INVESTIGATION OF ION-ACTIVATED IN SITU GELLING SYSTEM OF TIMOLOL MALEATE FOR GLAUCOMA THERAPY

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Article History: **Received: 28.03.2023** **Revised: 20.04.2023** **Accepted: 06.05.2023**

Abstract:
Introduction- Glaucoma is a condition where a series of progressive visual neuropathies are identified by the cavity of the optic disc, the apoptotic degradation of retinal ganglion cells (RGC), and the consequent loss of vision. Timolol maleate is a non-selective beta-adrenergic antagonist which is used mainly as an ocular preparation to treat glaucoma and ocular hypertension.
Methods- Three formulations were formulated by ion activated in situ gel system. The formulation was prepared by changing the concentration of gelling system. Gellan gum concentration used were 0.5% for F1, 0.8% for F2, 1% for F3 formulation. These formulations were evaluated.
Results- FTIR spectra showed all the functional groups represent in the compounds and there was no physical incompatibility observed. F1 formulation was found to have a good drug content of 87.3%. Gelling capacity was found to be good in F1 formulation. All formulations had good viscosity around 2300 to 3400 and all the formulations were found to be sterile.
Conclusion- the prepared formulation F1 was sterile, with good viscosity, maximum drug concentration and with good gelling capacity.

Keywords: In-situ gel, timolol maleate, glaucoma, gellan gum and formulation.

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DOI: 10.48047/ecb/2023.12.si5a.0387

INTRODUCTION:

Glaucoma: Glaucoma is a series of progressive visual neuropathies identified by the cavity of the optic disc, the apoptotic degradation of retinal ganglion cells (RGC), and the consequent loss of vision. Axons, which make up the optic nerve, are used by retinal ganglion cells to send visual information to the brain. Retinal ganglion cell axons congregate in the optic disc, turn 90 degrees, and infiltrate the sclera and lamina cribrosa (highly structured, multilayered, perforated connective tissue loaded with astrocytes) before emerging as the optic nerve from the globe. The cup is the optic disc's central depression[1]. In glaucoma, the Lamina cribrosa is damaged and loss of retinal ganglionic cell axons which results in progressive enlargement of the cup. Increased intraocular pressure damages the lamina cribrosa, which results in loss of the normal structure and metabolic support of the RGC axons and damaged axoplasmic transport[(2.3)]. Apoptosis occurs when neurotrophic signalling to the RGC is reduced. The only method that has been shown to stop or reduce glaucoma progression is intraocular pressure reduction. The equilibrium of aqueous humour production, aqueous humour outflow, and episcleral venous pressure determines intraocular pressure. The ciliary body generates aqueous humour, which supports the metabolic activities of the avascular tissues in the anterior portion of the eye. Although 21 mm Hg is frequently cited as the upper limit of normal intraocular pressure, many glaucoma patients have intraocular pressures that are lower than this, and not all people with higher intraocular pressures (also known as ocular hypertension) go on to develop glaucoma[(4,5)].It's crucial to consider intraocular pressure as a constant risk factor throughout the overall physiologic range. The onset and evolution of the disease may also be influenced by poor optic nerve perfusion. There are several methods for estimating ocular perfusion pressure (OPP). It is, in general, the distinction between intraocular pressure and systemic blood pressure.

Ophthalmic in-situ gel: In-situ gels for the eyes are made up of polymers that are sensitive and will have their structure changed by even little variations in the environment's pH, temperature, and ionic strength. When instilled into the eye, in-situ forming gels are liquids that quickly undergo gelation in the eye to generate viscoelastic gels in reaction to environmental changes. Release the medication gradually under physiological conditions last[6]. As a result, the in-situ gel's residence duration will be prolonged and the drug will be released gradually. This will increase bioavailability, reduce systemic absorption, and allow for less frequent dosage, all of which will improve patient compliance[7]. Additionally, in-situ gelling systems have demonstrated several additional potential benefits such as an easy manufacturing process, convenience of administration, and delivery of a precise dose.Several mechanisms, such as those involving temperature, pH, and ion-activated systems, can result in in-situ gel formation[8].

Temperature-triggered in-situ gel systems: The oldest, most widely studied, and most prevalent kind of stimuli-responsive gel is temperature-sensitive in-situ gel. Without causing discomfort or blurred vision, can be precisely and simply injected into the eye in liquid form. After administration, the gel is created at precorneal temperature (35 °C) to withstand lachrymal fluid dilution without causing rapid precorneal drug removal[9]. A good thermo-responsive ocular in-situ gel has been advised to have a gelation temperature above room temperature and undergo gel-sol transition at a pre-corneal temperature to avoid being stored in a refrigerator before installation, which could occasionally cause eye irritation due to the cold nature of the gel. Chitosan- Chitin, which is present in the exoskeletons of arthropods like crustaceans, is converted into the amino polysaccharide chitosan through partial deacetylation and depolymerize- tion[10].

pH-triggered in-situ gelling systems: This in-situ gelling system uses polyelectrolytes with acidic (carboxylic or sulfonic) or basic (ammonium salts) groups that receive or release protons in response to changes in the pH of the environment. The formulation exists as a regular solution at 4.4 pH, however, at pH 7.4, the pH of tear fluid goes through gel formation. Polyacrylic acid, polycarbophil, and cellulose acetate phthalate are the pH-responsive polymers used in ophthalmic preparation the most frequently (CAP)[11]

Ion-activated in-situ gel system: Ion-activated in-situ gelling systems create a crosslink with the Na+, Ca2+, and Mg2+ cations found in tear fluid, leading to the formation of a gel on the ocular surface and extending corneal contact time[12].

Polymers used in Ion-activated in-situ gel system: Gellan gum (Gelrite®), hyaluronic acid, and sodium alginates are the three most often used

ion-activated polymers in ocular formulations[13]. Gellan gum: Polysaccharides called Gellan gum can be utilized to create ion-sensitive hydrogels. It is a tetra saccharide repeating unit comprising glucose, glucuronic acid, and rhamnose in the ratio of 2:1:1 and is a linear anionic heteropolysaccharide (Fig. 5). Gellan has hydroxyl and carboxylic functional groups, which may engage in hydrogen bonding or electrostatic interactions with other polymers[14]. Gelrite®, a low-acetyl Gellan gum, is commonly marketed and it undergoes gelation in the presence of mono- or divalent cations[15]. When injected as a liquid solution into the cul-de-sac, the electrolytes in the tear fluid, particularly the Na+, Mg2+, and Ca2+ cations, are specifically known to cause gel formation of the polymer[16].

Timolol maleate is a non-selective beta-adrenergic antagonist which is used mainly as an ocular preparation to treat glaucoma and ocular hypertension. The most effective and safest medication for decreasing intraocular pressure is timolol maleate, which is given once or multiple times daily through conventional eye drops[17]. In the case of primary open-angle glaucoma, it has unique antioxidant properties with an impact on the trabecular meshwork cells of the ocular tissues[18]. The in-situ gelling system is one of the widely studied methods that could prolong the precorneal resident time and provide sustained release of the drug, thereby improving optical bio-availability, therapeutic response and lowering systemic absorption and toxicity of the drug. Additionally, because of its ability to sustain the release of drugs and reduced number of administrations, the patient compliance of in situ gel can be improved[19].

MATERIALS AND METHOD

Preparation of Simulated tears fluid

Accurately weigh 0.67g of NaCl, 0.2g of $NaHCO_3$, 0.008g of $CaCl_2$and dissolve in a little amount of distilled water and make up the volume up to 100ml[20].

a) Appearance: The color and appearance of the timolol maleate were assessed[21].

b) Determination of the Melting Range: The capillary method was used to estimate the drug Timolol Maleate's melting point. A capillary tube was taken and sealed on one edge of it using heat. The drug was filled to a height of 2.5–3 mm from the closed edge to the open end. The thermometer and tubing were both placed inside the melting point apparatus. Temperature knob was turned on, and as the temperature increased, the range was recorded as decreasing. It was noticed at what temperature; the drug entirely melts[22].

c) Drug estimation by spectroscopy: Timolol maleate standard plot preparation in STF (pH-7.4) calculating the maximum wavelength A glass funnel was used to transfer 10 mg of timolol maleate, which had been precisely measured on an analytical balance, into a 100 ml flask[23]. The simulated tear fluid is used to make up the volume to adjust the pH to 7.4. This is called a stock solution. The stock solution and buffer were used to prepare concentrations of 100 µg/ml and 10µg/ml. These solutions were put in the cuvettes, and the solutions' spectra between 200 and 400 nm were observed[24].

d) FTIR: By employing the Shimadzu FTIR 8400S model, IR Spectroscopy was used to determine whether the drug and its excipients were compatible. According to the formulation, drug and excipient mixes were created. The drug's spectrum peaks were compared with those of other formulation ingredients that had distinctive spectral peaks. (John RD.2007; Robert M S and Francis S W, 6th edition)[25].

e) UV-Spectrophotometry: For a week, all of the ingredients were held at high temperatures and room temperature after being combined according to the necessary weights and ratios (500C and respectively). They are removed after a week, dissolved in appropriate solvents, and examined with a UV Spectrophotometer[26].

Pre-formulation studies:

2. Formulation of Timolol maleate in situ gel

Table No. 3:- Formula for the prepared In-situ gel.

Ingredients	F1	F2	F3
Timolol Maleate	0.25%	0.25%	0.25%
Chitosan	0.2%	0.2%	0.2%
Gellan gum	0.50%	0.80%	1.0%
Buffer (Sodium citratedihydrate)	0.29%	0.29%	0.29%
Benzalkonium chloride	0.01%	0.01%	0.01%
Purified water	(q.s) 100%	(q.s) 100%	(q.s)100%

Timolol maleate eye drops are often administered as a 0.25–0.5% w/v drug solution for antiglaucoma action. As a result, 0.25 per cent of the dose was used to make the formulation. Timolol maleate in the prescribed quantity was added to a volumetric flask and dissolved in distilled water[27].
As a preservative, benzalkonium chloride was added in an amount of 0.1 per cent. The formulation's osmolarity was measured using an osmometer (Fiske Associate, USA). The solution was then put into amber-coloured bottles, each of which had a fitting top and a dropper with a teat. The resultant solutions were autoclaved for 20 minutes at 121°C, and 15 psi to sterilize them[28]. 3 formulations were prepared with different combinations of Gellan gum. Formulation F1 was prepared with 0.5g of Gellan gum, F2 was prepared with 0.8g of Gellan gum and F3 was prepared with 1g of Gellan gum.

Timolol maleate eye drops are often administered as a 0.25–0.5% w/v drug solution for anti-glaucoma action. A specified amount of Gellan gum was added to 100ml ultra-pure water in a beaker and it is kept in a magnetic stirrer. After the Gellan gum was dissolved completely, chitosan was added in an amount of 0.2g and dissolved. Subsequently, 0.9g of sodium chloride and 0.029g of sodium citrate dihydrate (buffer) were added after dissolving each ingredient[29]. After obtaining a clear solution, a prescribed amount of timolol maleate drug was added to the formulations. As a preservative, benzalkonium chloride was added in an amount of 0.01%. The solution was then put into amber-coloured bottles, each of which had a fitting top and a dropper with a teat. The resultant solutions were autoclaved for 20 minutes at 121°C, and 15 psi to sterilize them.

3. Evaluation of formulated in situ gel

The prepared *in situ* gel solution was assessed for appearance, pH, clarity, gelling capacity, and drug content estimation

a) Clarity, pH, Appearance: By visually observing the formulations under light alternately against the B&G background, it was possible to compare the clarity of the formulations before and after gelling. The prepared formulation was placed in a flask, and 1M Sodium Hydroxide was added drop by drop while stirring continuously to determine the gelation pH. Equiptronics' digital pH metre was used to measure pH, and Brookfield's RVT model viscometer was used to measure viscosity at a speed of 20 rpm in a small volume adaptor. The pH that caused an abrupt shift in viscosity was documented as the gelation pH[30].

b) Gelling capacity: By adding a drop of the created formulation to a vial containing 2 ml of freshly made simulated tear fluid and visually observing the results, the gelling capability of the formulation was determined. Its gelling process' duration was recorded.

c) Rheological studies: The Brookfield viscometer LVDV-E model was used to measure viscosity. The sampling tube contained the in-situ gel formulations. Before each measurement, the samples were examined by a circulating bath attached to the viscometer adaptor at 37°C +/- 0.5°C. [7–10] The spindle's (Spindle number- 64) angular velocity was increased by 1 to 4 while the formulation's viscosity was assessed[31].

d) Drug content estimation: 1ml of the formulation was taken in 10ml of STF to make a concentration of 0.25µg/ml. the drug content was then analyzed by using a UV visible spectrophotometer against the blank reagent at an absorbance of 296nm.

e) Sterility tests: By using the cup plate technique, an agar diffusion test was used to measure antimicrobial activity. Through a solid agar media, the drug was allowed to diffuse. The developed formulations containing timolol maleate and the control formulations were made with the standard minimum inhibitory concentration (MIC) of 2 µg/ml. The above medium, which is kept at a temperature of 52°C to 58°C, received 0.5 ml of the microbe suspension after being prepared and sterilized in an autoclave at 15 lb/sq-inch pressure for 18 minutes. This will be carried out in sterile circumstances. Each Petri plate was filled with 20 ml of the microbial agar suspension immediately. The created formulations were diluted appropriately with sterile distilled water to form test solutions, which were then added to the cup of sterile nutrient agar Petri plates once the medium had solidified. An earlier seeding of test organisms was done here (Escherichia coli and Staphylococcus aureus).
The agar plates were incubated at 37°C for 24 hours after allowing the solutions to diffuse for two hours. Each cup's Zone of Inhibition (ZOI) was measured and compared with a control. A laminar airflow device was used for the entire process. Three copies of each formulation solution were tested. Throughout the investigation, both positive and negative controls were maintained[32].

RESULTS AND DISCUSSION

A. Pre-formulation studies

1. Appearance: The characteristic physical appearance of timolol maleate was found to be a white-colored, odorless, crystalline powder as shown in table 4.

Table No. 4:- Physical evaluation of Timolol maleate

Formulation	Colour	Odour	Nature of powder
Timolol maleate	white	Odourless	crystalline

2. Determination of melting point:

Table No. 5: - Melting point of ingredients

Sl. no	compounds	Melting point range	Actual melting point
1	timolol	203-205	203
2	chitosan	214-216	214
3	Gellan gum	70-80	73
4	Sodium citrate dihydrate	150-160	152

The melting point of all the active Ingredients was done by the capillary method.

All the ranges of the melting point of compounds used in the formulation were the same as that in the literature.

3. Standard calibration

Table No.6: - Standard calibration data of Timolol Maleate

Concentration(µg/ml)	Absorbance(nm)
0	0
0.2	0.171
0.4	0.361
0.6	0.569
0.8	0.798
1	0.99

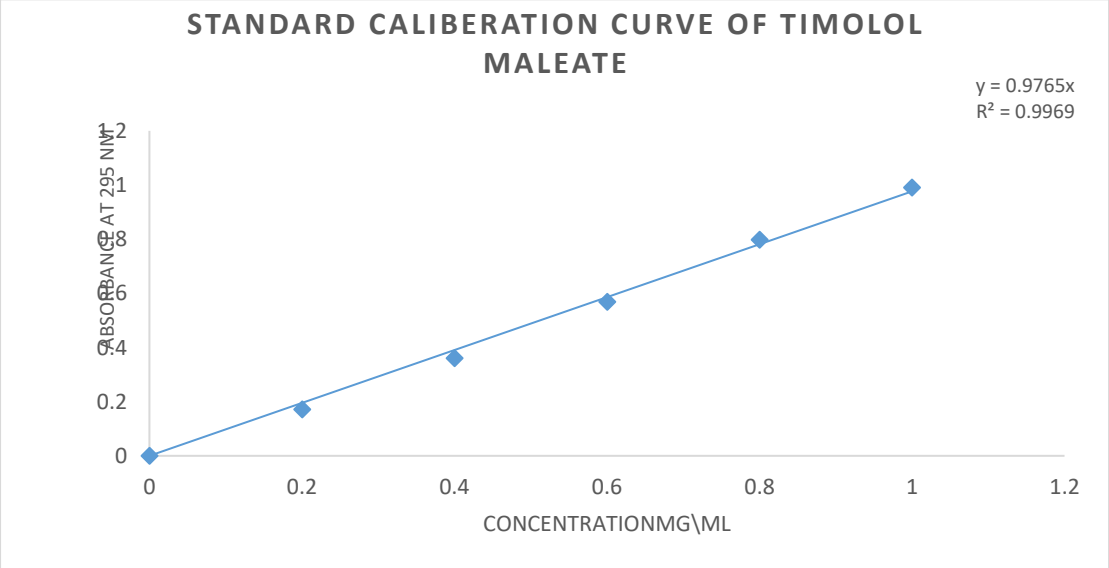

Figure 2: - Standard calibration curve of timolol maleate in STF buffer (pH- 7.4)

4. FTIR spectroscopy

The chemical structure of the samples of the formulated in-situ gel was investigated by using Fourier Transform Infrared spectroscopy. The FTIR spectrum was recorded over the range of 600 - 4000 cm-1.

Table. No. 7: - Interpretation of FTIR spectra of the compounds in the formulation

Sl.no	Functional groups	Assessment of peaks 1	Assessment of peaks 2	Assessment of peaks 3	Assessment of peaks 4	Assessment of peaks 5	Assessment of peaks 6	Assessment of peaks 7
1	C=O stretching	1390	1068.60	1190.08	1089.62	1675.95	1460.12	1119.74
2	O-H stretching	3461	3365.94	3350.92	3415.34	3290.99	3385.31	3380.85
3	C-H stretching	2825	2939.6	2895.89	2941.86	2945.43	2853.14	2897.86
4	C=N stretching	1574	2365.14	1634.07	1556.93	1568.46	1637.39	1589.6

5	N-H stretching	3399	3457.43	3549.09	3386.04	3426.88	3454.38	3347.52

From the interpretation of the FTIR study, it was confirmed that the drug Timolol Maleate was compatible with the components used in the formulation of in-situ gels. This shows that there is no physical incompatibility and chemical interaction between the drug Timolol Maleate and the components used. FTIR spectra of Timolol Maleate, other ingredients and physical mixture are represented in the following figures.

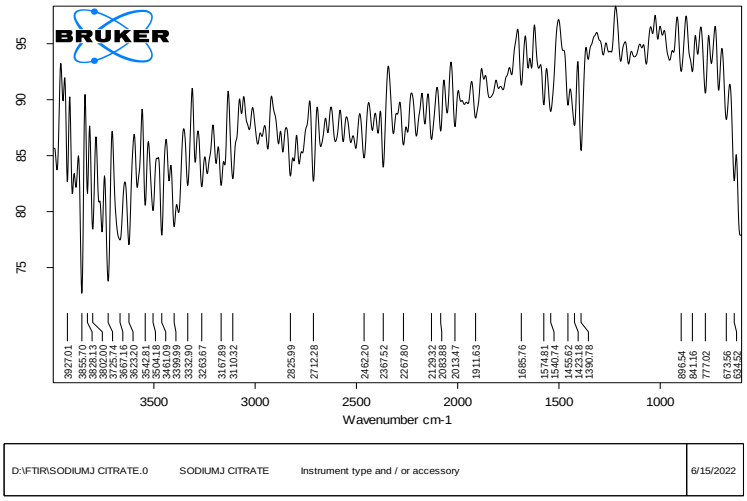

Figure 3: - FTIR spectra of sodium citrate

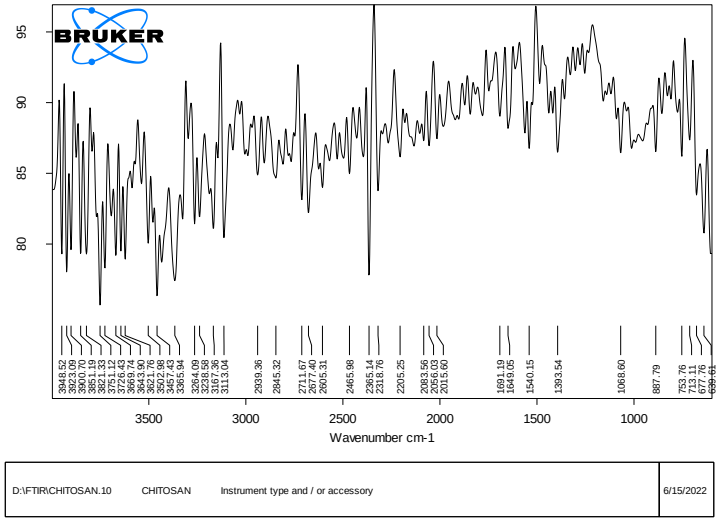

Figure 4: - FTIR spectra of chitosan

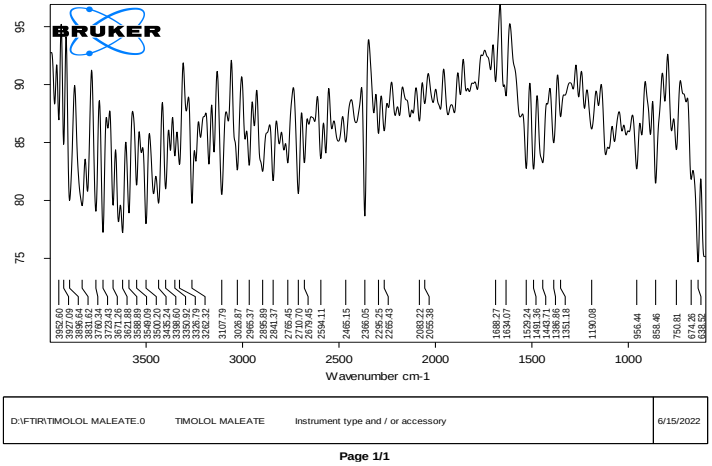

Figure 5: - FTIR spectra of Timolol maleate

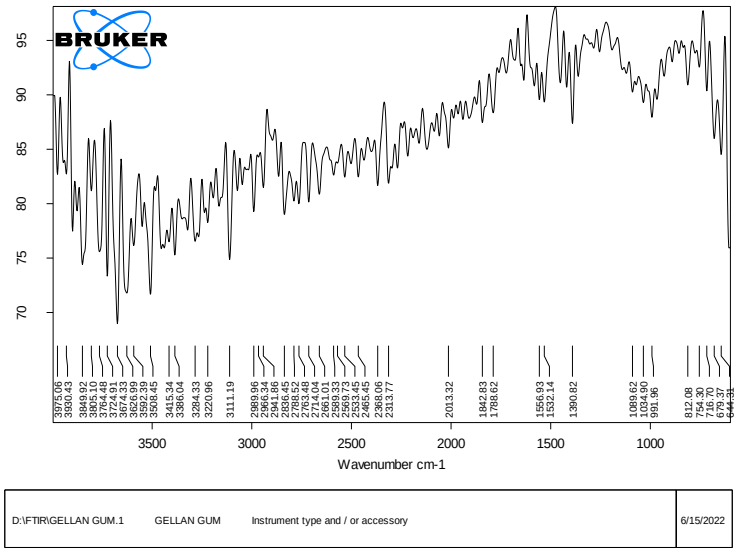

Figure 6: - FTIR spectra of Gellan gum

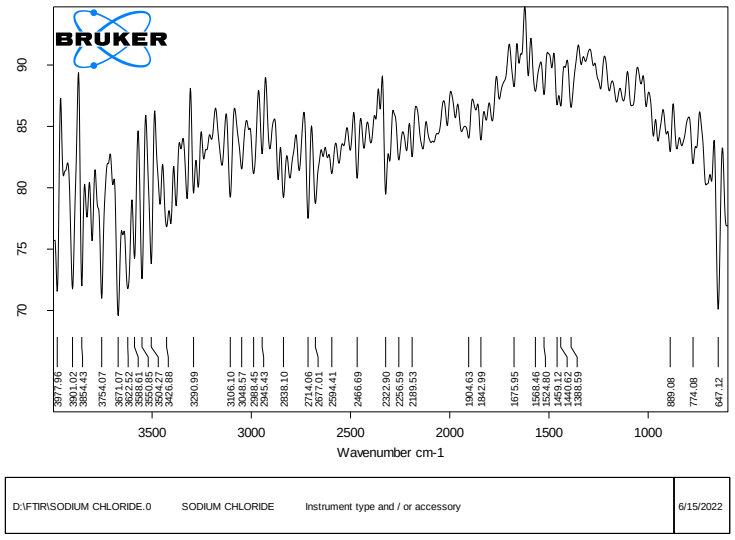

Figure 7: - FTIR spectra of Sodium chloride

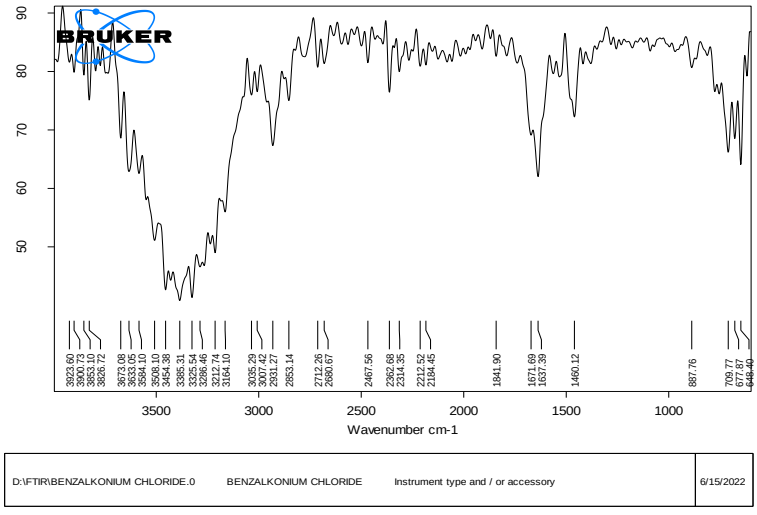

Figure 8:- FTIR spectra of Benzalkonium chloride

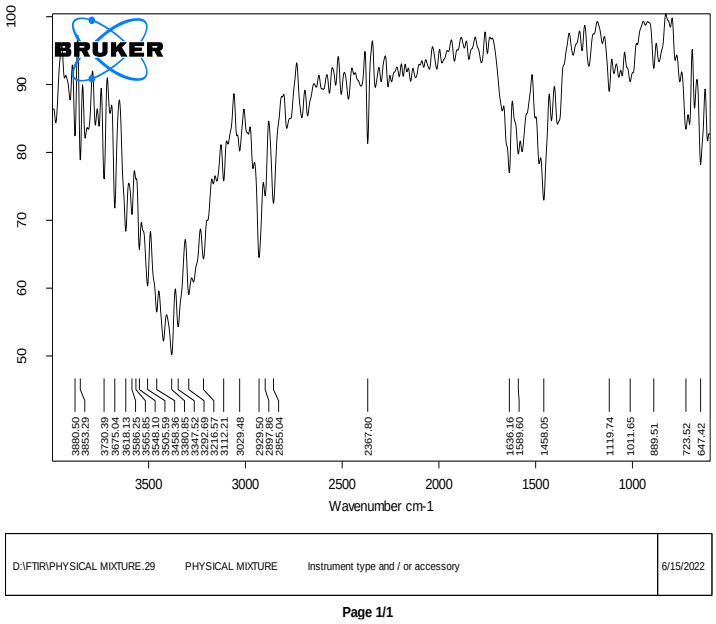

Figure 9: - FTIR spectra of Physical mixture

The peaks which were found in the individual analysis of compounds were also present in the analysis of the physical mixture. Hence there is no physical incompatibility between the components of the formulation.

5. UV-Spectrophotometry

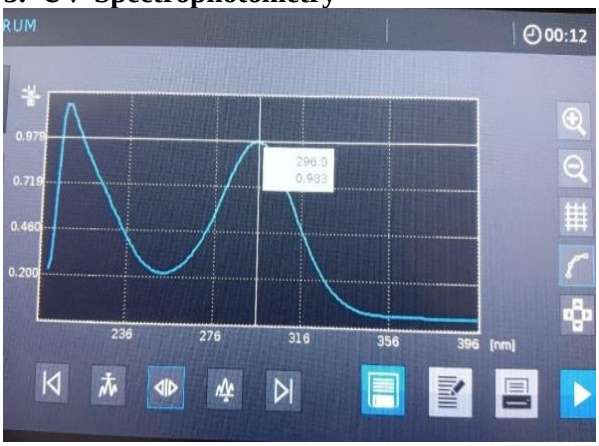

Figure 10- $\lambda_{max}$of timolol maleate

Determination of λ_{max} max of timolol maleate; the product timolol maleate was scanned in a UV spectrophotometer and the maximum absorption was found to be 296nm. This absorption peakwas used for further analysis and is represented in figure 10.

B. Evaluation parameters

1. Clarity, pH, appearance: The appearance of all the 3 formulations was found to be transparent and clear with free-flowing properties. There was no presence of any particles and particulate matter in the Timolol Maleate in-situ gel. The pH of the prepared formulation was found to be in the range of 6.9- 7.4 which is similar to the range of the physiological range of the human eye

2. Gelling capacity: All the formulations showed instant gelation when incorporated into simulated tear fluid (STF) and remained for an extended period as shown in table no. 8

3. Rheology studies: It was found that the formulations showed viscosity ranging from 15.4 cps to 20.3 cps after gelation. The increase in the viscosity was dependent on the concentration of Gellan gum as shown in table no. 8

4. Drug content estimation: The drug content of the formulated timolol maleate in situ gel was found to be in the range of 80% to 87% by using UV spectroscopy at 296nm against blank as shown in table no. 8

Table No. 8: - Evaluation parameters of In-situ gel

Formulation	Gelling capacity	Viscosity in cps	Drug content
F1	+++	20.3±0.4	87.3 ± 0.54
F2	++	17.5±0.3	82.4 ± 0.24
F3	++	15.4±0.5	80 ± 0.54

++ shows formulations are retained as gels for 6 hours.

+++ shows formulations are retained as gels for 8 hours.

5. Sterility tests: Sterility test was checked for both bacterial and fungal contamination and kept for 14 days. All the products were found to be sterile as shown in table 9 and figure 11 and 12

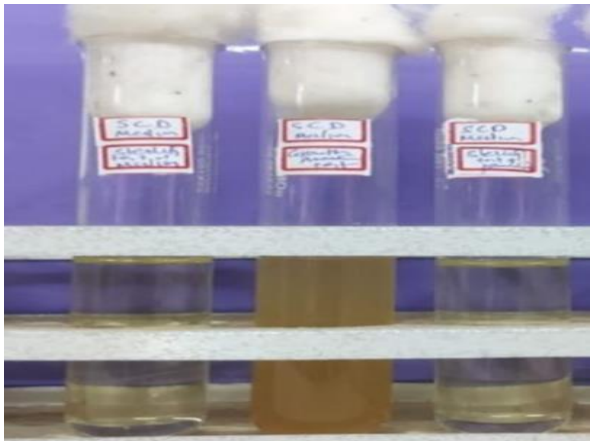

Figure 11: - Soybean casein mediu

Figure 12: - Thioglycolate medium

Table No. 9: - Soyabean casein digest medium and fluid thioglycollate medium

Test	Test	Days													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
FTG	sterility of medium	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	growth promotion test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	sterility test of the formulation	−	−	−	−	−	−	−	−	−	−	−	−	−	−
SCD medium	sterility of medium	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	growth promotion test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	sterility test of the formulation	−	−	−	−	−	−	−	−	−	−	−	−	−	−

CONCLUSION:

The formulation was developed to keep the gelling agent at a low concentration. FTIR studies revealed that there was no physical incompatibility between the components. F1 formulation was found to be the best formulation with 0.5% of Gellan gum which showed gelling capacity at optimum viscosity. Drug content when estimated was high in F1 formulation at 87.3%. All three formulations were found to be sterile.

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