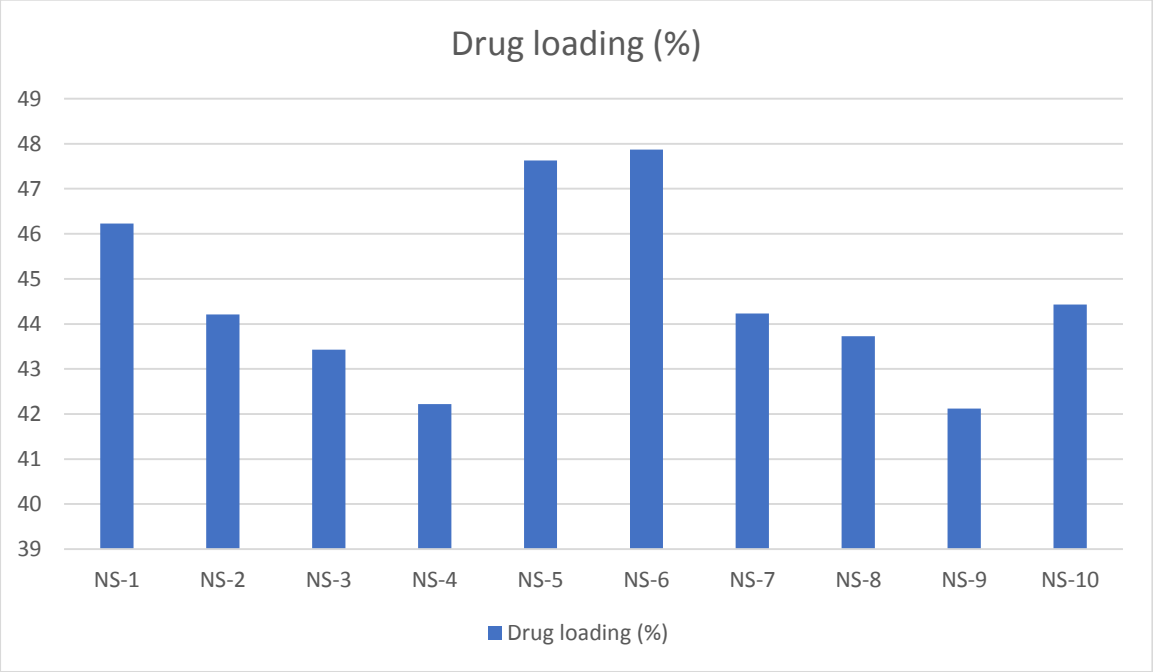


Figure 14 Graph of Drug loading

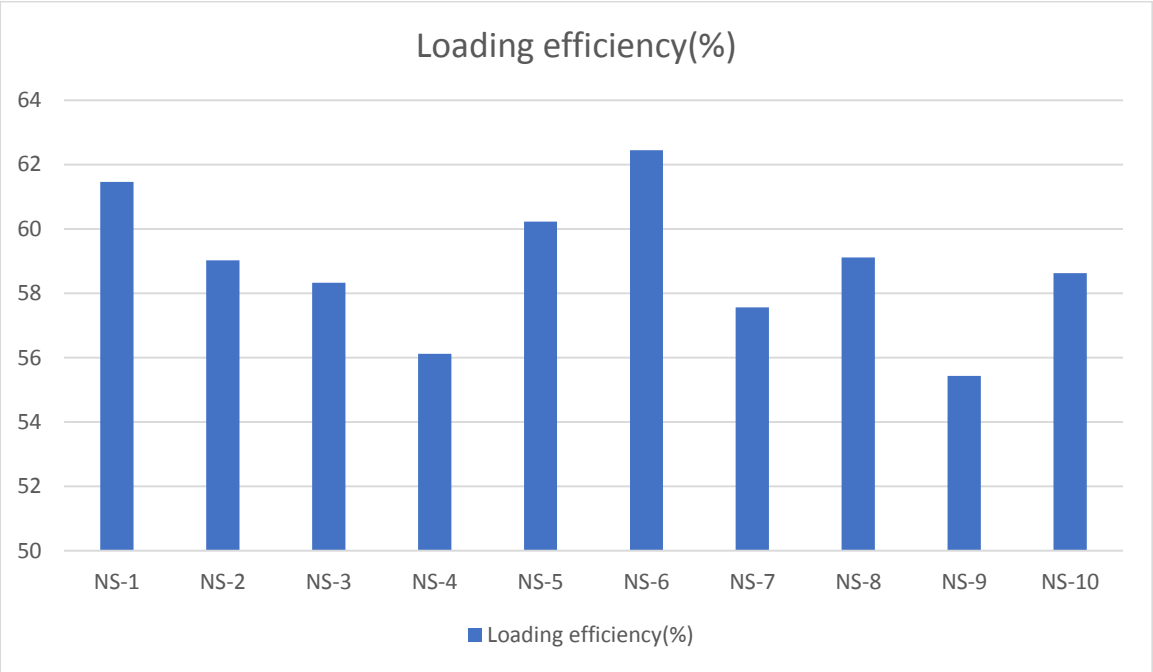


Figure 15 Graph of Loading efficiency

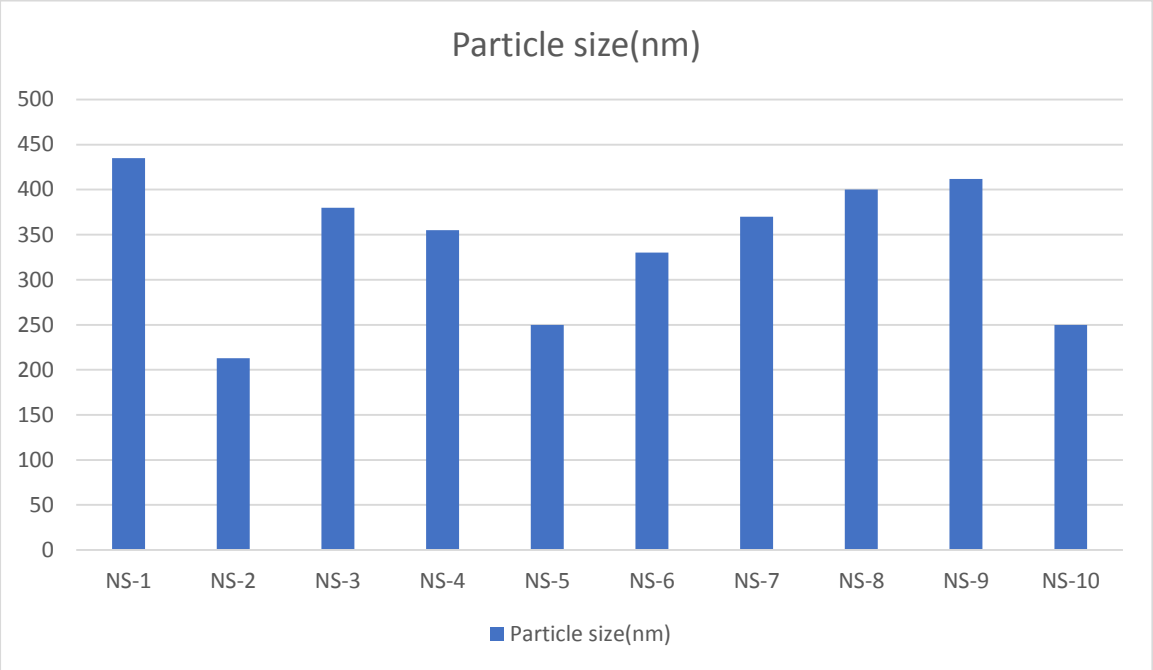


Figure 16 Graph of Particle size

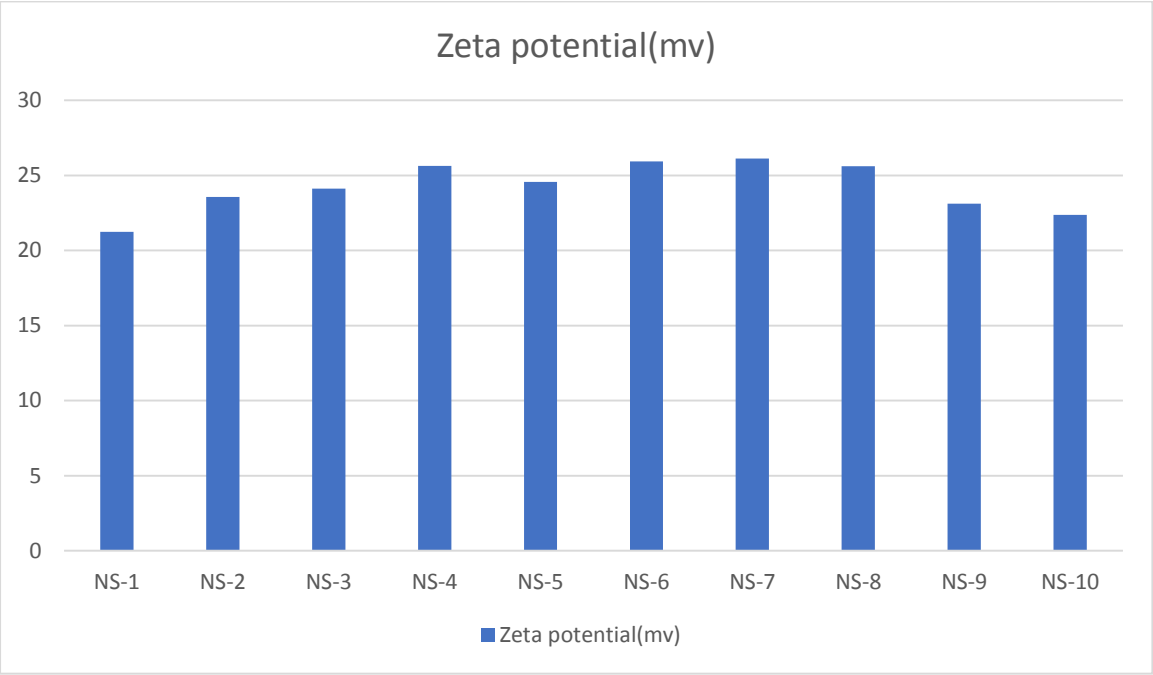


Figure 17 Graph of Zeta potential

Evaluation of Gel Formulation

Gel formulations are used to deliver the drug topically because of easy application, increase contact time and minimum side effects as compare to other topical preparation and oral administration. The evaluation parameters of gel formulation are shown in table 15 and table 16

Table 15 Evaluation of Gel Formulation

Formulation	pH	Consistency(mm)	Homogenecity
NS-1	6.6	5.1	Homogenous
NS-2	5.3	5.4	Homogenous
NS-3	5.2	5.3	Homogenous
NS-4	6.3	6.0	Homogenous
NS-5	5.2	4.2	Slightly Homogenous
NS-6	5.3	5.5	Homogenous
NS-7	5.4	4.5	Slightly Homogenous
NS-8	6.1	3.0	Slightly Homogenous
NS-9	5.3	3.2	Slightly Homogenous
NS-10	5.4	5.7	Homogenous

Table 16 Evaluation of Gel Formulation

Formulation	Spreadability(g.cm./sec.)	Viscosity

NS-1	5.3	8480
NS-2	5.5	8550
NS-3	5.2	7605
NS-4	4.7	7444
NS-5	5.7	8125
NS-6	4.0	7891
NS-7	6.5	8321
NS-8	5.4	7328
NS-9	4.3	6233
NS-10	5.6	8121

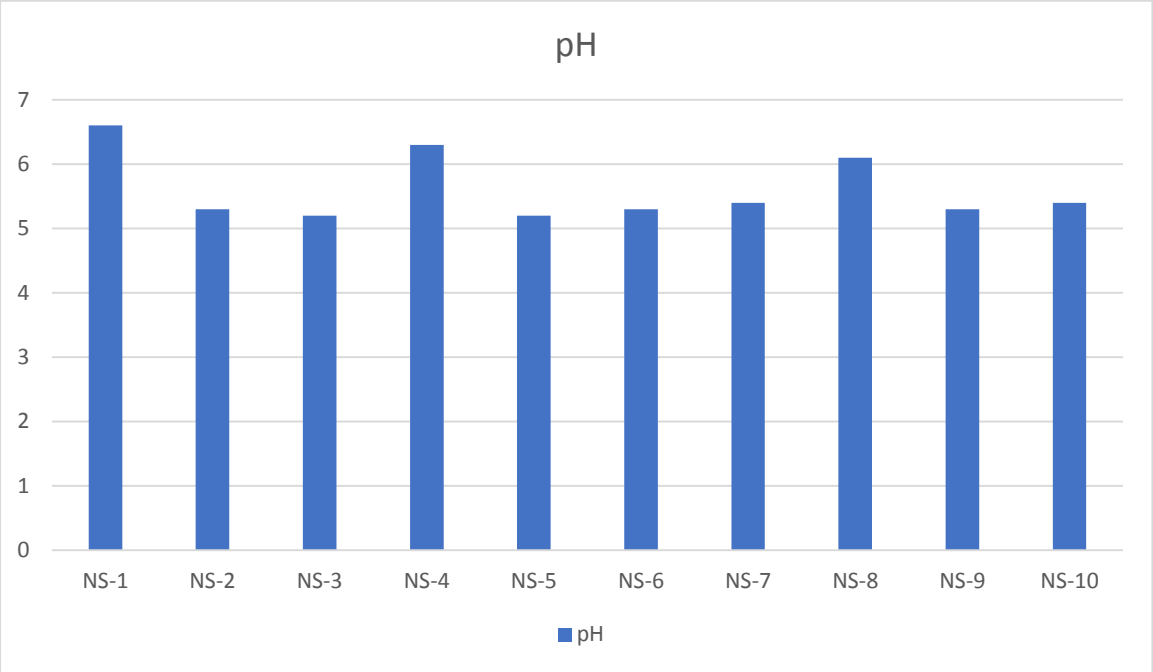


Figure 18 Graph of pH

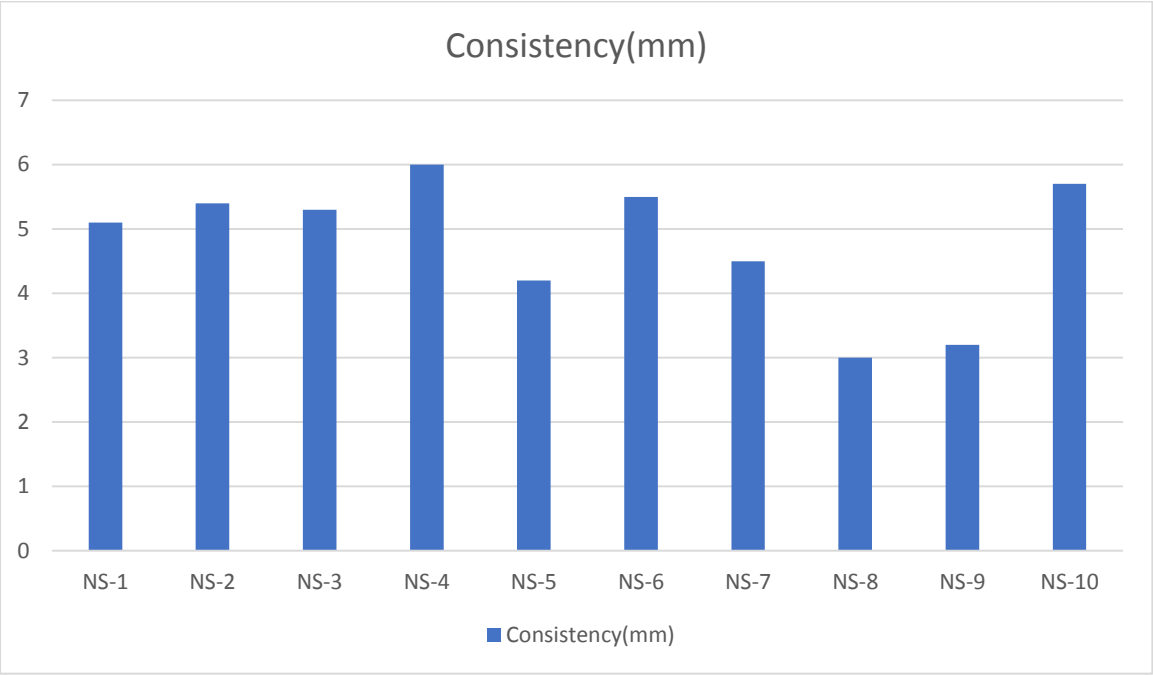


Figure 19 Graph of Consistency

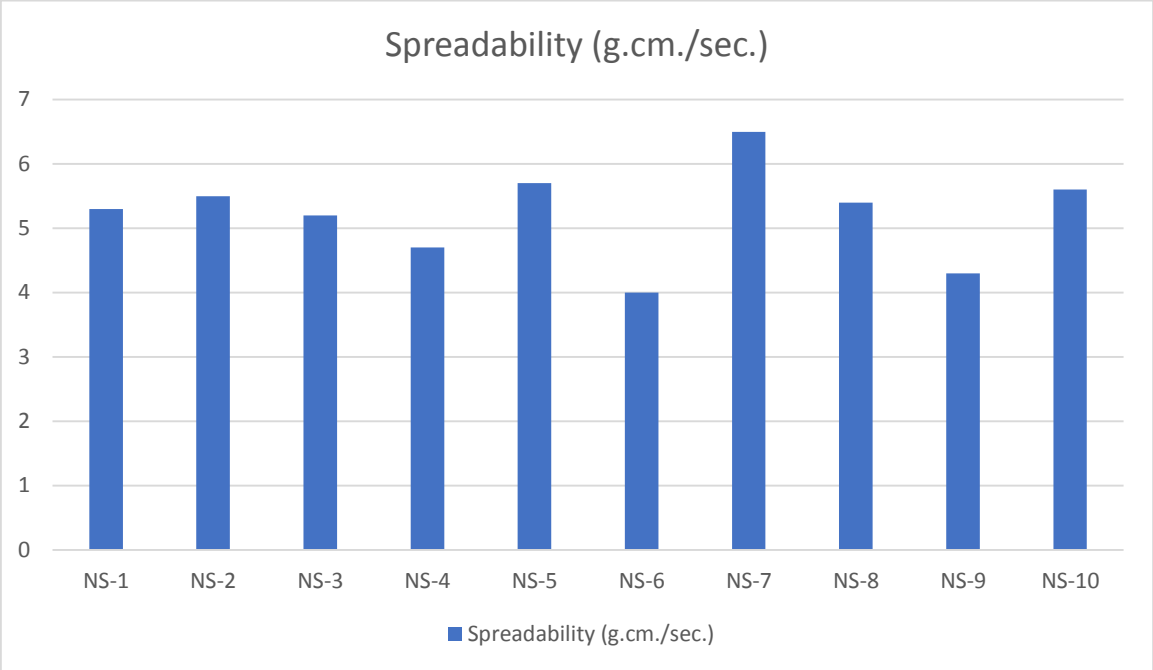


Figure 20 Graph of Spreadability

Figure 21 Graph of Viscosity

Conclusion

The nanostructures used in TDDS may promise endless opportunities regarding stability, penetration, encapsulation efficiency, controlled release, targeted drug delivery, decreased side effects, and improved patient compliance. The Organoleptic properties of γ- Oryzanol were as follow-a combination of off-endlessly white to off white powder, crystalline, odourless, powder in texture and tasteless.γ- Oryzanol melting points was discovered to be 136.5^{0}C. Solubility of γ- Oryzanol in different solvents is recorded in table 5. UV spectrum of γ- Oryzanol is shown in figure 7. Partition coefficient was determined to be 0.28. The standard curve of Phosphates buffer pH 7.4 γ- Oryzanol was found to be in range 1-25 µg/ml of drug in phosphate buffer pH 7.4 showed linearity at λmax 319 nm Standard curve of γ- Oryzanol in 7.4 phosphate buffer was found to be in the range 1-25 µg/ml. The drug loading study is shown in 10. The bulk density and tapped density were found to be 0.68 and 0.56 respectively. The compressibility index, hausner ration and angle of repose were found to be 6.68, 1.44 and 27.38^0 .Drug loading %, drug loading efficiency, yield percentage, particle size and zeta potential are shown in table 13 and table 14. The evaluation parameters of gel formulation are shown in table 15 and table 16

Acknowledgement

I appreciate the administration of this excellent professional institution for giving the top lab facilities I needed to do my study. The conception and writing of this page were greatly assisted by all of the above authors.

Conflict of interest

The Authors declare no conflict of interest.

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