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Formulation and Development of Thermosensitive in Situ Gel of Aspirin Containing Nanoparticles for Opthalmic Use

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Polymers, In-Situ Gel Formulation, Gels, Hydrogel, Bioavailability, Drug Delivery Systems.

ABSTRACT:
A lot of researchers have recently become interested in aqueous polymer solutions that become gels due to changes in environmental factors like pH and temperature. This process, entitled "in situ hydrogel," is of scientific significance and has potential uses in the medicine or healthcare industries. When the hydrogel is produced under physiological conditions and keeps intact for the desired length of time, the technique may offer a number of advantages over conventional hydrogels. Drugs that are hydrophobic can be solubilized and delivered with controlled release using the in-situ gel formulation. These advantages derive from the ease of drug formulation through solution mixing biological compatibility with biological processes, and simple administration. Once the compatibility prove, the produced gel will give further benefits for in vivo applications that need breakdown, resulting in non-toxic degradation products. A summary of polymer systems that go through solution-gel formation, particularly those triggered by temperature variations, is useful right now. The emphasis should be on the underlying processes of these transformations and the various delivery options.

1. Introduction

One of most delicate organs, eye is shielded from the outside world by several defenses and barriers. For instance, because of its unique structure and due to availability of the blood-aqueous barrier (BAB) and blood retinal barrier (BRB) it is tough to deliver the medicine in to eye and treat ocular disease. Furthermore, drug bioavailability after administration may be less than 5%, which is frequently insufficient. Recently various pharmacological formulation used in the treatment of eye such as ocular drops, ointments and gels. There are numerous barriers that limit medicine's bioavailability to the eye site, including the tear film, corneal, conjunctival, scleral, blood-aqueous, and blood retinal barriers. Due to the fact that creating innovative drug delivery methods for ocular particulates with enhanced permeability or delayed release effects has recently become a research priority. Particulate drug delivery systems (DDSs) that are most frequently used are microspheres, liposomes, emulsions, dendrimers, and micelles. The primary site of drug delivery system is eye surface, intraocular tissues and periorbital tissues. Every single area of eye has different barrier so that key factors for drug administration also changed. To effectively distribute medications to various areas of the eye, the creation of a unique ocular DDS has emerged as a key concern. Previously,

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the primary focus of nanomedicine was on treating glaucoma and disorders of the ocular surface. However, over the past ten years, fundus lesions and ocular cancers have been included in the rapid development of nanomedicine in ophthalmology. Therefore, when a medication is placed into an ocular nano-level DDS, its solubility, stability, and permeability are enhanced; this extends the drug's residence time and boosts its efficacy.[1]

Eyeball's Structure

The mature eyeball, which is composed of the ocular wall, has a diameter of roughly 24 mm. The anterior segment of the eyeball is composed of the cornea, conjunctiva, iris, ciliary body, sclera, aqueous humour, and lens, whereas the posterior segment is made up of the vitreous body, retina, choroid, and sclera. One unique anatomical characteristic of the eyeball that makes it difficult to achieve an effective medicine concentration is the blood-eye barrier. Because of the hydrophilic inner layer and lipophilic outer layer of the cornea, ointments cannot be both fat- and water-soluble. This indicates that getting an effective intraocular concentration after applying topical medication difficult. The main element in the therapy eye illnesses is the therapeutic intraocular drugs concentration. Thus, the main variables influencing the increase of intraocular drugs concentration are the numerous dosage forms and drug administration routes.[1]

Conventional Ophthalmic Dosage Form

Treating eye problems through traditional treatments involves either local or systemic injection (Figure 1.1). Periocular (subconjunctival, subtenon, posterior juxtascleral, retrobulbar, and peribulbar) and intravitreal injections are examples of local administration. However, there are certain restrictions on these drug delivery techniques. Treatment of eye diseases in the clinic usually involves local application of medication to the cornea, conjunctiva, sclera, and anterior uvea. Usually, eye drops seep out of the surface of the eye quite fast. Additionally, because of developments in technology and medication research, intravitreal injections are used to treat diseases of the retina and vitreous. Just 1% to 2% of drugs that are administered systemically actually reach the target region as a result of BAB and BRB's activity. Excessive dosages and repeated systemic injections might also have negative effects.[1-3]

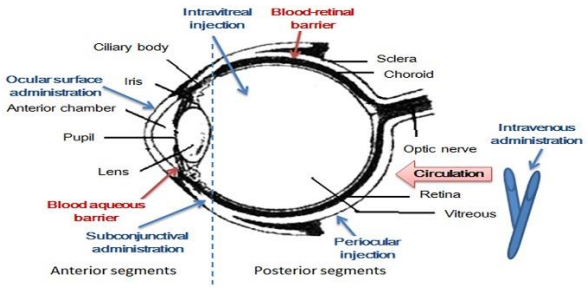

Figure 1: Structure of the eye.

Novel ocular drug delivery system

Recent studies have shown that hydrophilic or lipophilic pharmaceuticals can be administered to the eye using nano-carriers, which can also boost the bioavailability of certain medications, such as peptides and proteins, and prolong the half-life of medications in the vitreous. Moreover, polymeric nano-micelles have been studied as potential therapies for both intraocular and surface eye disorders. Additionally, the medicine is shielded against deterioration by nanotechnology throughout storage and in vivo circulation. As a result, a novel ophthalmic DDS is required that has great penetration, prolonged release, and long half-life in the eye.[4-6]

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Drug delivery systems in situ gelling

A polymeric systems that experience sol-gel transitions, especially those caused by temperature changes, is appropriate now. The focus should be on the underlying processes of these transformations and possible delivery methods.

Over the past 20 years, in situ gelling drug delivery technologies have drawn increased attention from the ophthalmology.

Prior to administration, these delivery systems are in a solution form and have the ability to form gels in response to various stimuli [7] and prolong the duration of drug residence on the eye surface (Figure 2).[8]

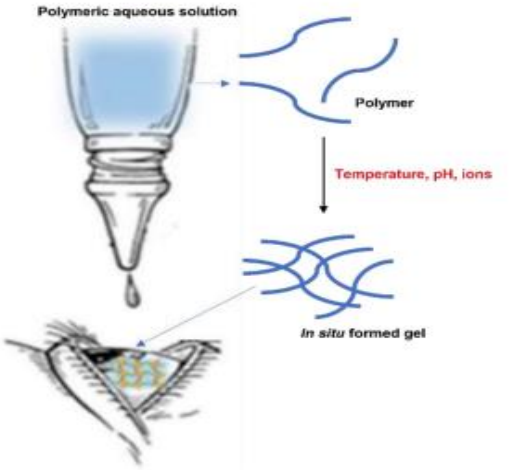

Figure 2: In situ gelling system

2. Methods

Collection of drug

Acetylsalicylic acid (Aspirin) was received as gift sample from Wellona Pharma Private Limited. All excipients and reagent was received from well-known vendor. All excipients are pharmacologically inert and all reagents are analytical grade.

Preformulation studies

Preformulation studies examine the physical and chemical properties of a drug substance. The preferred drug was aspirin, which was evaluated for the following physical attributes: organoleptic melting point, partition coefficient, solubility, UV-visible spectra, and FT-IR spectra.[9]

Organoleptic characteristics

Physical characteristics such as color, look, and odor were noted for the drug sample. Aspirin's physical study were found to be in line with published research.[9]

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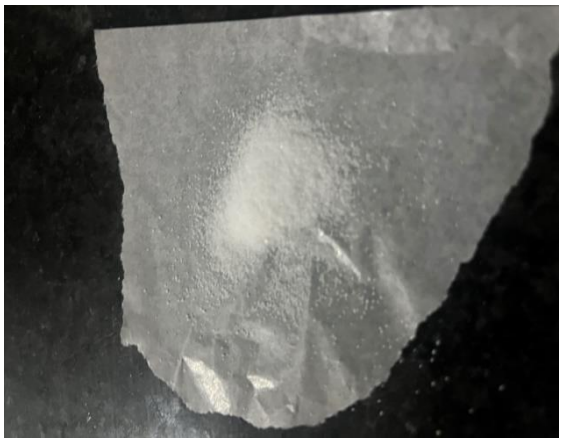

Figure 3: Raw material

Table 1. Physical study of Aspirin

Properties	Inferences
Color	White
Form	Crystalline
Taste	Slightly bitter

Melting point (M.P.)

Melting point is the temperature at which substance changes state from solid to liquid at one atmosphere of pressure.[10] the drug's purity is implied by the melting point measurement. Aspirin's melting point has been found via the capillary tube method to be quite similar to the stated melting point.

Table 2: Aspirin's Melting Point

Material	Standard M.P.	Observed M.P.
Aspirin	135.0°C	134.3±0.577°C

UV spectrum of Aspirin

Typically, the UV spectrum is plotted against wavelength to record absorbance. An UV-visible double beam spectrophotometer was used to measure the drug's λmax. between 200 and 400 nm, an aspirin solution containing 90µg/ml in methanol was analyzed.[11]

Utilizing a UV-visible spectrophotometer to estimate aspirin

Stock Solution preparation

Standard stock solution of Aspirin was prepared by dissolving 10mg of Aspirin in 100ml of methanol which gives 100µg/ml.

Working solution preparation

In methanol, the aspirin standard stock solution (100µg/ml) was made. Methanol was used to dilute this solution, yielding several dilutions ranging from 10 to 90 µg/ml. Using a UV-visible spectrophotometer, the absorbance of these solutions was measured at 236 nm against methanol as a blank, and a standard curve was drawn against concentration. The intercept, slope, straight line equation, and correlation coefficient of the calibration curve were determined.

Identifying methanol absorption maxima

The drug was evaluated quantitatively using a double beam UV-visible spectrophotometer.

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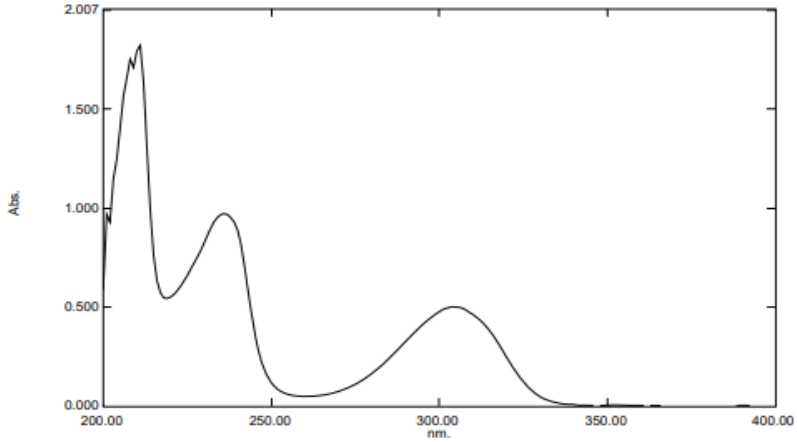
A 90 µg/ml aspirin solution in methanol was detected between 200 and 400 nm. The figure 3 indicates the aspirin's UV spectrum result. Aspirin's maximum wavelength was found to be 236 nm, in line with previous findings (table 3).



No.	P/V	Wavelength	Abs.	Description
1	↑	390.00	0.001	
2	↑	351.00	0.003	
3	↑	304.00	0.500	
4	↑	236.00	0.916	
5	↑	211.00	1.824	
6	↓	380.00	-0.003	
7	↓	347.00	-0.000	
8	↓	261.00	0.046	
9	↓	219.00	0.542	

Figure 4: Aspirin's UV spectrum in methanol

Table 3: Aspirin's absorption maxima (λ max) in methanol

Name of drug	**Absorption maxima (λ_{max})**	
	Observed	**Reference**
Aspirin	236	236

Table 4: Aspirin's calibration curve in methanol (λmax = 236 nm)

Concentration.(µg/ml)	**Mean ± SD**
10	0.135±0.001
20	0.242±0.002
30	0.342±0.001
40	0.442±0.001
50	0.532±0.001
60	0.620±0.001
70	0.728±0.001
80	0.829±0.001
90	0.916±0.001

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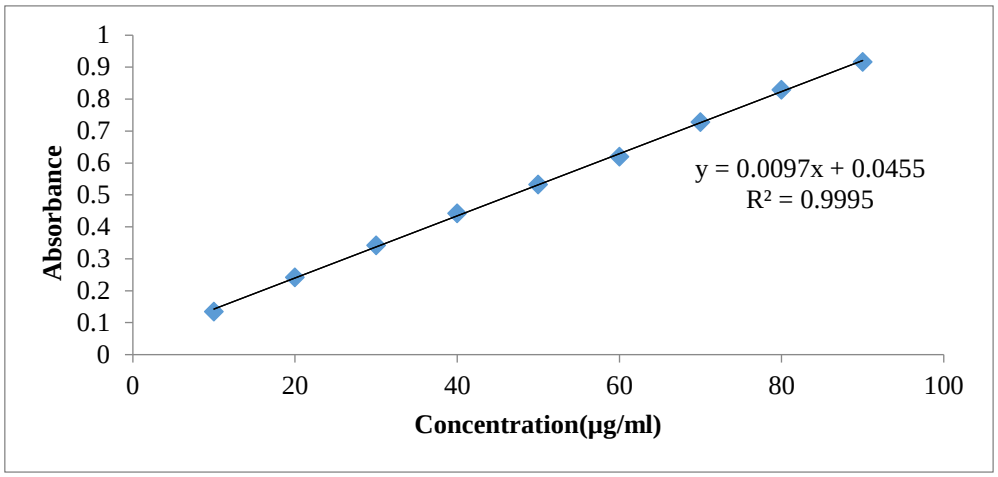

Figure 5: Aspirin's standard calibration curve in methanol

Table 5: Outcome of UV method's regression analysis

Statistical parameters	Results
λ_{max}	236 nm
Equation of regression (y = mx + c)	y = 0.0097x + 0.0455
Slope (m)	0.0097
Intercept (C)	0.0455
coefficient of correlation (R^2)	0.9995

Using aspirin concentrations ranging from 10 to 90 µg/ml in methanol, the calibration curve for aspirin was obtained. At 236 nm, the absorbance was measured. Table 5 and Figure 5 demonstrate the good linearity of the aspirin calibration curve which is represented by the regression equation y = 0.0097x + 0.0455 and R^2 value 0.9995.

Studies on solubility

Tests for drug solubility in various solvents were conducted to determine the constituents that would be employed during the formulation developing procedure.[12]

Tests for drug solubility in various solvents were conducted to determine the constituents that would be employed during the formulation developing procedure.12 For the quantitative solubility study results, the drug was put in thoroughly cleaned test tubes together with 1 ml of different solvents (methanol, ethanol, chloroform, 0.1N HCl, water, and simulated tear fluid). The test tubes were then sealed tightly. On a water bath shaker, these test tubes were shaken for a whole day at room temperature. The supernatant was extracted from each sample after it had been centrifuged for 24 hours at 15,000 rpm. The filtrates were then suitably diluted and quantified using spectrophotometry after the supernatant was filtered. Aspirin was examined with a UV spectrophotometer that was calibrated to 236 nm.

Aspirin is freely soluble in methanol, soluble in ethanol and chloroform, and slightly soluble in water, 0.1 N HCL, and Simulated TF Fluid (figure 6 and table 6), as can be observed from table 6.

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Table 6: Aspirin's solubility analysis in various solvents

Sr. No.	Name of Solvents	Solubility (mg/ml)	Solubility
1	Methanol	114.050±0.773	Freely soluble
2	Ethanol	56.057±0.773	Soluble
3	Chloroform	50.644±0.773	Soluble
4	Water	5.791±0.015	Slightly soluble
5	0.1 N HCL	3.997±0.015	Slightly soluble
6	Simulated TF Fluid	3.915±0.009	Slightly soluble

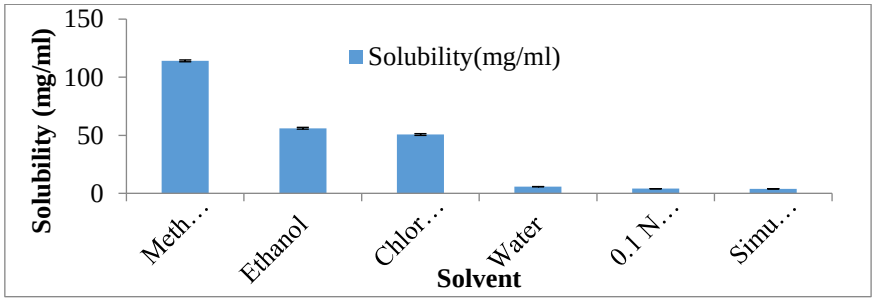

Figure 6: Aspirin solubility analysis in various solvents

Estimation of partition coefficient

The Aspirin's partition coefficient has been calculated using n-octanol and water. If a drug's log P value is more than one, it is lipophilic; hydrophilic medicines have partition coefficients that are less than one[13]. This proved the drug's purity and lipophilicity. Aspirin's partition coefficient in n-octanol:water was determined to be 1.36±0.039, which is consistent with the literature and suggests that the aspirin is lipophilic in nature (table 7).

Table 7: Aspirin's partition coefficient determination

Material	Solvent system	Log P	Standard
Aspirin	n-octanol:water	1.36±0.039	1.19

Aspirin's FTIR

The Fourier Transform Infrared (FT-IR) spectra of any given substance can be used to determine which groups are present in that particular substance.[14] For the structure analysis, FT-IR spectroscopy was employed. A sample of about 2-3 mg was taken, and it was scanned between 4,000 and 400 cm^{-1}**.** Aspirin's spectra showed the main IR absorption peaks at 1682.12 cm-1 (C=O stretching), 1456.73 cm-1 (O-H stretching), and 1012.54 cm-1 (C–O stretching).The primary peaks that were observed validated the purity of the aspirin.

FTIR Compatibility Study of Drug-Excipients

The drug's compatibility with the excipients was verified using FTIR. FTIR was used as a technique to identify any physical or chemical interactions between the active materials and the excipients. The drug was well mixed in a 1:1 ratio with several excipients. The samples were scanned in the 400–4000 cm-1 range using FTIR. The spectra of the drug without excipients and the drug with excipients were compared to search for any physical changes or incompatibilities.[15]

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The physical mixing and observed major peaks of the excipients attested to the genuineness and purity of the raw ingredients. **Technique for Formulation Preparation** The following two stages were taken in order to develop a thermosensitive aspirin in-situ gel: 1. Aspirin nanoparticle formulation Making a thermosensitive gel with drug-loaded nanoparticles in it

1. Formulation of Aspirin Nanoparticles[16]

A 0.1M hydrochloric acid solution was gradually mixed with an appropriate quantity of aspirin and chitosan (CS) powder to prepare the nanoparticles. Subsequently, chitosan solution was cooled in an ice bath to 4°C. Sodium Tripolyphosphate at various concentrations (0.5%, 1% and 2%) was dissolved in purified water and placed in an ice bath with the CS solution. Subsequently, the CS solution was subjected to vigorous agitation as the 1 mL Sodium Tripolyphosphate solution was included dropwise. The solution was constantly agitated for 30 minutes to create a CS/TPP solution. To obtain aspirin-loaded nanoparticles, the solution was then sonicated for 4 and 10 minutes at 40 amplitude (with the pulse set to be on time for 2 and 3 seconds off) using a probe-type sonicator at 100 W in an ice bath. After that, the nanoparticle dispersion was subjected to 4 °C probe sonication for every 5 minutes of cycle, with a 1-minute break in between each cycle to prevent excessive heat generation that could cause product degradation.

Table 8: Different trial designs to screen for the creation of nanoparticles.

Formulation code	Drug (mg)	Condition			
		Chitosan (mg)	Sodium Tripolyphosphate (%w/v)	Amplitude (%)	Time (min.)
F1	100	50	0.5	40	4
F2	100	100	0.5	40	4
F3	100	150	0.5	40	4
F4	100	200	0.5	40	4
F5	100	150	1.0	40	4
F6	100	150	1.5	40	4
F7	100	150	2.0	40	4
F8	100	150	1.0	60	4
F9	100	150	1.0	80	4
F10	100	150	1.0	60	10
F11	100	150	1.0	80	10

2. Preparation of thermosensitive gel incorporating drug loaded nanoparticles[17]

The cold method was used to prepare thermosensitive gel. In summary, the drug-loaded nanoparticles dispersion was gently mixed for three hours at 4°C with a slow addition of Carbopol 934 (0.3%, w/v) to allow it to hydrate. After adding Poloxmer 407 (15%, 18%, and 20% w/v) to the CP 934P solution, it was left to dissolve at 4 °C for the entire night. Poloxamer 407 and CP 934P were the polymers used in the development of the gel. Carbopols are popular gelling agents that have been demonstrated to possess mucoadhesion qualities. Poloxamer 407 is a thermosensitive polymer that demonstrates exceptional solubility in water, favorable drug release

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properties, minimal toxicity and irritation, and cross-compatibility with additional excipients.

Table 9: Composition of different formulation aspirin loaded nanoparticles in thermosensitive gel

Composition	Formulation Code		
	F10 (A1)	F10 (A2)	F10 (A3)
Aspirin nanoparticle dispersion (ml)	10	10	10
Carbopol 934 (%w/v)	0.3	0.3	0.3
Poloxmer 407 (%w/v)	15	18	20

Evaluations of Aspirin loaded nanoparticles[16]

Visual Appearance: The visual appearance of aspirin nanoparticle is shown in table 10 & table 11.The results indicate that formulating the nanoparticle show good appearance and no phase separation were produced in all batches. All trial formulations were selected for further evaluation based on a visual appearance test.

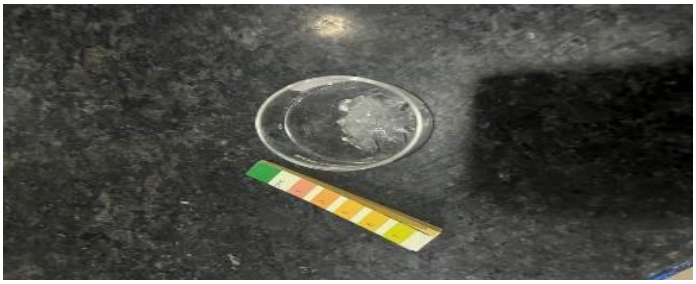

Figure 7: Visual appearance of aspirin nanoparticles

Table 10: Visual appearance of different nanoparticle formulations

Formulation code	Visual Appearance
F1	Homogeneous uniform dispersion
F2	Homogeneous uniform dispersion
F3	Homogeneous uniform dispersion
F4	Homogeneous uniform dispersion
F5	Homogeneous uniform dispersion
F6	Homogeneous uniform dispersion
F7	Homogeneous uniform dispersion
F8	Homogeneous uniform dispersion
F9	Homogeneous uniform dispersion
F10	Homogeneous uniform dispersion
F11	Homogeneous uniform dispersion

Drug entrapment efficiency/ Percentage Drug loading

Ten milliliters of phosphate buffer were used to soak 10 mg of known weight nanoparticles for thirty minutes. Centrifuging the prepared nanoparticles (NPs) was done. Methanol was used to centrifuge the supernatant. A UV spectrophotometer was used to measure the amount of unentrapped aspirin at the drug's λmax. Before measuring absorbance, samples taken from the supernatant were diluted. The supernatant provides us with the entire amount

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of unentrapped medication, free aspirin. When creating drug-loaded nanoparticles, drug loading is an essential factor to consider. Comparing different nanoparticle systems directly is very challenging because drug loading and encapsulation efficiency are defined differently in different studies. Drug loading = (amount of drug)/ (amount of nanoparticle) ×100 % Entrapment Efficiency = (Added drug-free drug)/ (Amount of drug added) ×100

Table 11: Aspirin nanoparticles drug loading and entrapment percentages

Formulation Code	Entrapment percentages	Drug loading Percentage
F1	43.12±0.231	30.23±0.195
F2	64.50±0.222	34.55±0.168
F3	86.54±0.170	35.86±0.146
F4	79.76±0.064	27.86±0.154
F5	87.87±0.064	36.21±0.209
F6	74.31±0.170	36.21±0.209
F7	60.72±0.222	30.46±0.215
F8	87.46±0.170	35.99±0.256
F9	86.87±0.170	35.41±0.118
F10	88.76±0.170	35.89±0.143
F11	87.17±0.111	35.82±0.269

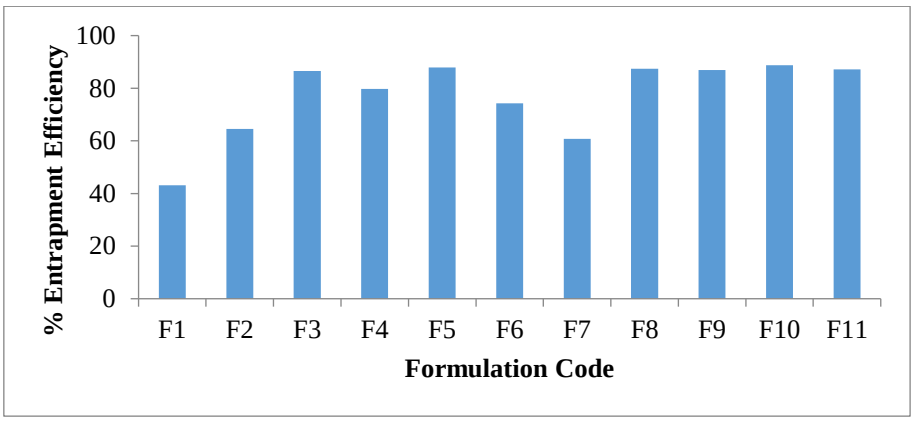

Figure 8: Aspirin nanoparticle drug entrapment percentage

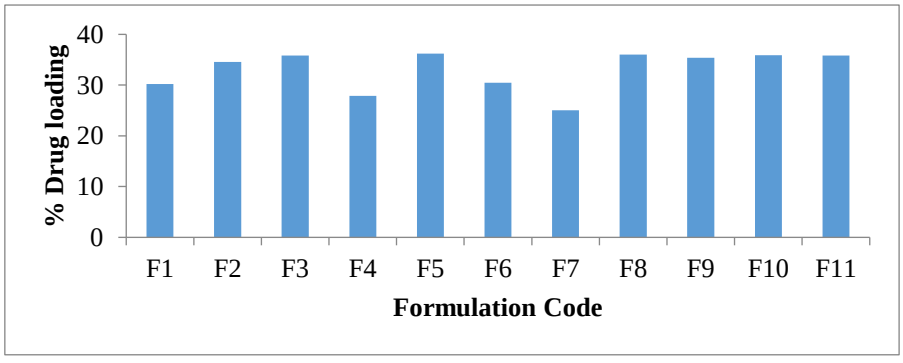

Figure 9: Aspirin nanoparticle drug loading percentage

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Table 11 showed that the range of drug entrapment percentages for all formulations was 43.12±0.231% to 88.76±0.170%. Formulation F10 showed the highest percentage of drug entrapment, measuring 88.76±0.170%. The best formulation, out of all the formulas, was determined to be F10. **Table 11** showed that the drug loading percentage for all formulations fell between 30.46±0.215% and 35.99±0.256%.

Percentage practical yield

Following filtration, the NPs were dried for a whole night in vacuum desiccators, and the weight that was obtained represented the practical yield of NPs. The theoretical yield is equal to the sum of the drug and the total amount of lipids.

Percentage practical yield = (Practical mass of NPs)/ (Theoretical mass) ×100

Table 12: Percentage yield of aspirin nanoparticles

Formulation Code	% Yield
F1	93.47±0.384
F2	91.44±0.762
F3	93.41±0.399
F4	92.07±0.508
F5	91.77±0.609
F6	90.39±0.828
F7	90.74±0.229
F8	92.30±0.230
F9	93.23±0.398
F10	93.63±0.398
F11	93.09±0.609

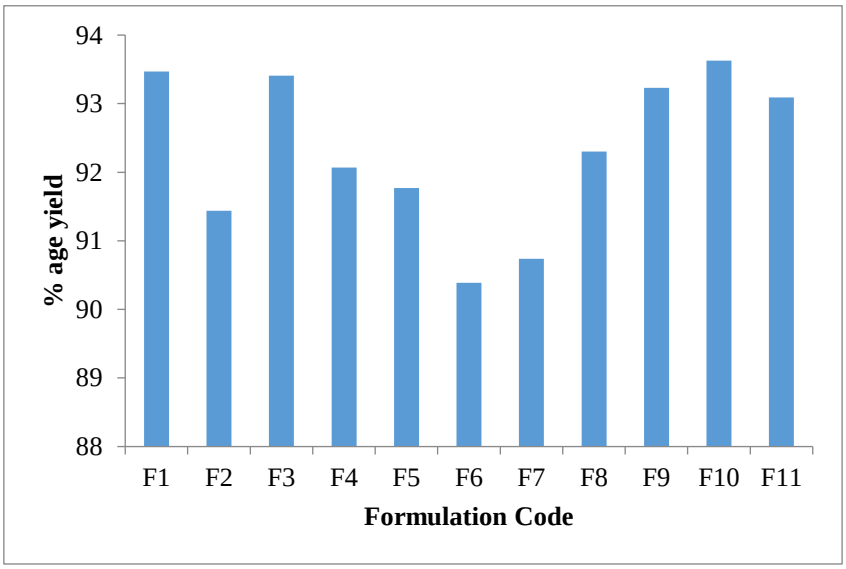

Figure 10: Percentage yield of aspirin nanoparticles

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Table 12 showed that each formulation's percentage yield fell between 90.39±0.828% and 93.63±0.398%.

Particle size and Zeta potential

Using a Zetasizer nano device, photon correlation spectroscopy, and electrophoretic mobility, respectively, the formulation's zeta potential and particle size were determined. To assess the zeta potential and particle size, the produced formulation was diluted before being put to the sample cell and placed in the sample holding unit.The aspirin formulation F10's particle size was determined to be 129.58 nm, and Figure 12 showed that the formulation's stability was indicated by the zeta potential of -15.6 mV for the formulation.

Particle size & Zeta Potential

Aspirin nanoparticles particle size

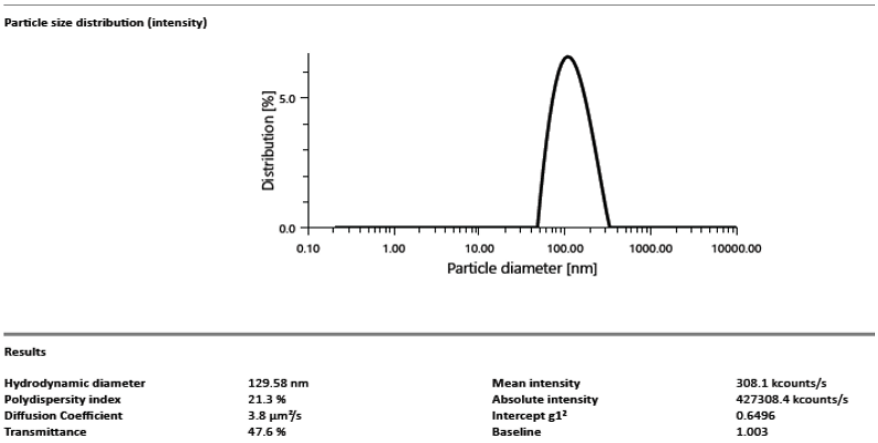

Figure 11 Particle size of aspirin nanoparticles formulation (F10)

Aspirin nanoparticles Zeta Potential

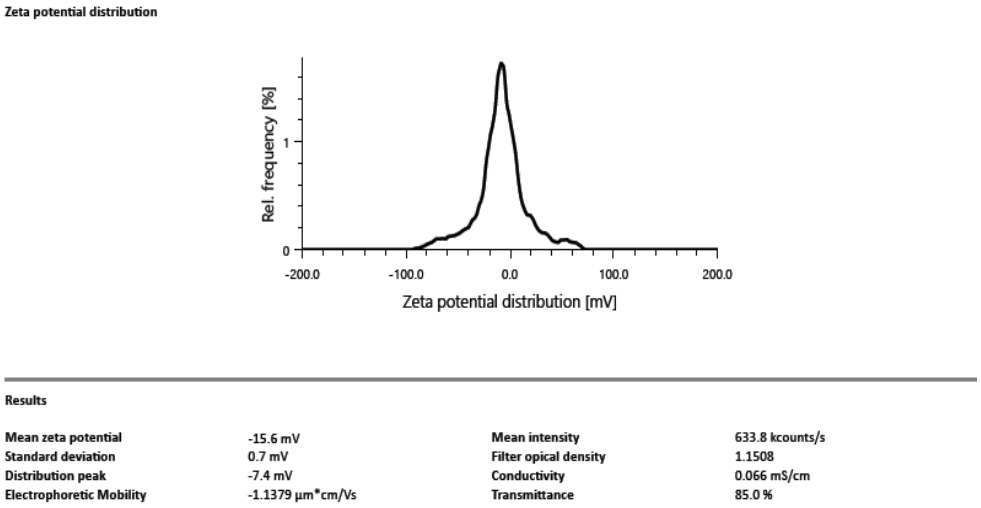

Figure 12: Zeta potential of the aspirin nanoparticles (F10)

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Evaluation of thermosensitive in situ gel[18-19]

Physical Appearance & Clarity

One of the most important factors in ophthalmic preparations is transparency. By visually examining each prepared formulation against a black and white backdrop, their clarity was assessed.

Visual inspection was done to make sure the manufactured gel had a smooth appearance and consistency. All of the developed formulations showed excellent homogeneity, no lumps, clarity, and free flow at room temperature. Therefore, the gel formulation F10 (A1) to F10 (A3) was chosen for further investigation.

Figure 13: Visual Appearance of thermo-sensitive in situ gel

Table 13: Visual appearance of thermo-sensitive in situ gel

Formulation Code	Appearance	Clarity
F10 (A1)	Free flowing liquid	Clear
F10 (A2)	Free flowing liquid	Clear
F10 (A3)	Free flowing liquid	Clear

PH determination

The pH of the gel formulations was measured using a pH meter. 1 g of gel was dissolved in 100 milliliters of pure water, and the pH of the mixture was measured using a pH meter. After coming into touch with the gel, the meter was allowed to acclimate for one minute. The pH was measured three times, and the averages were calculated.

Table 14: pH study of gel (F10 (A1)-F10 (A3))

Formulation code	pH (Mean ± S.D)
F10 (A1)	7.25±0.01
F10 (A2)	7.40±0.02
F10 (A3)	7.43±0.02

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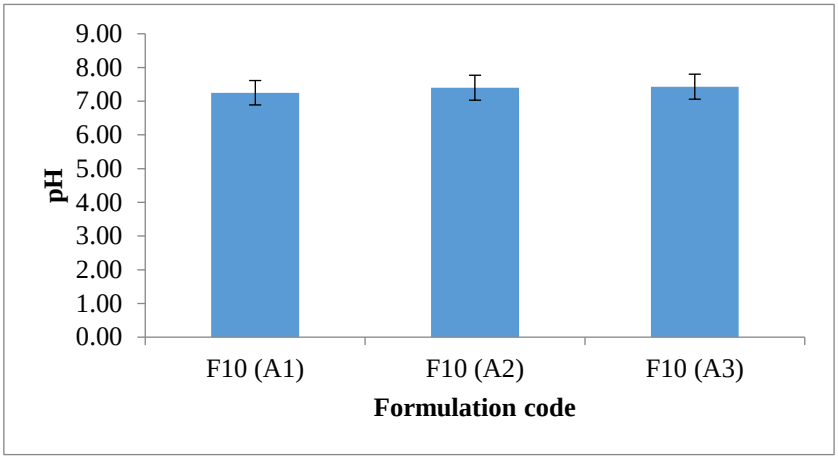

Figure 14: pH of gel (F10 (A1)-F10 (A3))

The physiological pH range for the eye is 7 to 7.4. All formulations' pH values were found to be between 7.25±0.01 and 7.43±0.02 (Table 14), which is within the physiological range of the eye and compatible with eye.

Gelling strength

25 gram ready-made formula placed in a 100 ml graduated cylinder by using a thermostat and the mixture was gelled at 37 °C.14 g weight is pierced through five centimeters of the gelled form. The gel strength is the amount of time required for penetration that is documented.

Table 15: Gel strength of formulations (F10 (A1)-F10 (A3))

Formulation code	Gelling strength (Seconds) (mean ± SD)
F10 (A1)	62.667±1.53
F10 (A2)	72.00±1.00
F10 (A3)	81.00±1.00

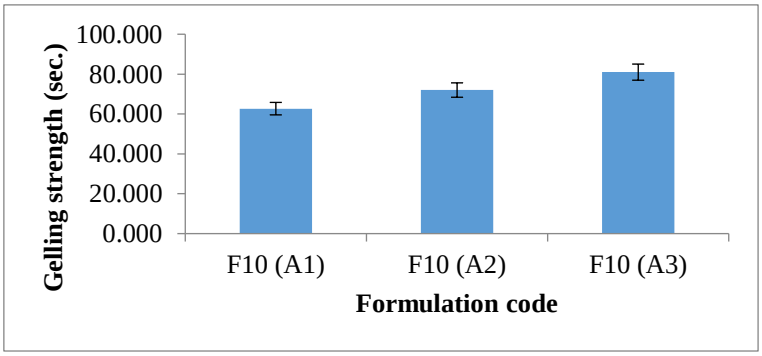

Figure 15: Gel strength of formulations (F10 (A1)-F10 (A3))

The gel's strength is measured at the eye's physiological temperature, which is 37°C ± 1°C. The gel formulations' observed gel strengths are displayed in Table 15. As Poloxamer 407 concentration increased, formulations' gel strengths gradually increased as well.

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Measurement of gel viscosity

A viscometer was used to measure viscosity. Gelling liquids were prepared in accordance with the earlier guidelines and heated to 37 °C in a water bath until full gelation was seen. Spindle T-4 was used to measure the viscosity at 10 RPM. Every measurement was conducted thrice, and the mean ± standard deviation is given**.**

Table 16: Viscosity Study of formulations (F10 (A1)-F10 (A3)

Formulation code	Viscosity at non physiological condition (cps)
F10 (A1)	147±7.09
F10 (A2)	193±7.57
F10 (A3)	255±1.00

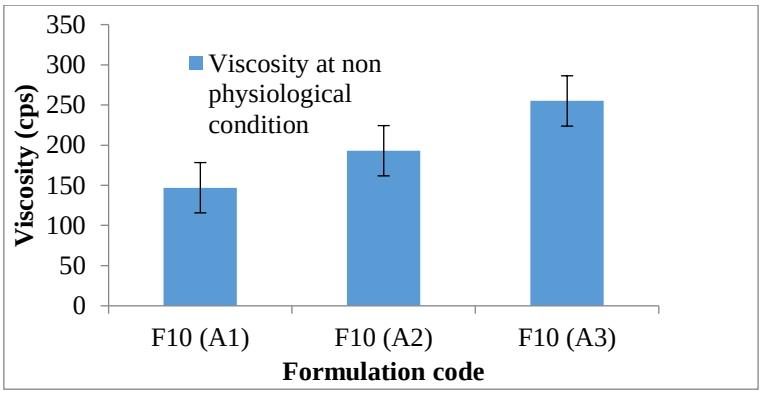

Figure 16: Viscosity study of formulations (F10 (A1)-F10 (A3))

Table 16 lists range of viscosity under non physiological conditions was first tuned for pourability and quick drug release.

Drug content

The gel formulation, which is around to 30 mg of drug was taken and prepared 100 ml freshly Simulated Tear Fluid (Composition: Nacl-0.67 g, NaHCO3 -0.20, CaCl2, 2H2 O-0.008 g, and distilled water to 100 ml). One milliliter was collected and diluted with STF to get ten milliliters. The absorbance was measured with a UV-Visual spectrophotometer, with STF serving as the blank.

Table 17: Drug content of formulations (F10 (A1)-F10 (A3))

Formulation code	% Drug Content
F10 (A1)	98.580±0.428
F10 (A2)	96.852±0.741
F10 (A3)	97.716±0.566

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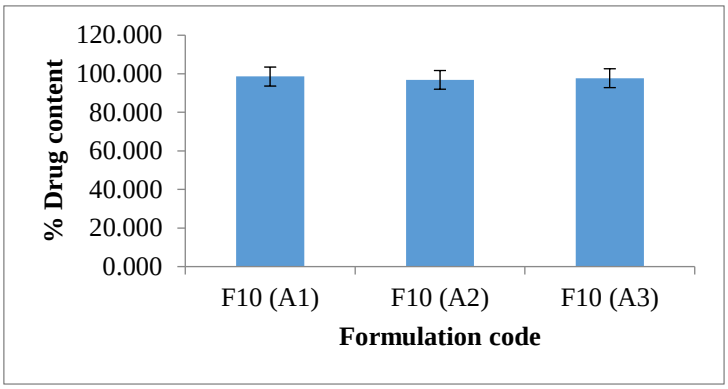

Figure 17: Drug content of formulations (F10 (A1)-F10 (A3))

The gels containing drugs had respective contents of 96.852±0.741% and 98.580±0.428%. It was determined that all formulations had a satisfactory percentage of drug content. Thus, it was determined that the technique used for gel formulations was appropriate.

Gelation temperature (GT)

Ten milliliters of the sample solution were placed in a transparent beaker on a water bath. The complete assembly was mounted on a magnetic stirrer, and the temperature of the water bath was progressively increased while stirring constantly at the lowest feasible speed. The gelation temperature is defined as the temperature at which the magnetic bar no longer moves due to gelation. Three measurements were taken for each sample, and the average ± standard deviation is shown.

Table 18: Gel temperature of formulations (F10 (A1)-F10 (A3))

Formulation code	Gelation Temperature oC (Mean ± S.D)
F10 (A1)	45.93±0.14
F10 (A2)	39.67±0.32
F10 (A3)	35.83±0.22

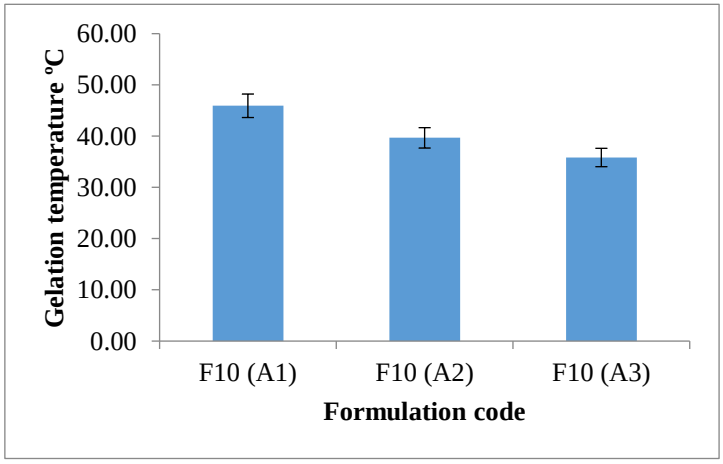

Figure 18: Gel temperature of formulations (F10 (A1)-F10 (A3))

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FTIR Spectra:
The final formulation's FTIR spectrum was evaluated using FTIR spectroscopy.

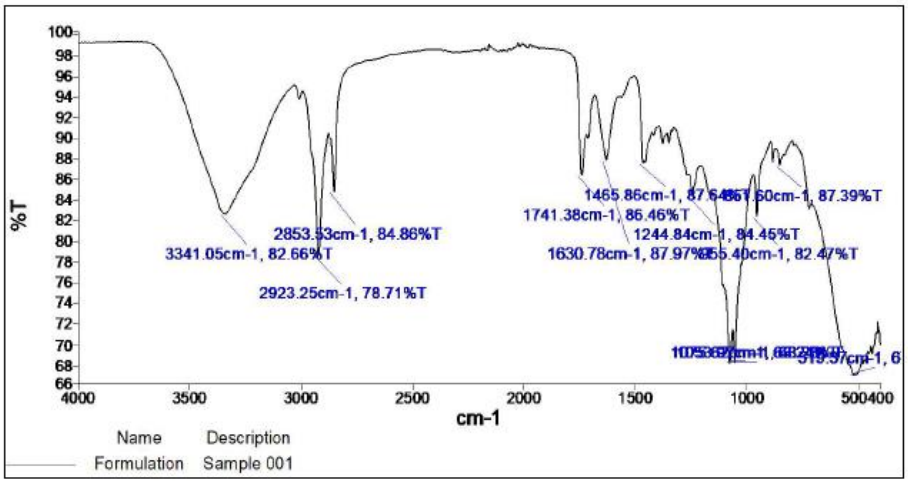

Figure 19: Infrared spectra of formulation F10 (A1)

Table 19: Interpretation of formulation F10 (A1)

Peak	Reference (cm^{-1})	Observed (cm^{-1})
N-H stretching	3291	3341.05
C=O stretching	1692	1630.78
stretching of the C-O-C bridge	1028	1075.62

Figure 19 and table 18 show the spectra of formulation F10 (A1), with peaks at 3341.05cm-1 (N-H stretching), 1630.78cm-1 (C=O stretching), and 1075.62cm-1 (stretching of the C-O-C bridge). The Infrared spectra of final formulation F10 (A1) showed some aspirin peaks with modest shifts.

In-vitro study of drug release
The dialysis bag approach was used in the in vitro studies. In dialysis bags, only one milliliter of formulation was used. Simulated tear fluid (40 mL; 6.78 g sodium chloride, 1.38 g potassium chloride, 2.18 g sodium bicarbonate, 0.084 g calcium chloride dihydrate, and 1000 mL pure water) was utilized as a releasing medium. To replicate the eye temperature, the shaking temperature was maintained at 35±0.5°C and the stirring rate was set to 50 rpm. At intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 hours, the samples (3 mL) were taken from the release media and substituted with an equal volume of brand-new artificial isotonic tear solution. Aspirin concentration was determined using a UV spectrophotometer. The percentage of cumulative release was determined, and a graph of percent cumulative release vs time was plotted. The receptor phase was replenished with an equal volume of simulated tear fluid at each time of sample withdrawal.

Table 20: Aspirin nanoparticle loaded thermo-sensitive in situ gel in vitro release

Time (Hr)	Drug Release: Pure Drug (%).	Drug Release: Formulation F10 (A1) (%)
0	0	0
0.25	1.830±0.068	14.037±0.093

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0.5	5.415±0.068	20.319±0.143
1	11.030±0.112	26.200±0.089
2	18.096±0.093	32.667±0.444
3	22.689±0.118	36.963±0.679
4	29.667±0.089	42.741±0.257
5	32.022±0.118	49.704±0.679
6	40.222±0.089	56.104±0.185
8	40.741±0.257	61.793±0.136
10	40.704±0.679	73.296±0.679
12	40.504±0.679	73.148±0.679

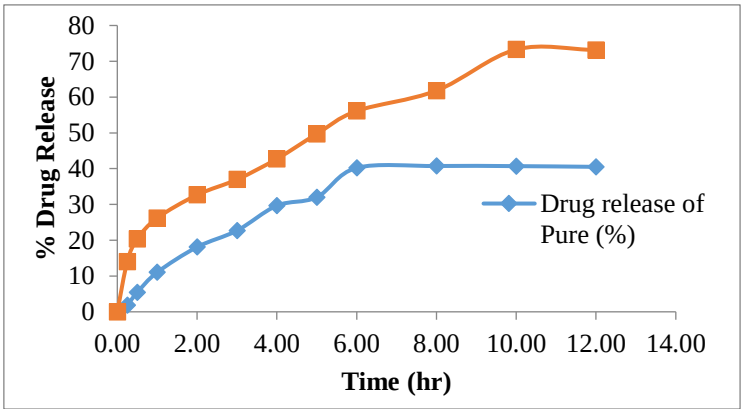

Figure 20: Percentage drug release of Aspirin nanoparticle loaded thermo-sensitive in situ gel

Table 20 and Figure 20 show the in-vitro drug release of Aspirin nanoparticle-loaded thermosensitive in situ gel and pure drug. When compared to the release of pure drugs, the formulations significantly prolonged drug release. The pure drug release rate was 40.504±0.679 in 12 hours. Formulation gel F10 (A1) had a release rate of 73.148±0.679% in 12 hours. As a result, it can be shown that the release formulation F10 (A1) provides a sustained drug release.

3. Summary and Conclusion

Aspirin is both an NSAID and a salicylate. It can also be used to treat arthritis by reducing pain and swelling. It enhances the osteogenic and anti-inflammatory characteristics of human mesenchymal stem cells.-1 Peptide-decorated substrates.

Prior to nanoparticle development, preformulation experiments were conducted. The infrared spectra of drug samples was found to be consistent with the reference chemical groups found in the structure of Aspirin. The UV spectra of Aspirin in methanol showed a wide band at 236 nm. The melting point was calculated using the capillary method, which agrees with the melting point mentioned in the reference.

The solubility results showed that Aspirin freely soluble in methanol and soluble in ethanol and chloroform and slightly soluble in water, 0.1N HCl and simulated TF fluid. The solubility profile of the drug in several solvents indicates that it is lipophilic in nature, which is supported by the partition coefficient analysis. Aspirin standard curves were produced in methanol, and the absorbance data acquired were analyzed using linear regression. The correlation coefficients for aspirin were found

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to be 0.999, which is close to one, indicating good linearity. The preformulation investigation results indicated that Aspirin was pure and of good quality, and the estimation process was deemed to be correct and appropriate for formulation development.

A nanoparticle formulation of aspirin was produced using the nanoencapsulation process. For nanoparticle optimization, numerous formulations (F1 to F11) were produced with varying excipient amounts. All batches of nanoparticles produced a good look with no phase separation dispersion. The percentage of drug entrapment in all formulations ranged from 43.12±0.231 to 88.76±0.170%. Formulation (F10) with optimum entrapment efficiency, good look, and size is regarded an optimal formulation. The shape of the optimized F10 formulation was checked by measuring particle size with a particle size analyzer, which revealed that the majority of the particles were accurately identified.

The best formulation F10 was chosen to prepare a thermosensitive gel for ocular medication delivery with varying concentrations of Poloxamer 407 and Carbopol 934P.The gel was checked visually for consistency and determined to have an even glance. All of the developed formulations were free-flowing liquids at room temperature, transparent, particle-free, and exhibited good uniformity with no lumps. The pH of the produced gel was found to be between 7.0 and 7.4, which is within the physiological range of the eye and will not cause irritation when administered. Gel strength is evaluated at the eye's physiological temperature (37°C ± 1°C). Gel strength for the gel compositions is within range.

Drug content in gel formulations ranged from 96.852±0.741 to 98.580±0.428%.Poloxamer 407 15% concentration in Aspirin-loaded nanoparticles gel gelled at 45.93±0.14°C, closer to human temperature, according to stirring magnet bar experiments. The dialysis bag method was used to investigate the medication release from gel formulations. The improved formulation beat the gel formulation by a large margin, achieving a maximum of 73.148±0.679 in only 12 hours. The aspirin nanoparticle-loaded thermosensitive gel was found to be an affordable and repeatable technique that offered good appearance and entrapment efficiency.

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Conflict of Interest: Nill

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