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DEVELOPMENT AND CHARACTERIZATION OF SILYMARIN WITH SILVER NANOSUSPENSION: A PHYTOCOSMETIC SUNSCREEN GEL FOR UV PROTECTION.

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Abstract *:* The aim of this study was to develop the topical gel formulation containing Silymarin and Silver Nanoparticles for sunscreen protection. The aim of this research was to formulate and evaluate characterization of formulated gel and determined SPF value of sunscreen gel formulation containing a combination of silymarin and silver Nanoparticles by a simple and reliable UV- visible spectrophotometry. All the sunscreen gel formulation was evaluated for visual appearance and homogeneity, PH, viscosity, spread ability, extrudability, centrifuge and in vivo evaluation of sun protection factor was determined &formulation were evaluated for PH, which was near to the skin PH. All the formulations were found to be homogeneous and there was no aggregate formation, there were no observable sediment in centrifuge test. It shows its pharmacological and therapeutically activities so far wide in every aspects of view. Silymarin has been used medicinally to treat liver disorder, including acute and chronic liver diseases, anti –oxidant, anti- inflammatory properties and immunomodulatory responses. It has UV – induced skin damage protection, along with the process molecular biology brought to studies related to the skin and its change, it also offers advantages for the development of more effective treatments to fight skin aging. Cosmetics ingredients are becoming increasingly in their level. In addition, the evolution that came in the wake of the genome project makes it possible to offer customized products to target each individual's personal needs, as well as to optimize the formula according to the individual response to the selected treatment. One of the downsides of aging is that we end up with dull, thin, sagging, rough skin with more and more wrinkles. Since the appearance of these wrinkles on the face and discernible visual indicator of biological age, everyone wants to preserve one's healthy and youthful looking skin, particularly on the face as long as possible. The effect smoking was noticed by DANIELL as early as 1971 and also studied the severity of wrinkles of 1104 subjects after adjusting age and outdoor sun exposure. The results showed that premature wrinkling is an important system of smoker's skin.

KEY WORDS: Skin damage protection, Antiaging properties, silymarin, use of sunscreen, evaluation of sun protection, homogeneity

INTRODUCTION

NANOTECHNOLOGY:

The nanotechnology field is gaining worldwide recognition because of a wide range of application, such as improving the properties of topical conventional formulations, overcoming problems such as low permeation and retention in the skin outer layers, instability of active ingredients. poor water resistance or ROS produced by inorganic filters research studies have shown that the incorporation of UV filters into Nano systems (Nano sunscreens) presents several benefits compared with sunscreen formulations containing non-

encapsulated UV filters. the development of Nano systems containing different ingredients in their composition has been optimized based on the toxicological effects of the components, exposure level, and chemical nature for example: topical formulations containing nanoparticles encapsulation Zno and Tio2 present a transparent appearance and compared, with nanoparticle can prevent skin whitening after sunscreen application because of the reflection? Scattering of the UV radiation. thus formulations containing these Nano systems are both more comfortable to apply and also cosmetically acceptable.

The various mechanism through which silymarin exerts its effects in various dermatological conditions, ultraviolet radiation, especially ultraviolet B (UVB), is strongly absorbed by cellular DNA. Resulting in DNA damage by the formation of cyclobutane pyrimidine dimers and 6-4 photoproducts. the oxidation stress involving generation of free radicals and reactive oxygen species. And depletion of antioxidant machinery are important factors with respect to the photo carcinogenesis of skin (1).

STRATEGIES FOR MARKETING NANO SIZED DRUG PARTICLES:

Nanoparticles can be prepared by two methods, namely, "bottom up technology "and top down technology. bottom up technology is a method to form nanoparticles like precipitation, micro emulsion and melt emulsification methods. Top-down technology involves the disintegration of larger particles into nanoparticles, examples of which are high-pressure homogenization and milling methods.

There are several strategies for making Nano sized drug particles and those are broadly classified in four categories as bottom up, top-down combination approaches and chemical synthesis.

Top-down process:

Essentially high energy process where drug particles are broken down to lower size particles using technologies such as pearl milling, high pressure homogenization includes piston gap homogenizer and jet stream homogenizer. Though there are several patents and marketed products available for top-down approaches, there is a far a smaller number of patents and products dealing purely with bottom up or a combination technology.

Though the top-down process is more universal and industrially more feasible than the other processes, It has its own limitation in term of particles size reduction efficiency. It often requires a long process time to reduce particles sizes below 100nm. hence using standard top-down process is not practical to reduce particle sizes beyond a certain limit, which in most of the caser is about 150-300nm. Besides this, there are issues related to the solid-state changes of the micronized product and chemical degradation, which may also occur during milling, also, the phenomenon off residual metal content (Zisolid – stateterium) is a potential issue with top-down processes (29)

BOTTOM-UP PROCESSES:

The advantages include that these are low energy processes, require simple instruments, are less expensive and can be operated at a low temperature, making them particularly suitable for thermopile drugs, However, the advanced techniques like processing with supercritical fluid are costlier. The particles size obtained by bottom up technology has a narrow size distribution, the bottom up process is broadly called a precipitation process, because the principle can be induced by processes that further increase the super saturation in the system, such as evaporation of the solvent, reduction of temperature or by mixing it with an anti-solvent. It has been shown that some of the controlled precipitation processes for producing both micro and Nano-sized drug particles can be scaled up as well. Nanoparticles with a target particle with this size range get new physical properties, and their permeation through various biological barriers improves.(2)

VARIOUS TECHNIQUES USED FOR NANO SUSPENSION:

Nano suspension is a sub-micron colloidal dispersion of drug particles which are stabilized by surfactants, polymers or a mixture of both. They can also be defined as the biphasic system consisting, of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1 micrometer in size. The particle size distribution of the solid particles in Nano suspensions is usually less than one micro with advantage particle size ranging between 200 and 600 nm. Nano suspension differ from nanoparticles and solid lipid nanoparticles. In Nano suspension technology, the drug is maintained in the

required crystalline state with reduced particles size, leading to an increased dissolution rate and therefore improved bioavailability. An increase in the dissolution rate of micronized particles (particles size <10 micrometer) is related to an increase in the surface area and consequently the dissolution velocity.

The top-down process follows disintegration approach from large particles, micro particles to Nano sized particles

Examples are

- High pressure homogenization
- Nanodegree
- Nano pure
- Media milling (Nano crystals)
- Bottom up process is an assembly method forms nanoparticles from molecules. Examples incudes
- Solvent –Anti solvent method
- Super critical fluid process
- Emulsification solvent evaporation technique
- Lipid emulsion/ Micro – emulsion template. (3)

HIGH PRESSURE HOMOGENIZATION:

It is most widely used method for preparing Nano suspension of many poorly aqueous soluble drugs. Different methods are developed based on this principle for preparation of Nano suspensions are Disso cubes, Nano pure, Nano edge and Nano jet.

HOMOGENIZATION IN AQUEOUS MEDIA (DISSO CUBES):

This technology was developed by R.H. Muller using a piston –gap type high pressure homogenizer in 1999. In this method, the suspension contain a drug and surfactant is forced under pressure through a Nano sized aperture value of a high pressure homogenizer. this method is based on cavitation principle, the dispersion present in 3cm diameter cylinder is suddenly passed through a very narrow gap 25 micrometer. Then water Starts boiling at room temperature, and forms gas bubbles, which implodes when the suspension leaves the gap (called cavitation) and normal air pressure is reached. the particle cavitation forces are sufficiently high to convert the drug micro particles into nanoparticles (30)

HOMOGENIZATION IN NONAQUEPUS MEDIA (NANOPURE):

Nano pure is suspension homogenized in water free media or water mixture like PEG400, PEG 1000 etc. The homogenization can be done at room temperature,00C and below freezing point (-200) hence it is known as “deep freeze” homogenization.

MILLING TECSHNIQUE:

This method was first developed and reported by riverside (1992) the nanosuspenstions by this method are prepared by high shear media mill. The milling chamber was changed with the milling media, water, drug and stabilizer and rotated at a very high shear rate under controlled temperature at least 2-7 days.

A. DRY-CO-GRINDING:

Recently many Nano suspensions are prepared by the dry milling technique. Dry co grinding can be carried out easily and economically and can be conducted without organic solvents. Physicochemical properties and dissolution of poorly water-soluble drugs are improved by co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug. (4)

B. EMULSIFICATION-SOLVENT EVAPORATION TECHNIQUE:

This technique involves preparing a solution of drug followed by its emulsification in another liquid that is a no solvent for the drug. Evaporation of the solvent leads to precipitation of the drug. Crystal growth and particle aggregation can be controlled by creating high shear forces using a high-speed stirrer.

3.PRECIPITATION:

Within the last decade, precipitation has been applied to prepare submicron particles, especially for the poorly soluble drugs. The drug is first dissolved in a solvent, then this solution mixed with a miscible anti

solvent in the presence of surfactant. Rapid addition of a drug solution to the and solvent leads to sudden super saturation of drug and formation of ultrafine crystalline or amorphous drug solids.

A. SUPERCRITICAL FLUID PROCESS:
B. MELT EMUSIFICATION METHOD
C. LIPID EMULSION/MICROEMULSION TEMPLATE
D. SOLVENT EVAPORATION
E. EVAPORATIVE PRECIPITATION TECHNIQUE

SUPER FLUID PROCESS:

The particle size reduction was achieved more by the solubilization and Nano sizing technologies through the super critical fluid process. Super critical fluids (SCF) are no condensable dense fluids whose temperature and pressure are greater than its critical temperature and critical pressure. This process allows the micronization of drug particles to submicron level. Recent advances in SCF process are to create nanoparticles suspension of particle size of 5 to 200 nm in diameter.

MELT EMULSIFICATION METHOD:

In this method drug is dispersed in the aqueous solution of stabilizer and heated sample holder was entrapped with a hearing tape fitted with temperature controller and the temperature of emulsion was maintained above the melting point of the drug.

LIPID EMULSION /MICROEMULSION TEMPLATE:

➢ This method is mostly applicable for drugs that are soluble in either volatile organic solvents or partially water miscible solvents. In this method, the drug was dissolved in a suitable organic solvent and then it is emulsified in aqueous phase using suitable surfactants. Then the organic solvent was slowly evaporated under reduced pressure to form drug particles precipitating in the aqueous phase forming the aqueous suspension of the drug in the required particle size.

SOLVENT EVAPORATION:

➢ In the solvent evaporation method, the solution of polymer is prepared in volatile solvents and emulsions, but in the past years dichloromethane and chloroform were used which was now replaced by ethyl acetate which has a better profile of toxicology. the emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion.

Sunrays consists of an array of wavelength ranges that vary in frequency and their energy profiles. The suns electromagnetic radiation consists of cosmic rays, gamma rays, x-rays, UV rays, microwaves and radio waves in decreasing order of energy. The effects of UVA radiation usually after a long exposure damages the skin by penetrating into the layers of skin and producing the reactive oxidation species. the enzyme that degrades the matrix protein's elastin and collagen, UVA radiation can be leads to inducing polymorphous light eruptions in sensitive skin. UVA can also cause exacerbation of cutaneous lupus erythematosus, where solar urticarial can be caused by both UVA and UVB radiation. (5)

UVA can lead to impairing the antigen presenting cell activity of the epidermal cells and thereby causes immune suppression, thus contribution to the growth of skin cancer due to the damage caused to DNA of human melanocytes. UVA radiation can cause nuclear and mitochondrial DNA of human melanocytes, DNA gene mutations. Ultra violet induced photo aging accounts for 80-90% of visible skin aging. The UVA radiation penetrates into the skin and reaches the dermis. provoking several damages such as immediate and delayed tanning reactions, loss of collagen, diminution in the quantity of blood vessels, alteration of the connective tissue of the dermis and skin photosensitization. UVB is responsible for the tanning and immediate damages to the skin, which results in erythema or sunburn. Although the UVC region has desirable properties such as germicidal activity, this spectral band also been reported to be erythemogenic, mutagenic and carcinogenic.(28)

In daily life, UV exposure is unavoidable: therefore, sunscreen should be used regularly, regular use of sunscreen has been shown to reduce the number of actinic or percutaneous keratosis and solar elastosis. photo protective agents protect the skin by preventing and minimizing the damaging effects of UV rays. (13)
They can be used as sunblock, which is opaque when applied over the skin. They can be used for sunblock, which are translucent and require frequent reapplication for optimum efficacy, photo aging manifested as sagging, wrinkling and photo carcinogenesis is caused by damage to cells and DNA Ultraviolet filters also referred to as sunscreen, interfere directly with the incident solar radiation through absorption, reflection or dispersion of energy. they are classified into two categories based on their mechanism of action: organic or chemical sunscreens (6)

BASICS ON PROTECTION:

ULTRA VIOLET RADIATION:

It has the long been known that UVB is the principal cause of acute sunburn and tanning as well as being immune suppressants, mutagenic, and carcinogenic. Meanwhile, the importance of the biological effects of UVA has been recognized UVA induces significant photo biological reactions, mostly of indirect nature and requiring the presence of oxygen such as immediate and delayed tanning reactions and new melanin formation. There is now considerable evidence that UVA definitely contributes to long- term degenerative changes of the skin, such as significant connective tissue damage (premature skin aging) and cancer formation, and may also contributes to UVB induced carcinogenesis, UVR (UVA and UVB) plays a role in photosensitive
diseases such a chronic actinic dermatitis, photo allergic (7)

UV FILTERS, SUNSCREEN AND PHOTOSTABILITY:

Protection against the effects of UVR in the skin is achieved by specially designed by molecules (I.e. UV filters) incorporated in suitable formulations (sunscreen) such as creams or lotions, oils, gels, sticks etc . However, in view of the growing photo biological knowledge about the mechanisms of UVR- induced effects. The European Commission recommends that the minimum level of UVA protection (UVAPF) should be at least one- third of the sunscreen protection factor (SPF) , and the newest FDA and Australian regulations requires sunscreen carryings a “ broad spectrum” label further , they may contain not only chemicals that absorbs , reflects or scatters UVR but also chemicals that interfere with secondary reactions such as generation of free radicals and reactive oxidative species (ROS) in the skin, generation of inflammatory mediators, photoexcitation of different molecules , etc. .and ultimately exert long – term protective effects on degenerative skin damage , sometimes called “secondary photo protection” .
Consequently, non UVR absorbing, reflecting, or scattering molecules with a pharmacological action in the skin may be also be incorporated in sunscreen, endowing, the corresponding products with expanded properties. UV filters used in cosmetics sunscreen formulations are roughly considered belonging to two groups organic molecules deliberatively, selected for their UVR absorbing capacities, (i.e. organic, UV filters) and particles that absorb, reflect, or scatter UVR, particles may be inorganic (i.e. metal oxide) or organic (micro fine polymeric molecules). (8)

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EVAPORATION PRECIPITATION TECHNIQUES:

Evaporative precipitation into aqueous solution(EPAS) is another modified solvent anti solvent precipitation method, which was first proposed by a research group at the university of Texas, Austin. Evaporative precipitation into aqueous solution (EPAS) utilize the bottom-up approach to nucleate and grow Nano or micro particles of water –insoluble drug compounds. It is a particle engineering technology reported to produce submicron to micron –sized drug particles stabilized by surfactants or polymers and dispersed in an aqueous medium. According to Noyes Whitney law, an effective way to increase dissolution rate and oral absorption of a substance is the size reduction of powder particles. For this purpose, anti-solvent precipitation may represent a valid processing technique. IN comparison to more popular micro and Nano carrier that will be discussed in the following section, this approach presents several advantages, such as easy realization and rapidly. Specifically, the drug is first dissolved in a water – immiscible, organic solvent with a low boiling point (e.g.: dichloromethane or diethyl ether) and the resulting solution sprayed through a custom-made atomizing nozzle into a heated aqueous solution representing the anti-solvent.(27)

The anti-solvent precipitation method was recognized as a promising strategy, because it is rapid Needs low equipment requirements, it can increase the dissolution rate and oral absorption of the drug. Can be easily scaled up, with respect to the processes commonly used for EA encapsulated, such as emulsion-diffusion-evaporation, spray drying, co-precipitation, rotary evaporation and ionic gelation (10)

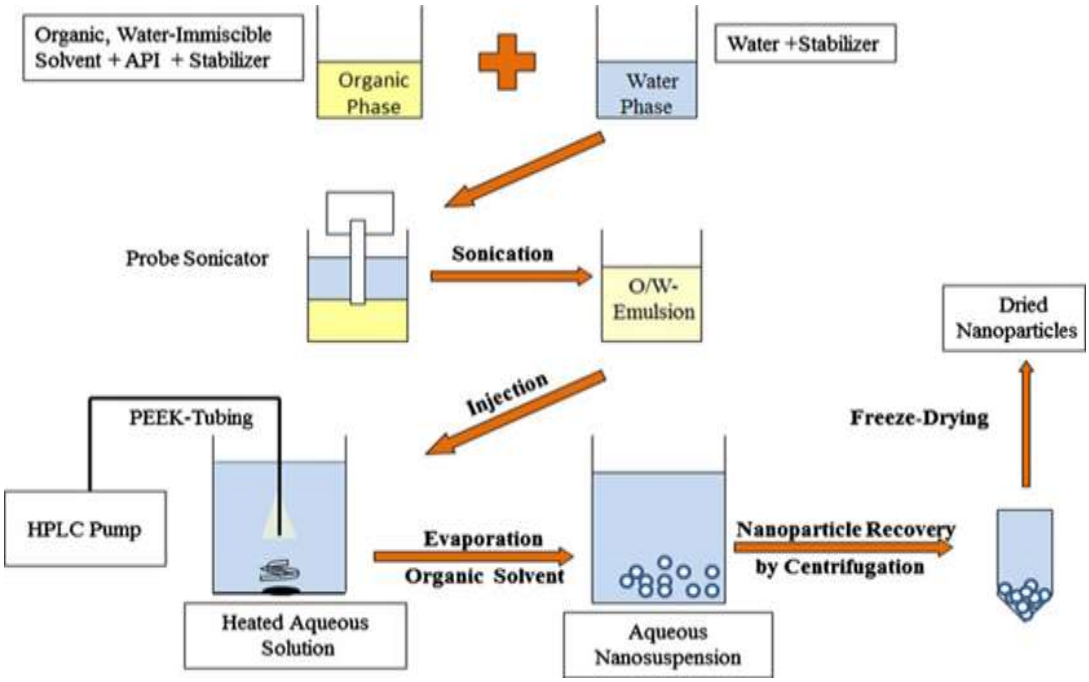


Figure 1: Preparation methodology of Nanoparticle Suncream

During EPAS, the active pharmaceutical ingredient is first dissolved in a low boiling liquid organic solvent, in this case dichloromethane, this solution is pumped through a tube where it is heated under pressure to a temperature above the solvent's boiling point and then sprayed through a fine atomizing nozzle into a heated aqueous solution. Elevated temperature of the heated aqueous solution ensures rapid evaporation of the organic solvent which in turn produces high super saturation levels and rapid precipitation of the drug in the

form of suspended particles. stabilizers added to the heated aqueous solution absorbs onto the newly formed particle surface decreasing surface energy and preventing particle growth. At the same time, those stabilizers are generally hydrophilic and therefore improve wetting and enable rapid dissolution of EPAS produced particles. The stabilizer adsorbs onto the newly formed drug particle surfaces consequently decreasing the surface energy and providing steric or electrostatic repulsion between particles. This process may be dictated by the thermodynamic and kinetic aspects of stabilizer adsorption: the stabilizer must absorb on the newly created surface and attain a confirmation that is conductive to steric stabilization. In addition, EPAS, is capable of producing particles with high drug loadings since excess, unbounded stabilizer can be removed by centrifugation of the aqueous suspension, particularly, high drug loadings of more than 90% (w/w) have been reported. (26)

The rapid evaporation of the organic solvent produces high super saturation and rapid precipitation of the API in the form of suspended particles that are stabilized by a variety of surfactants present in either or both the organic and aqueous phases. The surfactants adsorb onto the API surface and prevent particle growth during the spray process. EPAS produces systems with a high API- to surfactant ratio or payload relative to formulations in which the API is dissolved in micelles or liposomes, it may allow an effective dose to be administered consequently producing high blood levels with less potential interactions from the surfactant which may otherwise produce unwanted side-effects. Developed a concept to produce high potency, API's with API to surfactant ratios greater than 13 corresponding to potencies (wt. API+ wt. surfactant) as high as 93%. Typical potencies for API s dissolved in micelles or vesicles are lower by an order of magnitude or more. Aqueous dispersions formed by EPAS were centrifuged to remove the non-adsorbed. (11)

Despite these advantage characteristics EPAS produced particles, the EPAS process itself has certain limitations including optimization of control over particle size and size distribution and the necessity for consistent reproduction of a fine atomizing nozzle from stainless steel tubing. The purpose of this study was to develop a more robust precipitation process that combines the advantages of EPAS with tinplating (i.e. Advanced EPAS) to form nanoparticles of the poorly water soluble drug.

It was hypothesized that replacement of the organic feed solution with oil-in-water (o/w) allows for excellent control over the particle size without requiring an atomizing nozzle since the size of precipitated particles is regulated by the droplet size of the suspension. The stable aqueous drug suspension is dried by a variety of techniques including ultra-rapid freezing ultra-rapid freezing in conjugation with lyphoilization or spray drying, the dissolution rates are discussed as a function of the final particle size, crystallinity and morphology and the molecular interactions between the drug and stabilizing surfactant. The process utilizes the advantage of all these techniques resulting in drug particles that have a small primary size, low crystallinity and are intimately mixed with one or two surfactants.

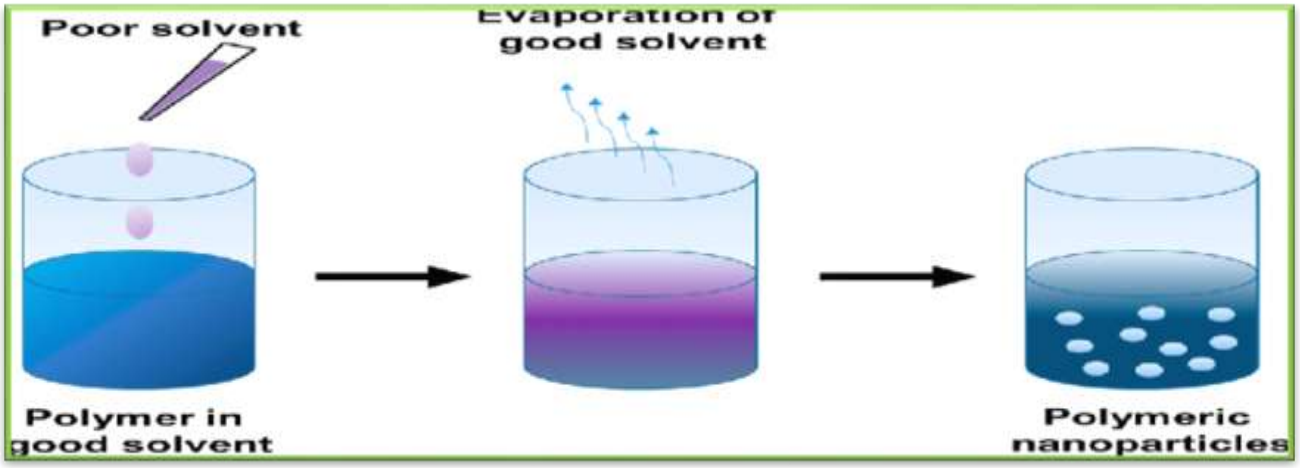


Figure 2: Diagram for polymeric Nanoparticle

Formulation development of sunscreen UV Protection gel with Silver with Silymarin Nano suspension

Flow chart - EPAS technology of Nano suspension Organic solution

(0.5g Silymarin + 10 ml Dichloromethane)
Preheated using heated plate submerged under water bath.

↓

Heated Aqueous solution at 80-85ºC.
(Tween 80 + Stabilizing solution+ 10 ml water)
Dissolved the Stabilizer on stirring.
Organic solution is sprayed into aqueous solution
& Stirred for few minutes in ultra-solicitor at 50ºC for 10 min

↓

Evaporate Organic solution forms Nano suspension.

↓

Rapid evaporation Precipitate of drug in form of colloidal suspension
Gel base

↓

(Carpool+ glycerine + Methyl Paraben)
Mixing of suspension +gel base adjusts pH (tri ethanol amine). (12)

In fact, controlled precipitation of volatile O/W containing poorly water-soluble drugs has previously been demonstrated to be a feasible approach for the preparation of submicron drug particles which were generally found to be in a comparable size range as the emulsion droplets. Two strategies to produce nanoparticles from O/w emulsions have been reported in the literature.

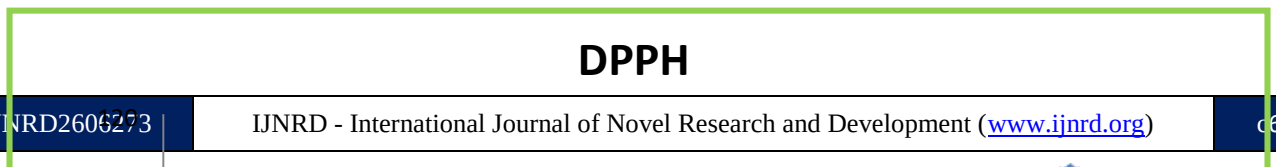
1. Removal of only the organic phase under reduced pressure resulting in a colloidal
2. Direct transformation of the emulsion into a dry powder by spray-drying

The approach taken in the present study involves rapid removal of the organic phase by spraying the O/W emulsion into a heated aqueous solution, which is different from the processes described. The present process allows for considerably shorter evaporation and consequently processing pressure. In addition, drug potencies of advanced EPAS produced particles are expected to be substantially higher compared with those particles obtained by direct conversion of the emulsion into a dry powder because unbound stabilizer may be easily removed by centrifugation of these aqueous colloidal dispersion. (13)

DPPH assay of Nano suspension containing of Silymarin and Silver:

In DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow-colored diphenyl-picrylhydrazine. This method is based on the reduction of DPPH in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH radical scavenging activity of ET-F-3 at 350µg/mL was found to be 96.9%. On the other hand, ET-F-2, ET-F-1and ascorbic acid exhibited 92.5%, 91.7% and 98.1% DPPH radical scavenging activity at the same concentration, respectively. These results exhibited that the DPPH radical scavenging activity of ET-F-3 was like ascorbic acid (14)

Table & Graph 1: DPPH Assay of Nanosuspension of concentration



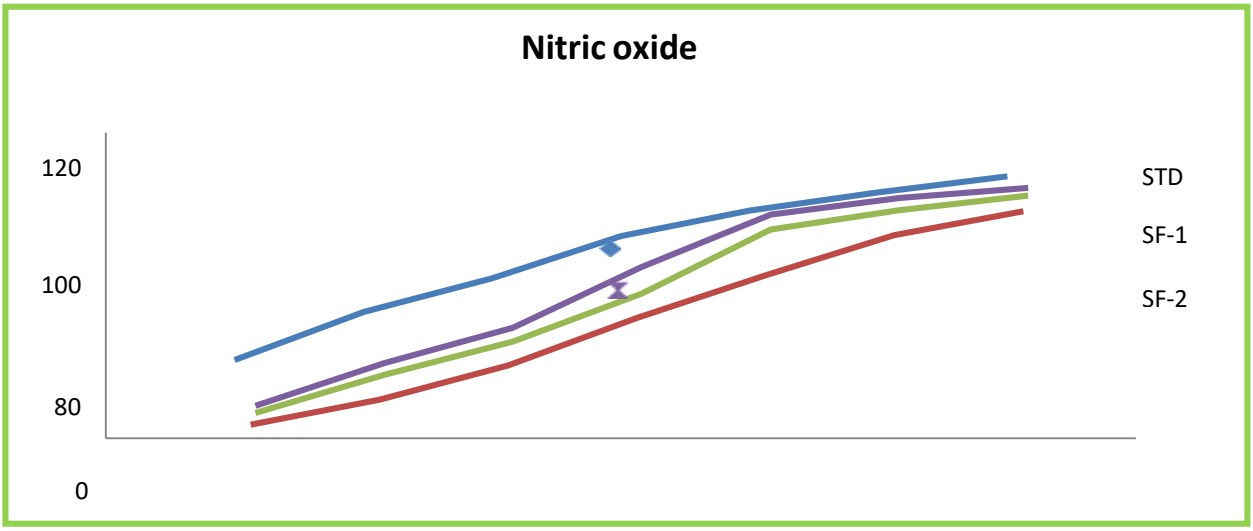
Nitric oxide assay of Nano suspension containing of Silymarin and silver:

The NO scavenging activity of the Nano suspension was 11.5±3% of the minimum concentration of 50 µg/ml, whereas maximum activity was 96.5±1.5% at 350µg/ml. Our IC50value (9.5µg/ml) of Nano suspension was in near agreement with IC50values (5.65±0.72µg/ml) given, whereas IC50values of AA was 9.5µg/ml, respectively. Figure:32 shows that the percentage of inhibition was increased with increasing concentration of the formulation. However, the activity of AA and formulation was more pronounced than that of the plain gel. *In vitro* inhibition of NO (NO) radical is a measure of antioxidant activity of gel. NO plays an important role in various inflammatory processes, but the overproduction of NO contributes to various diseases. The toxicity of NO increases greatly when it reacts with SO radical, forming the highly reactive per- oxy-nitrite anion. Nano suspension effectively reduced the generation of NO from sodium nitroprusside. The degree of inhibition of the NO free radicals was found to be increased with increasing concentrations of the Nano suspension and this indicates that the gel may contain compounds capable of inhibiting the generation of NO and offers scientific evidence for the use of Nano suspension in the treatment of various diseases. (15)

Concentration(µg/ml)	**STD (%)**	**SF-1(%)**	**SF-2(%)**	**SF-3(%)**
50	25.1±0.041	6.1±0.045	7.7±0.056	8.3±0.022
100	36.3±0.074	14.2±0.021	16.4±0.065	23.6±0.051
150	67.6±0.011	36.2±0.024	41.3±0.072	55.7±0.038
200	75.5±0.015	51.9±0.062	58.5±0.096	68.1±0.039
250	88.6±0.018	68.8±0.010	74.5±0.065	80.6±0.022
300	94.5±0.035	81.6±0.044	86.5±0.067	91±0.019
350	97±0.037	92.6±0.033	93.5±0.031	96.9±0.050

Table & Graph 2 : Nitric Oxide assay of Nanosuspension of concentration

Concentration(µg/ml)	**STD (%)**	**SF-1(%)**	**SF-2(%)**	**SF-3(%)**
50	26.5±0.028	6.3±0.086	8.18±0.03	13±0.012
100	45.5±0.021	14.9±0.04	23.27±0.013	26.7±0.021
150	55±0.065	30.3±0.077	35.38±0.010	4216±0.013
200	73.4±0.036	48.64±0.026	54.67±0.039	65.4±0.023
250	84.8±0.031	64.42±0.028	80.36±0.024	87.1±0.038
300	92.2±0.019	80.58±0.031	86.24±0.030	93.6±0.036
350	96.7±0.035	88.72±0.023	94.5±0.020	97.5±0.011

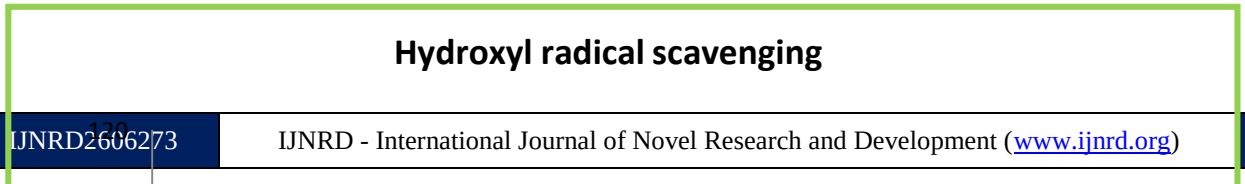


Hydroxyl radical scavenging activity of Nano suspension containing of Silymarin and silver:

Hydrogen peroxide has strong oxidizing properties. It can be formed in vivo by many oxidizing enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize several compounds. The hydrogen peroxide scavenging ability of Silymarin was shown in Figure:33 and was compared to that of SF-1, SF-2 and SF-3 and ascorbic acid which are reference compounds. Hydrogen peroxide scavenging activity of Silymarin gel at 350µg/mL was exhibited to be 97.7%. On the other hand, SF-2, SF-1, and ascorbic acid exhibited 94.6%, 90.4%, and 98.6% hydrogen peroxide scavenging activity at the same concentration, respectively. At this concentration, the hydrogen peroxide scavenging effect of Silymarin and standard compounds decreased in the order of BHT > EA >BHA > a-tocopherol > ascorbic acid. Addition of hydrogen peroxide to cells in culture can lead to transition metal ion dependent OH radicals mediated oxidative DNA damage. Levels of hydrogen peroxide at above or below about 20–50 mg seem to have limited cytotoxicity to many cell types.(16)

Table & Graph 3: Hydroxyl Radical Scavenging Activity nanosuspension of concentration

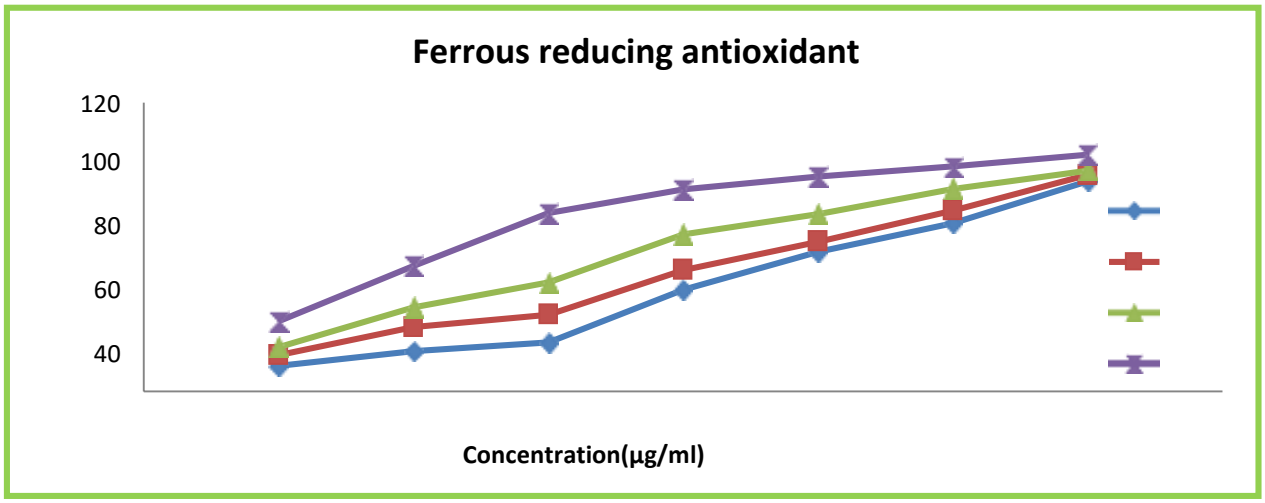
Concentration(µg/ml)	STD (%)	SF-1(%)	SF-2(%)	SF-3(%)
50	27.9±0.068	6.3±0.013	9.1±0.020	11.3±0.020
100	44.4±0.027	16.9±0.04	29.9±0.028	27.2±0.028
150	57.6±0.080	31.2±0.083	38.7±0.041	42.3±0.041
200	74.1±0.030	49.6±0.025	57.9±0.025	65.1±0.018
250	84.6±0.052	65.5±0.022	80.3±0.043	8.3±0.062
300	94.7±0.052	81.61±0.03	89.8±0.027	94.9±0.035
350	97.6±0.03	88.4±0.04	95.6±0.013	96.6±0.011



Ferric reducing powder assay of Nano suspension containing of Silymarin and silver:

The reducing capacity of a compound may serve as a significant indicator of its potential activity. Figure:34 shows that Nano suspension containing of Silymarin and Silver have effective reducing power using the potassium ferricyanide reduction method when compared to the standards. To measure the reductive ability of Nano suspension, the Fe3+–Fe2+ transformation was investigated using the method. At different concentrations (50–350 µg/mL), Silymarin and Silver in Nano suspension demonstrated powerful reducing ability. Ferrous reducing powder assay of Nano suspension at 350µg/mL was exhibited to be 91.8%reducing power of Nano suspension and standard compounds exhibited. The results demonstrate the electron donor properties of Silymarin and Silver in Nano suspension for neutralizing free radicals by forming stable products(17)

Table & Graph 4: Ferric reducing powder assay of Nanosuspension of concentration



DIFFUSION STUDY:

It was observed from the data that *in vitro* drug release of formulation was sharply increased up to 24 hr. It was found that S-F-3 formulation displayed maximum drug release that is **95.42%** after 24 hrs. at 1: 3 ratios of surfactant.(18)

Concentration(µg/ml)	STD (%)	SF-1(%)	SF-2(%)	SF-3(%)
50	29.2±0.020	10.6±0.043	15.2±0.018	18.5±0.014
100	52.2±0.066	16.6±0.031	26.8±0.032	34.9±0.081
150	74±0.047	20.2±0.069	31.9±0.070	45.3±0.039
200	84.1±0.021	42.3±0.015	50.6±0.049	65.2±0.052
250	89.4±0.080	58±0.018	62.3±0.022	73.7±0.038
300	93.5±0.025	69.9±0.047	75.2±0.048	84.3±0.031
350	98.4±0.015	87.4±0.011	90.1±0.095	91.8±0.017

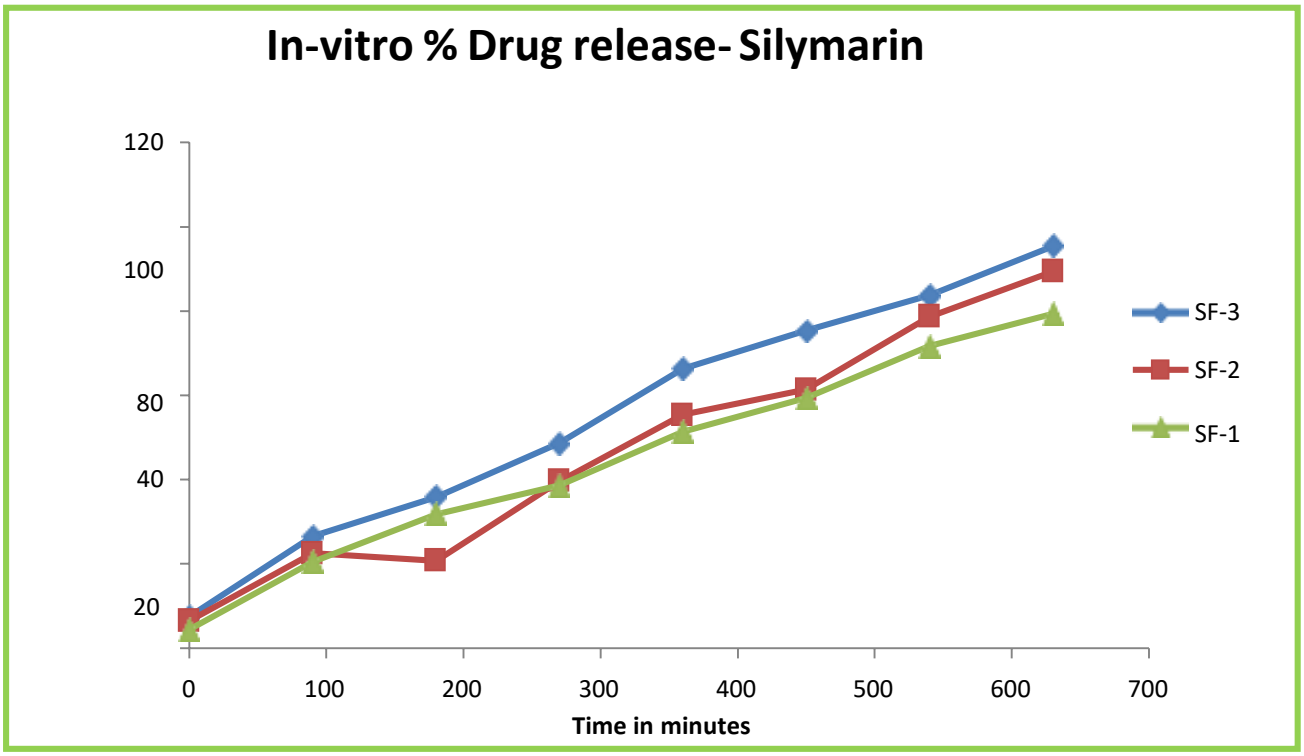


Figure 3: In- Vitro Drug Releases – Silymarin graph

Sun Protection Factor Estimation:

Sun Protection Factor results of sun screen gel formulation (SF1, SF2, SF3):

The evaluation parameter of sun screen gel formulation complies with the acceptance criteria and SPF of this cream found to be 18.68, 25.45, 32, 26 for SF1, SF2, SF3 respectively. This indicates that the prepared sun screen gel formulation can be considered as an efficient topical product. The above readings are averages of 3 replicate each consisting of 6 scans. SPF of sun screen gel formulation found to be higher, which indicates synergism and compatibility of excipients too. These results reveal that the prepared formulations have good SPF and good sun protection activity (20)

Table 5: Sun protection factor result

Scan Parameter	**SF1**	**SF2**	**SF3**
SPF	18.68±0.043	25.45±0.043	32.26±0.043
UVA/UVB	7.49	8.34	8.8
Critical Wavelength	388	388	388

UV ANALYSIS:

The calibration curve of silymarin was found to be linear in the range of **10-60 µg/ml** at 278 nm. Particle size of Nano suspension in EPAS was determined by Globolytics Nanoparticle analyzer. The Nanoparticle of presented a relative narrow size distribution with the mean particle size of about 40.06±1.89, 18.45±2.96 nm and **7.98±2.23nm. (21)**

Table 6: Evaluation parameter Ratio and results

EVALUATION PARAMETER	SF-1	SF-2	SF-3
Appearance	Clear	Clear	Clear
Homogeneity	Homogeneous	Homogeneous	Homogeneous
Grittiness	No particles	No particles	No particles
Particle size (nm)	40.25±1.68	18.94±1.24	8.05±2.31

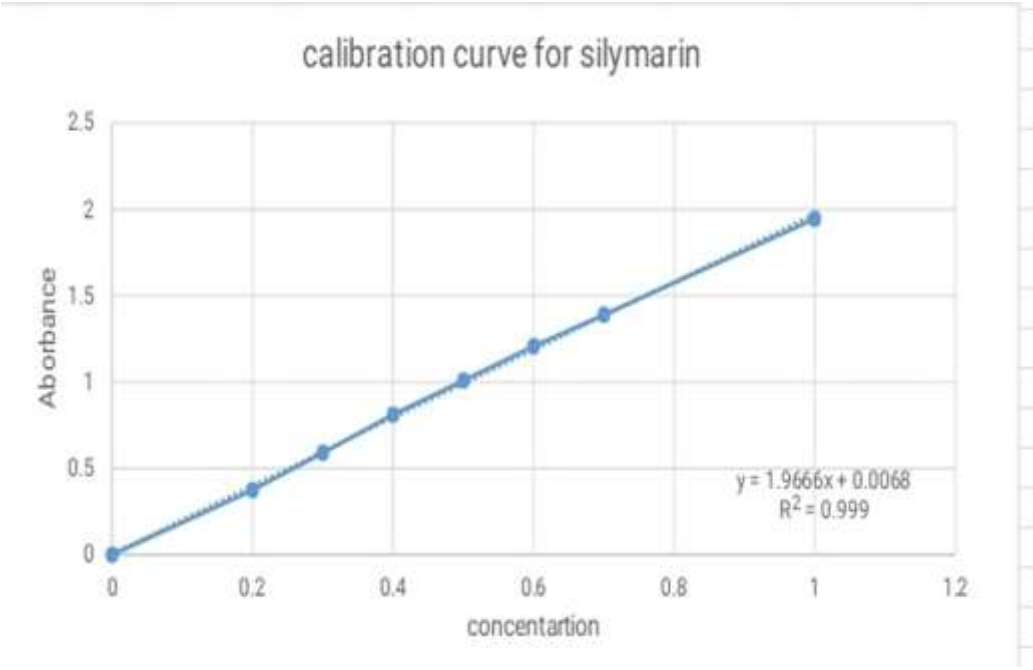


Figure 5: UV – Calibration curve for silymarin

PH DETERMINATION:

The gel could be used to test the Digital pH meter (Alpha 01, Lab matrix manufacturing LLP, Bengaluru) of the manufactured product, which was indicated to be between 6.4 and 7.3. It must arrive near the gel's alkaline Ph, which is suitable for skin application (22)

DRUG CONTENT:

Drug content of the Silymarin Nano suspension based gel were found to be 96.4 ± 0.08 to 98.8 ± 0.10% in the gel of different formulation, which showed good drug entrapment efficiency, and which was an essential requirement for the Nano formulation.(24)

VISCOSITY:

The viscosities of the gel formulations ranges from 5687 to 6860 are optimum meeting the requirement of sunscreen preparation and results were shown (23)

Spread ability:

Spread ability of the plain gel and gel formulation were found to be better as compared to plain gel. This could be because of loose gel matrix of gel due to presence of vesicles

Table 7: Evaluation Parameter for Spread ability result

EVALUATION PARAMETER	SF-1	SF-2	SF-3
Ph	6.84±0.24	6.76±0.44	6.28±0.17
Drug content	98.2 ± 0.16	96.4 ± 0.08	98.8 ± 0.10
Spread ability	6.3 ± 0.4	6.4 ± 0.8	6.6 ± 0.6
Viscosity in cp at 100 (rpm)	6860±2.7	5872±1.8	5687±3.0

FTIR SPECTRAL ANALYSIS:

The FTIR spectra of the pure drug showed prominent peaks at 3566.7 cm^{-1} due to O-H stretch, 2925.48 cm^{-1} due to C-H stretch, 2.360.44 cm^{-1} due to absorption of carbon dioxide, 1716.34 cm−1 due to C=O stretch cyclic ketone, 1684.52 cm^{-1} due to C=C stretch aromatic, 1508.06 cm^{-1} due to C-H bending, 1281.47 cm^{-1} due to C-OH stretch, and 1166.72 cm^{-1} due to C-O stretch. From the spectra (Figure 2), it was observed that there was no significant change in the original peak of the drug and the polymer when compared with the spectra of the physical mixture of the formulated gel. This indicates that there was no interaction between drug, polymer and other excipients.(25)

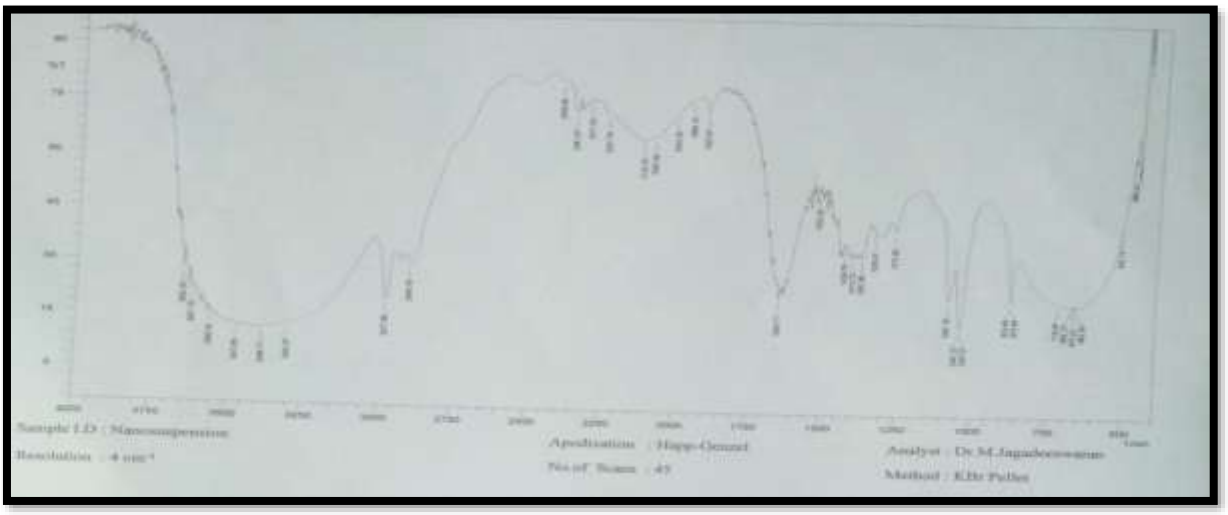


Figure 6: FORMULATION OF NANAOSUSPENSION OF SILYMARIN

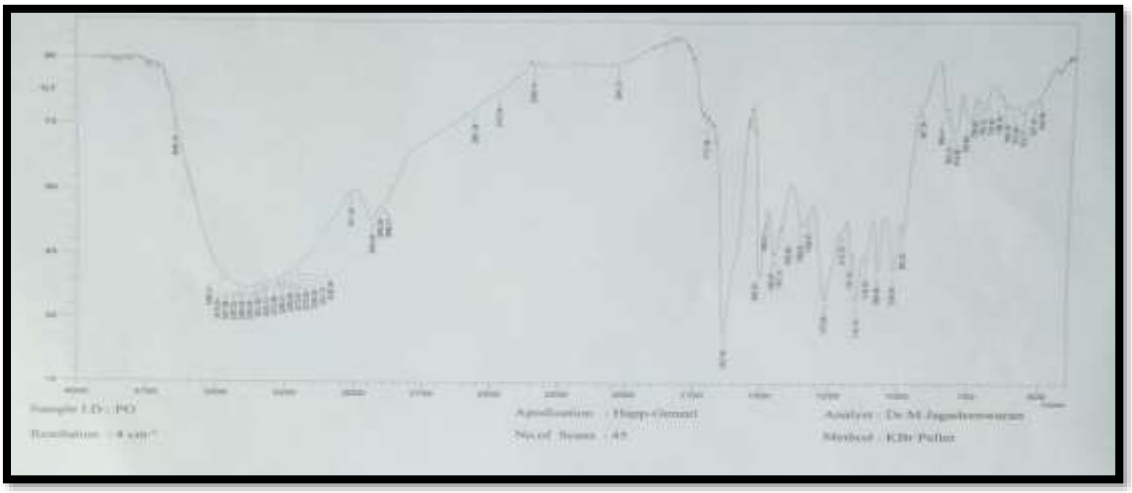


Figure7: FORMULATION OF SILVER NANOGEL ON SILYMARIN

CONCLUSION AND SUMMARY:

❑ It can be concluded that there is great market potential for sunscreen chemicals either synthetic / natural / in combination due to awareness of protection from hazardous UVA as well as UVB rays.

❑ Photo stable, uniform UVA /UVB protective sunscreen product with high SPF can be minimum ideal requirement but natural chemical, Silymarin and silver nanoparticles are more effective due to their long term beneficial effects especially against free radical generated skin damaging along with UV - rays blocking.

❑ It is concluded from present findings that Silymarin and silver containing gel formulation may contribute as a cosmetic ingredient for protection from UVB induced skin damage. Both Turpentine oil as well as Lavender oil having good possessed SPF (Sun Protection) and in vitro free radical scavenging activity (antioxidants activity).

Silymarin Nano suspension containing silver incorporated sunscreens gel might provide cost effective, truly broad spectrum sunscreen products with anti-oxidant, UV protection and many more skin protective effects. However, in vivo animal's studies for optimized formulation to be performed to establish its potential

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