Formulation Development and Evaluation of a Topical Nanogel Containing Kojic acid

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ABSTRACT- Topical drug administration is a localized method of delivering drugs to specific areas of the body via topical channels. The major route of topical medication delivery is through the skin, which is one of the most easily accessible organs on the human body for topical drug administration. The present investigation involves formulation of topical nanogel using Kojic acid for the treatment of hyperpigmentation, Kojic acid is an effective and well tolerated drug having melanin neutralising activity (Tyrosinase inhibitor). Topical nanogel of Kojic acid was prepared by using High molecular weight water soluble polymer Hydroxy propyl methyl cellulose such as K35M grade and other excipients including methyl paraben, Carbopol 940, glycerine and purified water were reported in the formation of nanogel. In the present investigation nanogel the formulated nanogel was evaluated for pH, viscosity, Spreadability, extrudability, conductivity, particle size, zeta potential, in vitro drug diffusion studies. Among the formulated nanogel batch 4 has met all the specifications and was formed to be optimized Efficient delivery of drug to skin application was found to be highly beneficial in localizing the drug to desired site in the skin.

KEYWORDS- Nanogel, Kojic acid, Particle size, Zeta potential, Drug Release.

INTRODUCTION

Nanogels are defined as nanoscale particles that, either physically or chemically, create crosslinked polymers. In order to transport polynucleotides, cross-linked bifunctional networks of a polyion and a non-ionic polymer were first developed [1]. Although soluble in water, nanogels differ from linear macromolecules with comparable molecular weights in their properties. These structures along with their larger equivalents [2]. Structures along with their larger equivalents [2]. Nanogels are typical formulations that typically range in size from 1000 nm, and their three-dimensional structure can be maintained by altering volume proportion and solvent quality. Nanogels have revolutionized the field of gene therapy because they have made it possible to deliver genes within cellular organelles for gene silencing therapy [3]. Nanogels are composed of ionic or non-ionic polymer chains that are hydrophilic or amphiphilic and

grow into nanoscale structures. Despite its use as a drug delivery system, nanogel has been studied for longer periods in the production of other substances like quantum dots, dyes, and other diagnostic agents [4]. The development of nano-sized microgels and hydrogels as a result of specific delivery system anticipation has been made possible by the wide range of polymer systems and the simple modification of their physico-chemical properties [5]. Transdermal delivery of drug is promising but challenging system is available for local as well as systemic effect of drug. The entry of drug through the stratum corneum may follow the intercellular, transcellular or appendageal route. The intercellular route is the more common pathway of the drug permeation through the skin [6].

Melanocytes create the biological pigment called skin melanin. Skin colour is primarily determined by melanin, which shields human skin from ultraviolet (UV) solar radiation's harmful effects. Hyperpigmentation is the term used to describe melanin over synthesis [7]. For most people, especially women, hyperpigmentation treatment is usually difficult and disappointing [8]. Kojic acid (KA) is a popular hydrophilic tyrosinase inhibitor with natural whitening properties that is used to treat hyperpigmentation. By chelating copper atoms, Kojic Acid inhibits the tyrosinase enzyme and prevents the synthesis of dopachrome. It synthesized several fungi species, including Aspergillus and Penicillium [9]. Despite KA and its derivatives distinctive qualities, the cosmetic industries hardly ever use them. Due to its hydrophilic nature and the two hydroxyl functional groups that make up its chemical structure, Kojic Acid is a hydrophilic component. Its skin absorption is inadequate [10].

The present study was conducted to design and evaluate Kojic Acid nanogel which provides prolonged release, increase the residence time of drug on the skin thereby enhance bioavailability.

MATERIALS AND METHOD

Kojic Acid was purchased from Arti pharmaceuticals, Mumbai. Carbopol 940 Research-lab Fine Chem Industries, Mumbai. HPMC K35M was obtained as a gift sample from Ashlands, Netherlands. Co., Methylparaben and Glycerine was purchased from Research-lab Fine Chem. Industries, Mumbai [11].

METHOD

Preparation of Kojic Acid Nanogel [12]

Preparation of 2% drug solution of KA- weigh 2 gm of drug Kojic acid dissolve in 100ml of distilled water.
2% of prepared kojic acid measure 2ml of solution mix with given quantity of HPMC K35m, mix well then add glycerine. (Organic phase). Weigh carbopol940 accurately, add 10ml distilled water (aqueous phase). Stir aq. phase on magnetic stirrer add organic phase dropwise. Add methylparaben (preservative). Batch B1, B2, B3, B4 was prepared at highest rpm 8000 with variation in composition.

Evaluation Parameters

Appearance: The prepared nanogel bases were inspected visually for clarity, colour and presence of any particles.

Homogenesity

All developed nanogels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates [12].

Measurement of particle size of formulation

Horiba sz-100 windows [z type] were used to investigate the particle size (PS) of the gel. Particle size and zeta potential were measured in triplicates after dilution with distilled water, and the average values ±SD were recorded [13].

pH measurement

The pH measurement was carried out by using calibrated digital type pH meter by dipping the glass electrode and the reference electrode completely into gel system so as to cover the electrodes.

Conductivity

A direct reading digital conductivity meter (Systronics model no. 304) and dipping type conductivity cell [14].

Drug content

For the estimation of the drug in nanogel, kojic acid was extracted from 1 gm of nanogel formulation with 50 ml of distilled water. From this, 2 ml was pipette out and made up to 10 ml. The absorbance of the sample was determined spectrophotometrically at 268 nm. The concentration of Kojic acid was estimated from the calibration curve [12, 24].

In vitro drug Release studies

The drug release from the formulation was determined by using the apparatus known as Franz Diffusion Cell, which consist of a cylindrical glass tube which was opened at both the ends. 1 gm of nanogel equivalent to 4 mg of Kojic acid was spread uniformly on the surface of cellophane membrane (previously soaked in medium for 24 hrs) and was fixed to the one end of tube. The whole assembly was fixed in such a way that the lower end of tube containing gel was just touches (1-2 mm deep) the surface of diffusion medium i.e., 100 ml of pH 7.4 phosphate buffer contained in 100 ml beaker. The assembly was placed on thermostatic hot plate with magnetic stirrer and maintained at temperature 37°±2° the contents were stirred using magnetic bar at 100 rpm for a period of 24 hrs, 5 ml of samples were withdrawn at different time intervals. This 5 ml was diluted up to 10 ml of fresh phosphate buffer (pH 7.4) and sample were analyse at 268 nm in UV-Visible spectrometer for KA. [12, 25-26].

Skin irritation test

Test for irritation was performed on human volunteers. For each gel, four volunteers were selected and 1.0 g of formulated gel was applied on an area of 2 square inch to the back of hand. The volunteers were observed for lesions or irritation [12].

Spreadability

Spreadability is determined by apparatus suggested by Mutimer. It consists of wooden block, which is provided by a pulley at one end. By this method, Spreadability is measured on the basis of "Slip" and "Drag". A ground glass slide is fixed on this block. A sample of 0.1 g of nanogel under study is placed on this ground slide. The gel is fixed on the beach formula was pressed between two slides and a 1 kg weight is placed on the top of two slides and left for about 5 min to expel air and to provide a uniform film of the nanogel between two slides. Excess of the nanogel is scrapped from edges. The top plate is then subjected to pull the weight. With help of string attaches to the hook and the time required by top slide to cover the distance is noted. A shorter interval indicates better spreadability, spreadability was calculated by using the formula,

S=M.L/T,

Where,

S= spreadability, L=Length of glass slide, M=weight tied to upper slide, T=Time taken to separate the slides [13, 15].

Extrudability

Measure the force required to extrude the material from tube. Extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds [16, 17]

+++ excellent ++ very good + average

Scanning electron microscopy

Scanning electron microscopy (SEM) provides high-resolution imaging that may be used to evaluate diverse materials for surface cracks, defects, contaminants, or corrosion. When a focused stream of secondary electrons interacts with atoms in the sample, multiple signals are produced that include information about the surface topography and sample composition using the Nova NanoSEM NPEP, all pictures were scanned at 10000x with a 5 m dimension scale 303 [18].

Zeta Potential

Zeta Potential of the prepared Nanosuspension was determined using Light Scattering method. The charge on the surface of particles is characterized by the HORIBA Scientific SZ-100 by measuring the zeta potential of a gel. The sample is injected into a disposable cell and a measurement of the particle electrophoretic mobility results in the calculated zeta potential [19-22].

Particle size

Horiba sz-100 windows [z type] were used to investigate the particle size (PS) of the gel. Particle size and zeta potential were measured in triplicates after dilution with distilled water, andthe average values ±SD were recorded [13, 23].

Content uniformity

Identification of pure drug

Identification of pure drug was carried was by Fourier Transform Infra-red Spectrophotometry (Shimadzu 8400s) scanned in the range of 200-400 nm.

Drug-excipient compatibility study

Studies of drug-excipient compatibility are important to ascertain drug and excipients are compatible with each other. IR spectra are used to study drug-excipient compatibility.

FTIR study

FTIR (Shimadzu 8400s) spectrophotometer were used in the range of 400-4000 cm^{-1} using potassium bromide discs (Mixing ratio1:1) The samples were hermetically sealed in aluminium pans and heated at a constant rate of 10°C/ min over a temperature range of 40 to 300°C.

FTIR spectroscopy

The FTIR spectrums of pure Kojic acid and physical mixtures of drugs and polymers were studied separately as per the excipients used in the formulation. It was observed that there were no major shifts in the main peaks of either drug. This indicates that there were no compatibility problems with the drug with the polymers and excipients used in the formulation. Kojic acid had peaks at 1715(C=O stretching), 3549 (O-H str.), 1620 (C-O), 2839 (C-H).

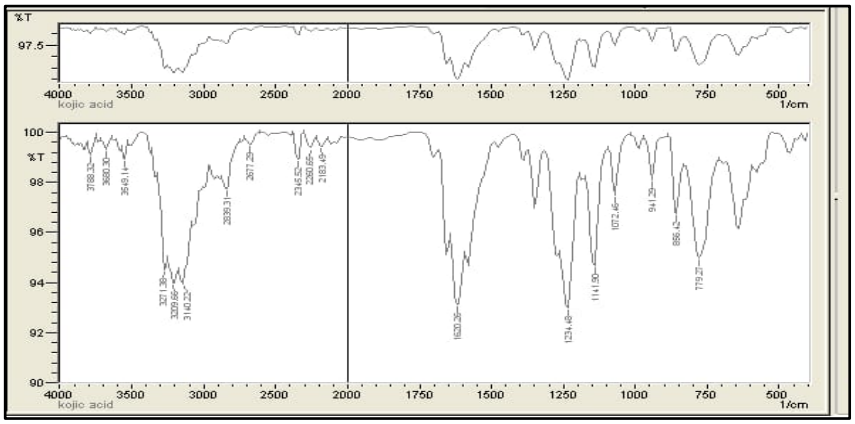
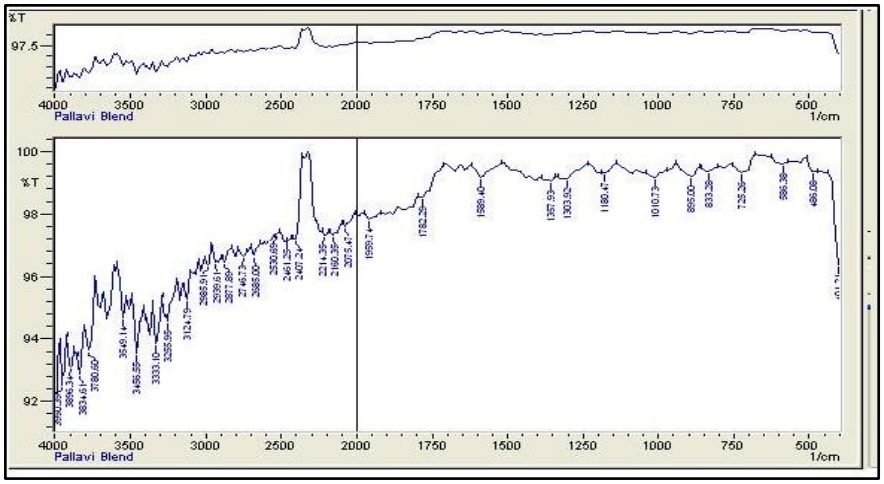


Figure 1: FTIR Studied of Kojic acid



UV spectroscopy:

The linearity of the response of kojic acid was verified at 2–10 µg/ml concentrations. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis. The equation of the linearity curve for kojic acid was y = 0.0646x +0.0036. The linearity curve was found to be linear in the a for mentioned concentrations (the correlation coefficient (r^2) of determination was 0.9978)

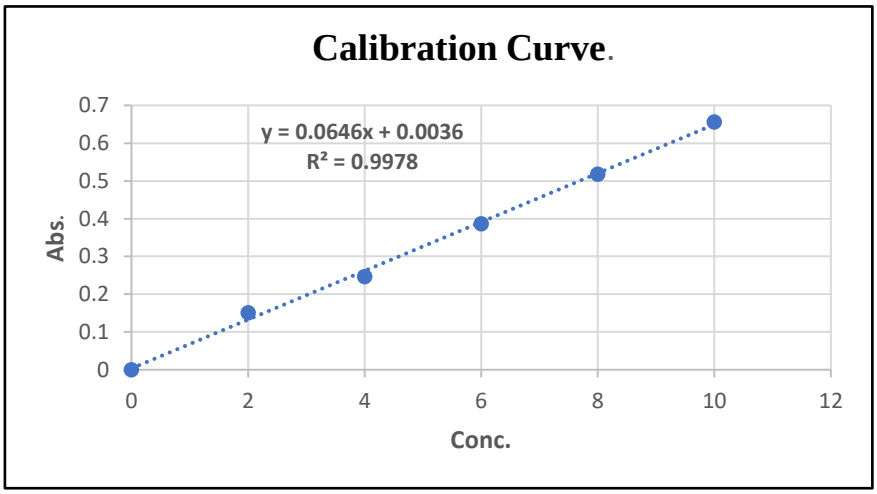


Fig 3: Calibration curve of Kojic acid

Composition of Nanogel: -

Table 1- Composition of nanogel (B1-B4)

Evaluation of prepared nanogel:

Appearance

Appearance of the prepared Nanogel was inspected visually and all the batches were white to Clear, and free from any particulate matters.

Particle Size Determination

Particle size of the prepared Nanogel was determined using Dynamic Light Scattering (DLS) method. Particle size determination results for all the prepared batches kojic acid nano are presented in the Table 3 and all the Graph obtained are reported in the Figure 4-7.

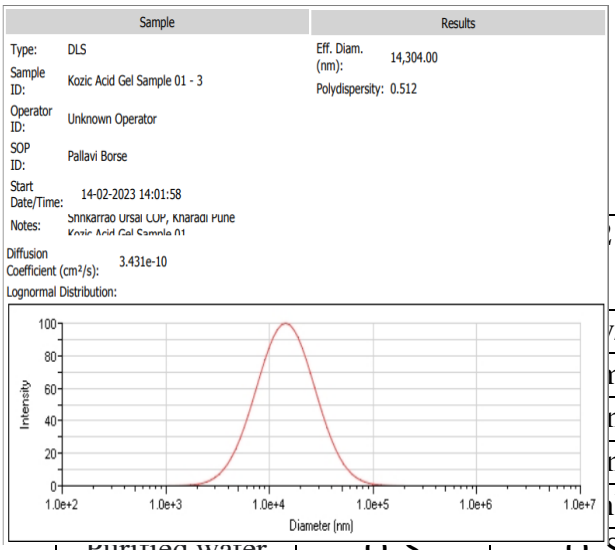


Fig 4 A: Particle size of formulation B1

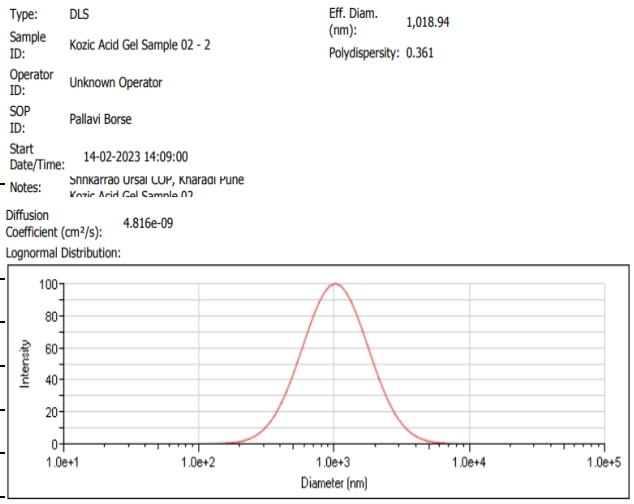
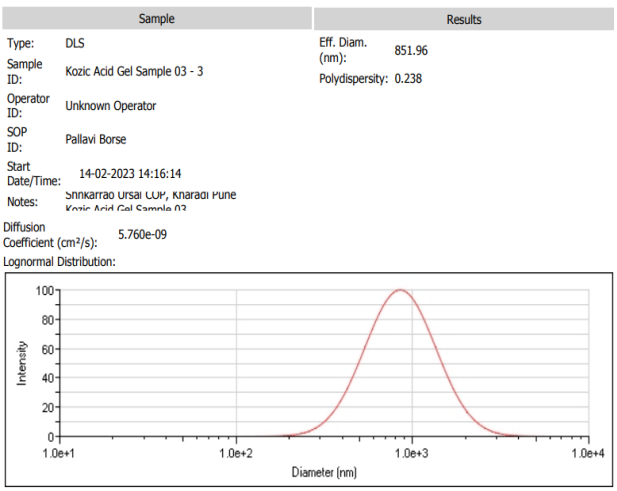


Fig 4B: Particle size of formulation B2

Fig 4D: Particle size of formulation B4



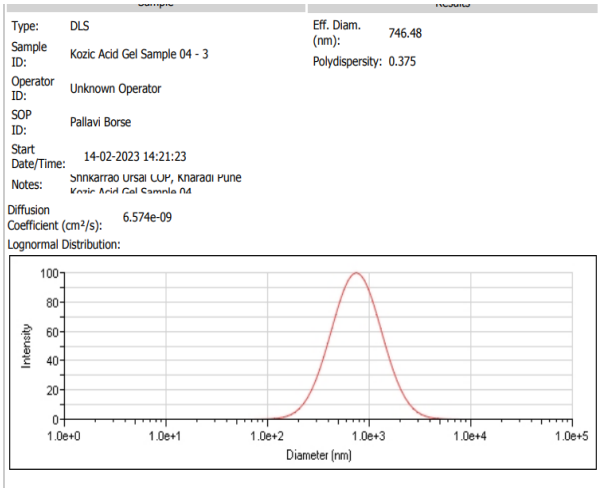
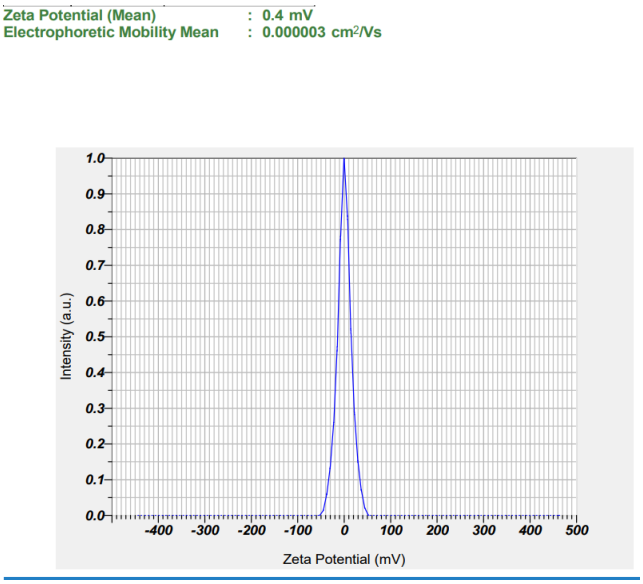


Fig 4C: Particle size of formulation B3

Particle size of batch 4 shows optimise size 746.48nm compare to other 3 batches. Batch4 is having optimum conc. of HPMC K35M and Carbopol 940.

Zeta Potential analysis

From the Graphical representation in following figures, it was observed that when the Nanogel was prepared using maximum conc. Of HPMC polymer compared to other batches is more stable.



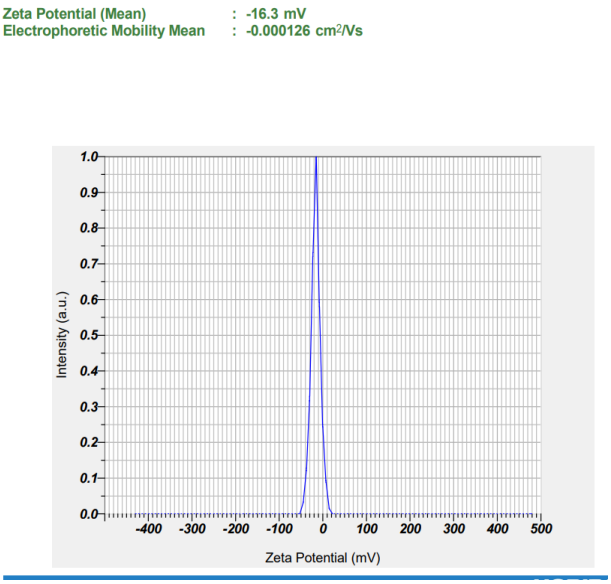


Fig 5A. zeta potential of formulation B1

Fig 5B. zeta potential of formulation B2

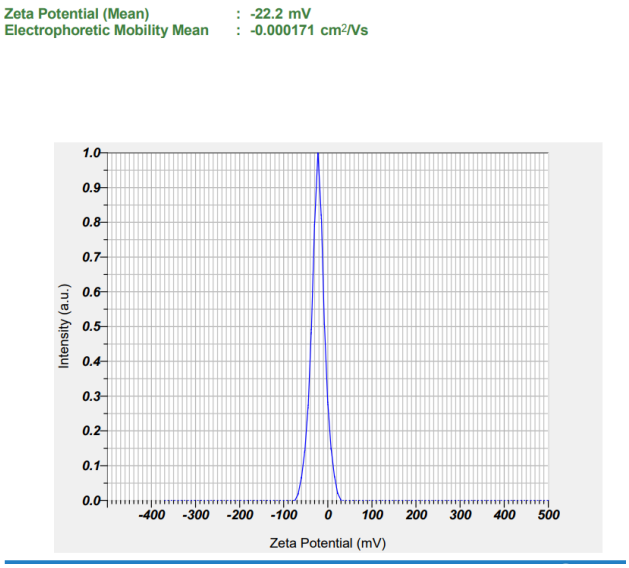


Fig 5C. zeta potential of formulation B3

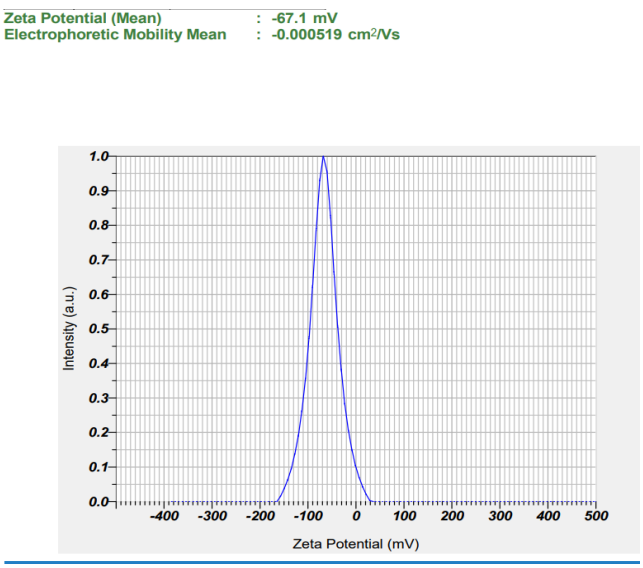


Fig 5D. zeta potential of formulation B4

To stabilize the Nanogel the Zeta Potential must be more that ±20 mV and it was observed from the above figure that batch 4 shows -67.1mV. From the Graphical representation the prepared Nanogel of B4 is more stable.

In-vitro Diffusion studies:

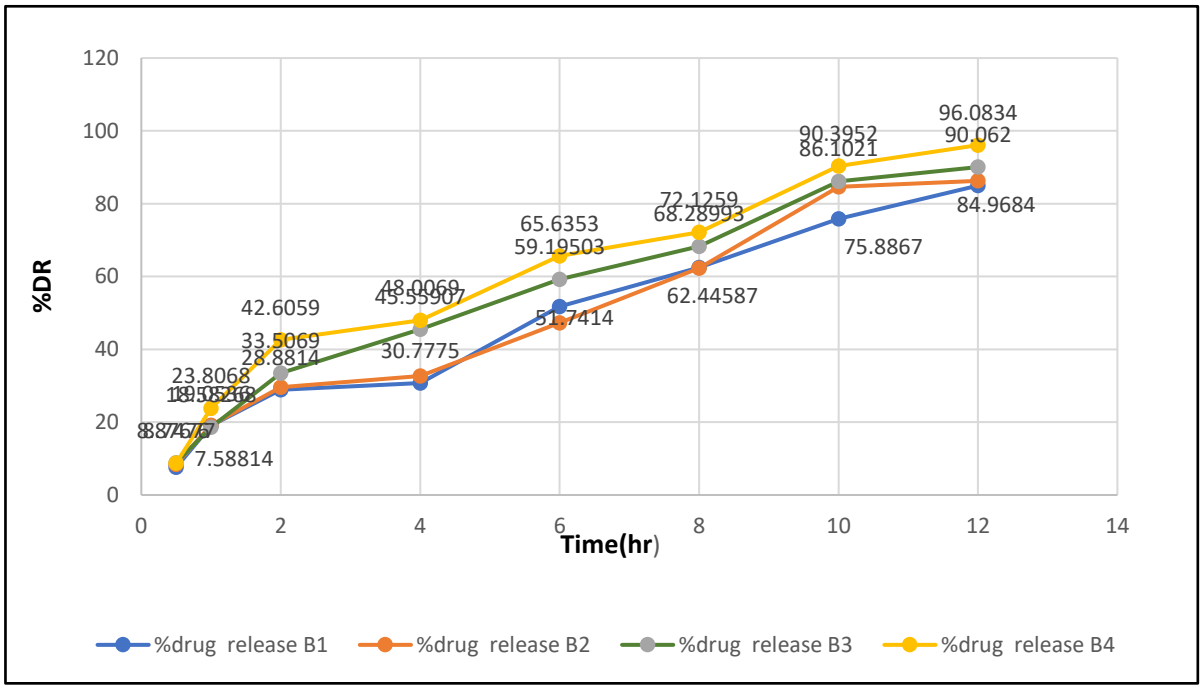


Fig 6: In-vitro drug release profile of nanogel of Kojic acid

From the above graph and % drug released readings batch 1 has 84.96% of drug release, batch 2 shows 86.27% drug release, batch 3 shows 90.06% drug release, batch 4 shows 96.83% drug

release in 12 hours. Formulation batch 4 shows maximum %drug release. Batch 4 have optimum concentration of polymer. Hence batch 4 consider optimise batch among 4 batches.

Viscosity study: (Spindle number-96)

Table 2: Viscosity results of B1

RPM	Surface (cP)	Middle (cP)	Bottom (cP)
10	27000	27560	28970
20	17530	19310	20480
30	14690	15250	16220
40	10200	10800	10930
50	9328	9469	1547

Table 3: Viscosity results of B2

RPM	Surface (cP)	Middle (cP)	Bottom (cP)
10	21940	21840	25590
20	15230	15940	14160
30	10650	10560	11120
40	7969	7406	7631
50	6688	6531	6625

Table 4: Viscosity results of B3

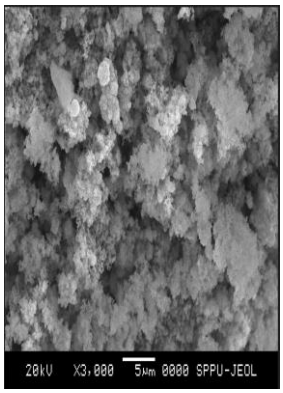
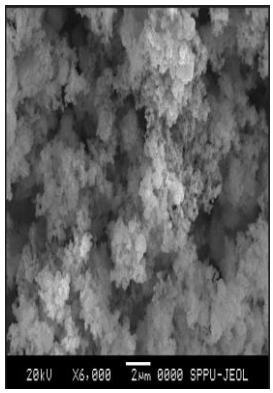
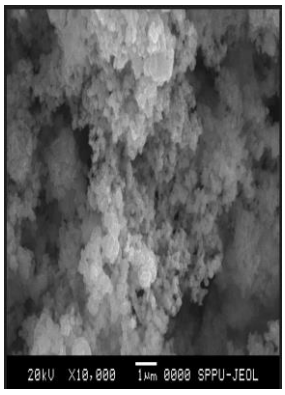
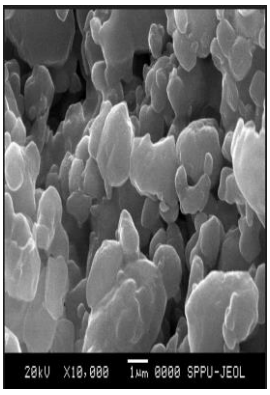
RPM	Surface (cP)	Middle (cP)	Bottom (cP)
10	22220	23110	24000
20	13550	13730	13880
30	10840	11090	11410
40	7388	7575	8231
50	6516	6531	6875

Table 5: Viscosity results of B4

RPM	Surface (cP)	Middle (cP)	Bottom (cP)
10	45000	50440	51000
20	30050	30560	31220
30	23380	23970	24560
40	15960	16560	16610
50	14200	14500	14690

Table 6: Evaluation For all formulated batches (B1-B4)

Particulars	**Batch1**	**Batch2**	**Batch3**	**Batch4**
Appearance	Whitish to colourless	Whitish to colourless	Whitish to colourless	Whitish to colourless
Fill volume (gm)	10gm	10gm	10gm	10gm
pH	5-7.5	5-7.5	5-7.5	5-7.5
Extrudability	++ (very good)	++ (very good)	+++ (excellent)	+++ (excellent)

Conductivity 1)200ms 2)20ms 3)2ms 4)200µs 5)20µs	 0 0 0.03 27 1.0	 0 0.2 0.05 057 1.0	 0 0.0 0.03 021 1.0	 0 0.1 0.12 123 1.0
Zeta potential (mV)	0.4mV	-16.3mV	-22.2mV	-67.4mV
Particle size (nm)	1754.20nm	1018.9nm	851nm	746nm
Spreadability (gm.cm/sec)	4.1 ±0.0264	4.6 ±0.0284	3.9 ±0.057	3.2 ±0.0156
Scanning electron microscopy				
Content Uniformity (%)	94.6%	96.1%	98.4%	98.7%

Conclusion:

It can be concluded from the experimental study carried out that the formulation of a Nanogel containing Kojic acid drug yields a formulation with a spherical and smooth surface, nano in the size range. The prepared nanogel was smooth without any lumps, particles and aggregates. So, all the formulations are homogenous. Based on all the factors the nanogel drug delivery system Batch-4 shows good drug content compared to others. The particle size of the nanogel formulation is optimum and it is less than 1000 nm. So, it concluded that the particles are in the tiny and nano in the size range. All nanogel formulations show pH in the range of 5.5 to 7. Based on the Spreadability diameter study it shows the nanogel is having good Spreadability. Nanogel formulations show a viscosity range from 5000-50000 cps. It concluded that they are stable in nature. Formulation Batch-4 showed the highest percentage of drug release compared to other formulations. In-vitro diffusion studies show Batch-4 formulation shows a controlled release pattern of drug from the formulation. The Zeta potential of batch Batch-4 showed -67.3mV. High zeta potential values show there will be no particles come together and no

flocculation. Hence it can be concluded from the experimental study carried out, that the formulation Batch-4 is an optimized batch with optimum HPMC K35M and Carbopol940.

ACKNOWLEDGEMENT

For the completion of the research work the authors would like to show sincere gratitude to PDEA'S Shankarrao Ursal College of Pharmaceutical Sciences & Research centre, Kharadi, Pune to provide with a lot of support and help whenever needed.

CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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