DESIGN AND DEVELOPMENT OF MOISTURIZING SKIN SERUM WITH HYALURONIC ACID BY USING NANO TECHNOLOGY

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A B S T R A C T

Nanotechnology Is widely used in skin care products,this technique is specially designed for target drug delivery.Nanoparticulate delivery system is more reliable and effective. Basically, nanoparticles are colloidal drug delivery system. Nanotechnology can be used in the product like lipstick, soap, antiwrinkle cream, perfume, toothpaste etc. serum are light weight moisturisers that penetrate deeper to deliver active ingredients into your skin. This study presents methods, characterization of hyaluronic acid nanoparticles, further formulation of skin serum and its evaluation at different parameters.

Keywords:Nanotechnology Hyaluronic, Acid, Skin Serum, Stability Testing

1.Introduction

Nanotechnology is fastest growing area for the maintenance of skin health as well as for the diagnosis and management of cutaneous disease. It enriches the study of particles smaller than 100 nm in size. The prefix “nano” from nanotechnology it is a Greek word, in which “nano” means small or little [1]. Nanoparticle is type of colloidal drug delivery system where the particle size ranges from 10—1000 nm in diameter. The sub particles are prepared from a variety of material and synthetic polymers that include gelatine, poly methacarylate some biopolymers etc. Drugs can be dissolved, entrapped, or encapsulated into the nanoparticl es, on simply absorbed on their surface. Nano sphere consists of a dense polymeric matrix in which the drug can be dispersed, whereas, Nanocapsules are constituted of a liquid core surrounded by a polymeric shell. Nanoparticles are formed by single layered shell and are filled with oil which tends themselves ideally as carriers for lipophilic agents [2]. Nanoparticles in cosmetic preparations are found to improve stability of various cosmetic ingredients such as unsaturated fatty acids, vitamins or antioxidants by encapsulating them, increase the efficacy and tolerance of UV filters on skin surface, make the product more aesthetically pleasing and enhance the penetration of certain active ingredients to the epidermis [3].

1.1 Nanoparticles under the skin in cosmetics

The important route is through dermal exposure. The dermis has a rich supply of blood and tissue macrophages, lymph vessels, dendritic cells, and five different types of sensory nerve endings. An increased inflammatory activity and epithelial translocation of manmade 20 and 30 nm solid particles was observed already 20 years ago. Broken skin represents a readily available entry even for larger (0.5-7 micro meter) particles, as evidenced by reports about accumulation of large amounts of soil particles in inguinal lymph nodes from people who runs or walks bare feet. However report shows that broken skin is not necessary for uptake of nanoparticles. Tinkle et al hypothesized that skin when flexed- as in wrist movements- can make the epidermis more permeable to nanoparticles and then favour uptake into lymphatic system and regional lymph nodes [4].

ADVANTAGES

1. Large scale production is possible.
2. Long term stability

3. Controlled and sustained release of active drug can be achieved.
4. Organic solvents can be avoided.
5. It can be lyophilized.
6. It can be freeze dried to form powder formulation.
7. By autoclaving and gamma radiation sterilization is possible.
8. It improves skin protection with organic compound.

DISADVANTAGES
1. Poor drug loading capacity.
2. High water content dispersion.
3. The low capacity to load hydrophilic drugs.

***OBJECTIVE*:**
The aim of my work was to prepare and investigate hyaluronic acid in skin serum by using nanotechnology.

In the first part of the investigation, nanoparticles were prepared with a method described below and further synthesizing its size.

The main steps are as follows:
- Reactions under the same conditions and with concentration of Hyaluronic acid, oxalic acid, sodium monostearate, 1-(3-dimethylaminoproyl)-3-ethylcarbodiimide hydrochloride. The standard solutions were prepared with appropriate concentrations, pH was adjusted, the preparation of hyaluronic acid, oxalic acid, sodium monostearate, 1-(3- dimethylaminoproyl)-3-ethylcarbodiimide hydrochloride solutions respectively , the mixing and stirring time the temperatures applied, maintain storage conditions.
- Confirmation of nanoparticles by LM 20 nanosight.

In the second part of the investigation, the formulation and evaluation of skin serum was studies as follows:
- Formula was set for the formulation of skin serum.
- Physical appearance and Stability testing of the batches
was studied with different concentrations of active. Serum was checked at different parameters and also microbial growth studies were done.

2. EXPERIMENTAL

2.1 Method Of Preparation

2.1.1 High pressure homogenization.
 2.1.1.1 Hot homogenization.
 2.1.1.2 Cold homogenization.
2.1.2 Micro emulsion technique.
2.1.3 Ultra sonication or high speed homogenization.
2.1.4 Double emulsion method.
2.1.5 Spray drying method.

***2.1.1 High pressure homogenization*:** In high pressure homogenization liquid is pushed at high pressure 100-2000 bar through a narrow gap. The fluid accelerates at very high velocity (1000 km/h). In this typical lipid contents in the range of 5-10% which represents no problem to the homogenizer. Higher lipid concentrations up to 40% have been also homogenized to lipid nano dispersions. It is widely used than any other method, because it is advantageous than other method. Following are some of the advantages of this method are that it is easy scale up and powerful techniques, short production times and more feasible [1].

2.1.2 Hot Homogenization: This method is similar to homogenization of an emulsion, because this is also carried out at temperature above the melting point of lipid. In the hot homogenization method the drug is dissolved or dispersed in melted solid lipid for SLN or in a mixture of liquid lipid (oil) and melted solid lipid for nano structured lipid carrier. This lipid melt containing drug is then mixed by high speed stirring in a solution of the hot surfactant at same temperature (5– 10 ºC) above the melting point of the solid lipid or lipid blend). This pre-emulsion is then passed through a high pressure. Homogenizer adjusted to the same temperature, generally applying three cycles at 500 bar or two cycles at 800 bars. This technique can be used for lipophilic and insoluble drugs as well as for the heat sensitive drugs because the exposure time to high temperature is comparatively short. The technique is not suitable for inclusion of hydrophilic drugs into solid lipid nanoparticle because of larger portion of drugs is in water during homogenization which leads to low entrapment capacity [5].

***2.1.1 Cold homogenization*:** This technique is developed to overcome the problems which are associated with hot homogenization like

temperature induced drug degradation and drug distribution into the aqueous phase during homogenization [1]. In the cold homogenization method, the lipid micro particles are obtained by melting and subsequent cooling of drug containing lipid melt followed by crushing, grounding and diffusing in cold surfactant to obtain a cold pre-suspension of micronized lipid particles. This suspension is then forced to pass through a high pressure homogenizer at room temperature applying typically 5–10 cycles at 1500 bar. This method is the first choice for hydrophilic drugs with good as well as low solubility (surfactants are added to improve solubility). This technique avoids and shortens melting process of lipid and hence it is appropriate for thermo sensitive and thermo labile drugs [5].

2.1.2 Micro emulsion technique This method is based on the dilution of micro emulsions. As micro-emulsions are two-phase systems composed of an inner and outer phase. Micro emulsions are clear, thermodynamically stable system composed of a lipophilic phase, water, surfactant and co-surfactant. Micro emulsions are produced at a temperature above the melting point of the lipids, so the lipid should have melting point above room temperature [1]. Solid lipid nanoparticles can also be prepared by micro emulsification of inner molten lipids phase (oil) which is preloaded with drug (at 65-70 ºC), followed by dispersion in cold aqueous phase with mechanical stirring (at 2-3 ºC). The dispersion is washed two times with distilled water by ultra filtration. After washing, the suspension is freeze dried. The diameter of the disperse phase droplet should be always below 100nm. There is no need of energy for this preparation [5].

2.1.3 Ultra sonication or High speed homogenization: Solid lipid nanoparticles were also developed by high speed stirring or sonication. The most advantage of this method is that, the Equipments that are used here are very common in every lab [1]. Solid lipid nanoparticles can also be prepared by sonication or high speed stirring. This is very general and simple technique and can be beneficial over other methods like hot and cold homogenization but with drawback of distribution of larger particle size ranging between micrometer range leading to physical instability such as particle growth upon storage and also metal contamination due to ultra sonication [5].

2.1.4 Double emulsion method: It is a novel method of preparation of solid lipid nanoparticles loaded hydrophilic drug moiety and is based on solvent emulsification evaporation by drug encapsulation in the outer water phase of w/o/w double emulsion along with a stabilizer to avoid partitioning of the drug to outer water phase during solvent evaporation [5]. For the preparation of hydrophilic loaded SLNs, a novel method based on solvent Emulsification-evaporation has been used. In double emulsion technique hydrophilic drugs was dissolved in aqueous solution, and then was emulsified in melted lipid. In this method the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion. Stabilized primary emulsion was dispersed in aqueous phase which contains hydrophilic emulsifier after that the double emulsion was stirred and was isolated by filtration [1].

***2.1.5 Spray drying method*:** It is an alternative procedure to lyophilisation in order to transform an aqueous SLN dispersion into a drug product. This method is cheaper than lyophilisation. This method cause particle aggregation due to high temperature, shear forces and partial melting of the particle. In this method short drying time and consequently fast stabilization of feed material at moderate temperatures make spray drying method suitable for producing nanoparticles of drugs that are thermo labile. The 20% trehalose in ethanol-water mixtures (10/90 v/v). Due to high temperature and shear force it may cause aggregation of particle [1].

Materials and Method

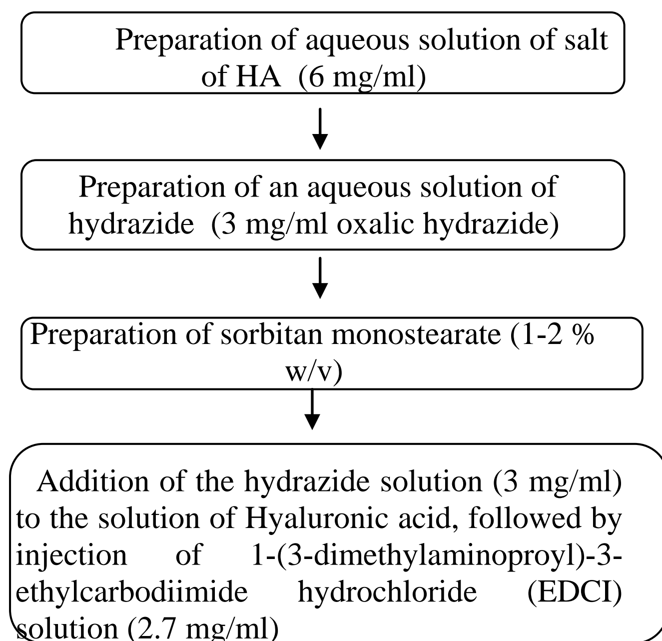
Hyaluronic acid Hyaluronic acid (HA) is a high molecular weight biopolysacharide, discovered in 1934, by Karl Meyer and his assistant, John Palmer in the vitreous of bovine eyes. Hyaluronic acid is a naturally occurring biopolymer, which has important biological functions in bacteria and higher animals including humans. It is found in most connective tissues and is particularly concentrated in synovial fluid, the vitreous fluid

of the eye, umbilical cords and chicken combs. It is naturally synthesized by a class of integral membrane proteins called hyaluronan synthases, and degraded by a family of enzymes called hyaluronidases [6]. Following are important points about hyaluronic acid. Hyaluronic acid derives from the Greek "hyalos", glossy vitreous and uronic acid. The molecule binds water and functions as lubricant between the collagen and the elastic fibre networks in dermis during skin movement. Effect on skin is that it hydrates viscoelastic film on the skin. The polymer may also be injected to obtain a smoother surface and reduce the depth of wrinkles. Properties: Most powerful moisturiser and humectants known so far provide smoothness and softening to the skin, reduce appearance of wrinkles. Ideal ingredient after skin peels. Usage typically used at 0.1-2%. Hyaluronic acid is not readily soluble in water as it binds water very quickly forming a gel [7].

2.2 Method of preparation of hyaluronic acid nanoparticles

The present study relates to the development of a hyaluronic acid nanoparticles for the administration of active molecules. These nanoparticles are made up of hyaluronic acid in salt form, preferentially the sodium salt of the polymers. In a typical experiment, the procedure comprises the following stages:

Flow chart 1: Method of hyaluronic acid nanoparticles

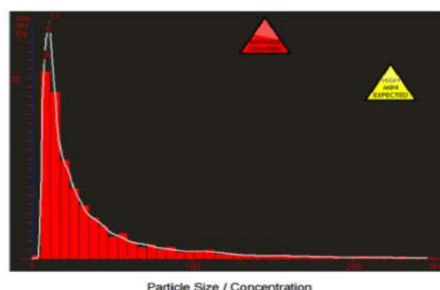


Addition of the surfactant solution and mixing under magnetic stirring, lowering the pH and maintaining the stirring overnight, which will produce the Nanoparticles.

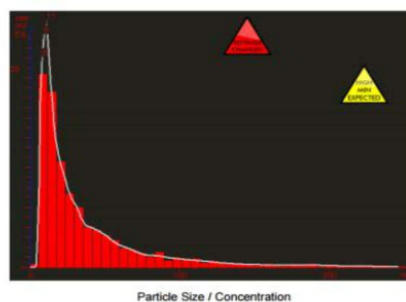
The work-up of the Nanoparticles was as follows: pH was increased to the range of 8-9, followed by the addition of alcohol to precipitate the Nanoparticles. The precipitated Nanoparticles were kept in drying oven at 25°C for six hours to dry. The resulting Nanoparticles can be kept in the refrigerator for storage [8].

2.3 Characterization of Nanoparticles

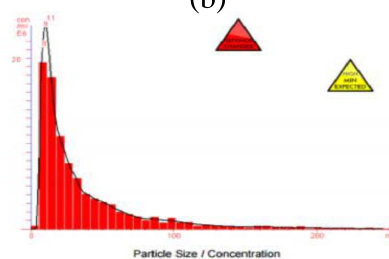
Estimation of particle size by LM 20 Nano Sight Nanoparticle tracking analysis divulges size of nanoparticles by tracking the Brownian motion of particles freely suspended in colloidal solution. Mean size of nanoparticles was calculated by tracking minimum of 1000 nanoparticles active in Brownian motion. The size histograms of hyaluronic acid are evident from Fig. 1 (a, b and c) respectively [9].



(a)



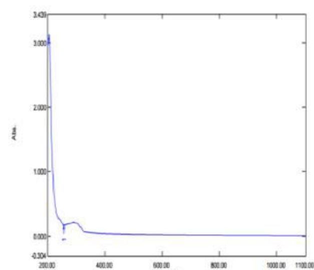
(b)



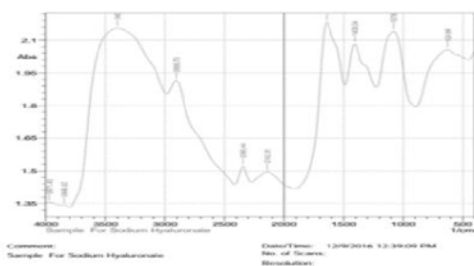
(c)

2.4 U.V. Spectrophotometry

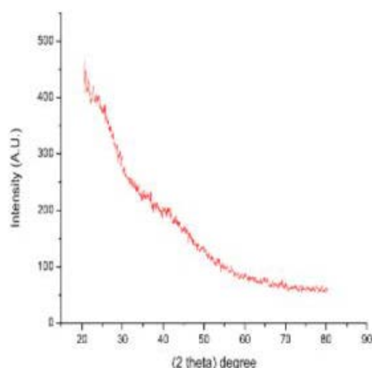
Ultraviolet (UV) and visible radiation comprise only a small part of the electromagnetic spectrum, which includes such other forms of radiation as radio, infrared (IR), cosmic, and X rays. Figure 2 (a), (b), (c) respectively [9].



(a)



(b)



(c)

2.5 Fourier Transform Electron Microscopy: A mathematical operation known as Fourier transform (FT) can separate the individual absorption frequencies from the interferogram, producing a spectrum virtually identical to that obtained with a dispersive spectrometer. This type of instrument is known as Fourier transforms infrared spectrometer [10].

2.6 X-ray Diffraction: X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d \sin \theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θangles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacing allows identification of the mineral because each mineral has a set of unique d-spacing. Typically, this is achieved by comparison of d-spacing with standard reference patterns. All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at the sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays. Powder and single crystal diffraction vary in instrumentation beyond this [11].

3. RESULTS AND DISCUSSION

The particle size mean of hyaluronic acid found to be 44nm whereas the mode is 11nm and standard deviation is 57nm. While the U.V. visible spectrophotometry shows that figure 2(a) absorbance is shown at peak of 257 nm. The XRD (X-ray Diffraction) result in figure 2(c) shows that the sample is of amorphous nature.

3.1 Skin Serum

A serum is a product typified by its rapid absorption and ability to penetrate into the deeper layers of the skin, together with its non-greasy finish and intensive formula with a very high concentration of active substances. Like many other skin products, serums are designed to focus on different actions – anti-ageing, brightening, acne prevention, etc. Because of the high concentrations of the active elements, it is common for cosmetic serums to contain only a few active ingredients which provide intensive nutrition for the deeper layers of your skin. The oil-free finish doesn't leave your skin feeling tight after use: instead it should feel velvety smooth because of the serum's intensive and deep-layer action. Since the active ingredients are so highly concentrated, a serum

will produce more visible results in less time than a simple moisturiser or other skin product. Sometime the high concentration of active ingredients can irritate sensitive skin [12].

3.2 Material and Methods

Table no. 1:formulation of serum

Sr. No.	Ingredients	Quantity for 100 gm			Uses
		F1	F2	F3	
1	Carbopol 940	0.5 gm	0.5 gm	0.5 gm	Gelling agent
2	Olive oil	1 ml	1ml	1ml	Emollient
3	Almond oil	1 ml	1 ml	1 ml	Emollient
4	Tween 80	1 ml	1.2 ml	1.3 ml	Emulsifier
5	Propylene glycol	2 ml	2.5 ml	2.8 ml	Humectant
6	Poly sorbate 60	1 ml	1.5 ml	1.8 ml	Solubuliser
7	Ethylenediaminetetraacetic Acid	0.1 gm	0.1 gm	0.1 gm	Chellating agent
8	Triethanaloamine	0.3 ml	0.5 ml	0.6 ml	Stabiliser
9	Iso propyl alcohol	q.s	q.s	q.s	Solubuliser
10	Glycerine	0.7 ml	0.8 ml	0.9 ml	Humectant
11	Perfume	q.s	q.s	q.s	Perfume
12	DM DM Hyadantoin	q.s	q.s	q.s	Preservative
13	Hyaluronic acid	0.8 gm	0.5 gm	1 gm	Active
14	DM water	To make 100 ml	To make 100 ml	To make 100 ml	Aqueous phase

Observation: From the above observation formula F2 was selected as it was stable and it shows consistency, spreadability and feel and active was added with different concentration and evaluated for in vitro study as per IS and in vivo study with human volunteers.

Procedure for base formula: Take clean apparatus. Weigh Carbopol 940 disperses in distilled water containing EDTA, DM DM Hyadantoin and glycerine. After proper mixing add TEA drop by drop to form a gel, then take another beaker to this add almond oil, olive oil, rose oil, Polysorbate 60, Tween 80, propylene glycol, stir it well and then pour it into gel under stirring slowly, allow it to stir for some more time and then fill it into suitable container.

Table 2: Optimization of serum base

Sr. No.	PARAMETER	F1	F2	F3
1	Appearance	**	***	***
2	Colour	**	***	**
3	Odour	*	**	**
4	Consistency	**	**	*
5	Feel	*	***	**
6	Spread ability	**	***	**

Good= * Better = ** Best = ***

3.3 Evaluation of skin serum

3.3.1 Determination of physical parameters

In physical parameters, appearance, consistency, colour, odour, and spreadability was taken into consideration. The Physical Parameters are determined by visual observation by taking small amounts of sample.

The Serum and lotion samples were kept at various temperatures such as room temp, at 45°C and at the elevated temp (freeze temp.).The formulations were checked after every 10 days for parameters such as colour, odour, consistency, spreadability and appearance [13].

3.3.2 Determination of pH (IS: 6608 - 1978)

For oil-in-water emulsion Serum Accurately 5±0.01 g of the Serum was weighted in a 100ml beaker. 45ml of water was added and the Serum was dispersed in it. The pH of the suspension at 270 C was determined using the pH meter [13].

3.3.3 Determination of total fatty substance content: (Indian Standard skin creams — specification, 2004)

For this the emulsion is broken up with dilute mineral acid and the fatty matter is extracted with petroleum ether. It is weighed after removal of the solvent. Accurately about 2g of sample was weighted into a conical flask, about 25ml of dilute HCl was added, reflux condenser was fitted into the flask and the content of the flask was boiled until the oil and water phases have separated. The content of the flask was poured into 300 ml separating funnel and it was allowed to cool to 20°C. The conical flask was rinsed with 50ml of ethyl ether in portions of 10ml. The ether rinsing was poured into separating funnel. The separating funnel was shaking well and leave until layers separate. Separate out with 50ml portions of ether twice. All the ether extracts was combined and washed them with water until free of acid. The ether extracts was filtered through a filter paper containing sodium sulphate into a conical flask which had been previously dried at temperature of 6°C ±2°C and then weighed. The sodium sulphate was washed on the filter paper with ether and the material remaining in the flask was dried at a temperature of 6°C ±2°C to constant mass [13].

3.3.4 Total fatty substance % by mass =100x M1/M2.

Where, M1 is Mass in g of residue and M2 is Mass in g of material taken for the test.

3.3.5 Determination of Thermal Stability:

A 20 mm broad and 5 mm thick strip was spreaded from the material to be tested on the internal wall of a beaker of 100ml capacity in its total height. The beaker was kept for 8 hrs. in the humidity chamber at 60 to 70% relative humidity and temperature 37± 1°C.

3.3.6 Microbial examination of Serum: T.

The test consist of pleating a known dilution of the sample on soya bean casein digest agar medium suitable for the total count of aerobic bacteria and fungi after incubating them for a specified period to permit the development of visual colonies for counting Pre-treatment of sample: To 10 ml of sodium chloride solution pH 7 or any other suitable medium add 1gm or 1ml of sample Total bacterial count: Pipette out in duplicate 1ml of pre-treated sample aseptically into 5 sterile Petri dishes. Pour 15 to 20 ml of molten soya bean casein digest agar maintained at about 45°C. Mix the content of the plate by swirling. Allowing the incubate the plates at 37°C +1°C in inverted position for three days Count the number of colonies in each plate. Determine the average number of colonies on plates and multiply by dilution factor. This will be the number of microorganisms per gm of the sample. If no colony was recovered from any of the plate it can be stated as less than 50 microorganisms per gm.

3.3.7 Total fungal count: Pipette out in duplicate 1ml of pre treated sample aseptically into 5 sterile petridishes. Pour 15 to 20 ml of molten sabouraud's chloranphenicol agar (SCA) maintained at about 45°C mixes the content of the plate by swirling. Allowing the plates to solidify, invert and incubated at 23+2°C for three days. Count the number of colonies in each plate.

3.3.8 Stability studies of Serum: Stability studies for Serums were carried out according to ICH guidelines. The Serum samples were kept on the 5°C, room temperature, and 40°C. The changes in the physical appearance, colour, odour etc and chemical changes such as change in pH, viscosity, pH separation were checked and thus. The formulation of Serum was optimized.

Table3: Evaluation Of Physical Parameters

Sr. No.	PARAMETERS	F1	F2	F3
	(A) Physical appearance			
1	Appearance	serum like	serum like	serum like
2	Colour	white opaque	white opaque	white opaque
3	Odour	pleasant	pleasant	pleasant
4	Consistency	semi liquid	semi liquid	semi liquid
5	Spread ability	good	good	very good
6	Oily/tacky feel	No	No	No

3.3.9 Accelerated stability studies: To ensure that a cosmetic remain stable till the consumers has used the entire cosmetic or has stopped using it, a number of special accelerated test procedures have been developed. The evaluation employs a combination of tests. This method of evaluation not only indicates stability of Base formulation but also indicates the stability of functional ingredient [13].

Freeze thaw cycle: These tests are not carried out at fixed temperature and humidity. In these tests, temperature was changed cyclically every day e.g. Low-high-low-high-low-high, to simulate changes in temperature daily [13].

Table 4: Freeze Thaw Cycle

Sr. No.	PARAMETERS	F1	F2	F3
1	Freeze thaw cycle	Stable	Stable	Stable

4. Result and Discussion

4.1 In Vitro-Study

Table No. 5: Determination of physical parameter of a Skin Serum containing hyaluronic acid as active. (Stability study after 10 days).

Sr. No.	PARAMETERS	F1	F2	F3
	Physical appearance	**	**	**
1	Appearance	**	**	**
2	Colour	**	**	**
3	Odour	**	**	**
4	Consistency	**	**	**
5	Spread ability	**	**	**
6	Oily/tacky feel	**	**	**

Change = * No change = **

Table No. 6: Stability study after 20 days

Sr. No.	PARAMETERS	F1	F2	F3
	Physical appearance	**	**	**
1	Appearance	**	**	**
2	Colour	**	**	**
3	Odour	**	**	**
4	Consistency	**	**	**
5	Spread ability	**	**	**
6	Oily/tacky feel	**	**	**

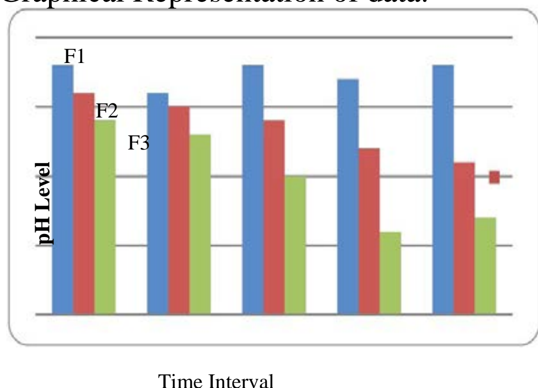
Table No. 7: Stability study after 30 days

Sr. No.	PARAMETERS	F1	F2	F3
	Physical appearance	**	**	**
1	Appearance	**	**	**
2	Colour	**	**	**
3	Odour	**	**	**
4	Consistency	**	**	**
5	Spread ability	**	**	**
6	Oily/tacky feel	**	**	**

Table No. 8: Determination of pH of serum containing hyaluronic acid as active. Standard value 5 to 9

Sr. No.	Time Interval	F1	F2	F3
1	0 Day	6.23	6.21	6.19
2	8th Day	6.21	6.2	6.18
3	16th Day	6.23	6.19	6.15
4	24th Day	6.22	6.17	6.11
5	30th day	6.23	6.16	6.12

Graphical Representation of data:



Time Interval

Table 9: Determination of Total fatty matter of serum containing hyaluronic acid as active (Standard value 5.0%)

Sr. No	Parameter	F1	F2	F3
1	TFM % by Mass	4.55%	4.65 %	4.60 %

Graphical Representation of data:

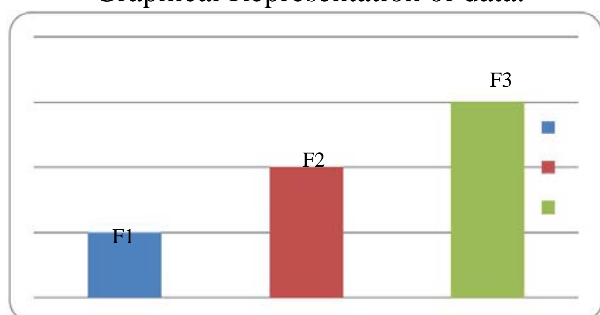


Table 10: Determination of Thermal Stability serum containing hyluoronic acid as active.

Sr. No	Parameter	F1	F2	F3
1	Thermal Stability	passed	passed	passed

4.2 Microbial examination of a Serum

Table No. 11: Microbial examination of a serum containing hyaluronic acid as active

Sr. No	Name of the test	Result	Specification	Unit
1	Total bacterial count	10 CFU/gm	NMT100 CFU/gm	CFU /gm
2	Fungal count	Nil	NMT10CFU/gm	CFU /gm

5. Conclusion

Nanoparticles are one of the promising drug delivery systems, which can be of potential use in controlling and targeting drug delivery. They possess better stability when compared with liposomes. They have various applications such as ophthalmic drug delivery, intravenous delivery as carriers for radio nucleotides in nuclear medicine, as cosmetics for the skin and hair care, sustained release formulations and many more. Nanoparticles formulated as amorphous spheres offer higher solubility than standard crystalline formulations, thus improving the poor aqueous solubility of the drug and hence its bioavailability. While serum on the other side, is a concentrated product widely used in cosmetology. The term itself comes from professional cosmetology. Cosmetic skin serum is a highly concentrated product based on water or all as any other cream. Serums are concentrates containing approximately 10 times more of biologically active substances than creams, therefore quicker and more effectively coping with cosmetic problems. The effect of serum when concentrates are that it immediately gets the necessary amount of active substances such form which assimilates easier. The active substances in high concentration act in the same way as cream they moisturise, rejuvenate, lift up, etc. The only difference is that in case concentrates are used correctly the noticeable result will be reached quickly.

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