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Formulation and Evaluation of Trimethoprim Loaded Nanosponge Ocular *In- Situ* Gel

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ABSTRACT

The objective of the work is to enhance the solubility of trimethoprim and prepare trimethoprim loaded nanosponge ocular *in-situ* gel by using temperature triggered method to improve bioavailability. β –Cyclodextrin based nanosponges were prepared by melt procedure. Optimization is done by design expert software 11.03(box Behnken design). Nanosponges are tiny sponges with a size of about a virus (250nm-1µm), which consist of cavities that can be filled with a wide variety of drugs. Nanosponges are solid, porous, biocompatible, and tiny in size with three-dimensional structures. In this work solubility of trimethoprim nanosponge was increased as compared to pure trimethoprim. The prepared nanosponge was characterized by scanning electron microscopy (SEM), drug content, solubility, in-vitro drug release study. The *in-situ* gel was prepared by cold method. The in situ gel was characterized by clarity and visual appearance, gelling temperature, and antimicrobial study. The nanosponge technique was found to increase the solubility and dissolution rate of trimethoprim as compared to pure drug. The anti-microbial activity of pure Trimethoprim, drug-loaded nanosponge and the *in-situ* gel formulations were showed antimicrobial activity.

INTRODUCTION:

Nanosponges are a new class of materials. The nanosponges carry drug molecules within its core. Nanosponge is a tiny their average size less than 1µm [(5)]. It carries lipophilic and hydrophilic substance [(6)].cyclodextrin-based nanosponge is biocompatible their crystalline or spherical in nature and improves stability, solubility. cyclodextrin-based nanosponge is prepared by melt procedure. In this method diphenyl carbonate as crosslinker[(1)]. Trimethoprim was a BCS class II drug. In this work to improve the solubility of trimethoprim Cyclodextrin are non-reducing, crystalline, water-soluble, cyclic oligosaccharide. Cyclodextrin has a well-defined cylindrical cavity exhibiting an apolar character, the peculiar shape of cyclodextrin are able to form stable inclusion complexes with molecules of suitable polarity and size in aqueous solutions[(1)].

In-situ gel forming systems have been widely investigated as vehicles for sustained drug release [(7)]. Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response because high tear fluid turnover and dynamics cause the rapid precorneal elimination of the drug. A high frequency of eye drop instillation is associated with patient non-compliance [(8)]. For enhancement of precorneal residence and improving the bioavailability of ophthalmic drugs an alternative approach has been used which is *in-situ* gelling systems that are instilled in a liquid form which converted into a gel form in a cul-de-sac of the eye [(9)]. The sol-gel transition can be induced by a shift in pH, temperature or ion activated systems. This type of gel combines the advantage of a solution (accurate and reproducible administration of the drug) and gels (prolonged residence time) for enhancing ocular bioavailability [(10)].

MATERIALS AND METHODS:

Materials:

Trimethoprim (Yarrow chem.), Beta cyclodextrin (chemco), Diphenyl carbonate (chemco), polaxomer

Table 1: Equipment used for the formulations

Sl no	Equipment	Company
1	Weighing balance	SHIMADZU AY120
2	FTIR spectroscopy	SHIMADZU A2137484
3	Scanning electron microscopy auto fine, coater.	JEOL MODEL JSM – 6390V
4	Magnetic stirrer	REMI EQUIPMENT
5	centrifuge	REMI EQUIPMENT
6	Uv spectrophotometer	SHIMADZU
8	France diffusion cell	MOORTHI'S LAB GLASSWARES HYDERABAD
9	Dissolution apparatus	ELECTROLAB -TDT -06L

Methods:

Preformulation studies

Preformulation studies were performed on the obtained sample of drug, polymer, and crosslinker such as physical appearance, solubility, melting point, Ultraviolet (UV) spectroscopic study.

Calibration curve of Trimethoprim

Accurately weigh 100 mg of Trimethoprim and dissolve 10 ml ethanol. Makeup to 100ml with phosphate buffer ph 7.4. The stock solution contains 1000 µg/ ml.

From the stock solution pipette out 1, 2, 3, 4, 5 ml to 100 ml with phosphate buffer ph 7.4 contains 10, 20, 30, 40, 50 µg/ml.

PREPARATION OF BETA CYCLODEXTRIN BASED NANOSPONGE

Melt Procedure:

Diphenyl carbonate, the crosslinker was melted along with Beta-cyclodextrin in a 50 ml beaker and heated around 80 -100°C the reaction was continued 4 hr with constant stirring under a magnetic stirrer. The reaction mixture was allowed to cool and solidified complex

was broken down to fine powder followed by repeatedly washed with ethanol to remove the unreacted product and excipients[1].

Loading of Drug into Nanosponge:

Nanosponge was further ground and triturated with water to avoid aggregates. Centrifuge the suspension and collected the supernatant solution containing nanosponge. Disperse the drug into this supernatant solution and maintained the suspension under constant stirring for a specific time for complexation. After complexation, separate the uncomplexed drug settle down by decantation. The supernatant solution was allowed to dry on a clean watch glass to obtain solid nanosponge powder[11].

OPTIMIZATION OF NANOSPONGE

Box Behnken design selected for the optimization of nanosponge.

Table 2: Box Behnken design applied for formulation

Sl no	Formulation code	Crosslinker ratio(coded)	temperature (coded)	Rotation (coded)	Crosslinker ratio (mg)	Temperature (°c)	Rotation (rpm)
1	F1	0	-1	+1	6	200	94
2	F2	-1	-1	0	3	200	87
3	F3	0	0	0	6	300	87
4	F4	+1	0	-1	9	300	80
5	F5	-1	0	+1	3	300	94
6	F6	0	+1	-1	6	400	80
7	F7	0	-1	-1	6	200	80
8	F8	+1	+1	0	9	400	87
9	F9	0	+1	+1	6	400	94
10	F10	+1	0	+1	9	300	94
11	F11	+1	-1	0	9	200	87
12	F12	0	0	0	6	300	87
13	F13	0	0	0	6	300	87
14	F14	-1	+1	0	3	400	87
15	F15	-1	0	-1	3	300	80

FORMULATION OF *IN SITU* GEL [7]

In-situ gel was prepared by the cold method. Weigh 10 gm of poloxamer 407 and was added to in a beaker and the volume was made up to 50 ml with distilled water, the solution was allowed to continuous stirring using a magnetic stirrer at a speed of 500 rpm for 2 h. The temperature of the water was maintained at 4±2 °C throughout the preparation. This solution was kept overnight in a refrigerator at 4 °C results in a clear solution. Trimethoprim loaded

nanosponge was weighed separately and dispersed in distilled water and added to the polymeric solution with continuous stirring until thoroughly mixed. Benzalkonium chloride (0.01%) was added as a preservative.

CHARACTERIZATION OF NANOSPONGES

1. Scanning electron microscopy(SEM)

Surface morphology was studied using a scanning electron microscope.

2. Solubility study

The solubility of drug loaded nanosponge was performed by distilled water. A weighed amount of trimethoprim loaded nanosponge was placed in a beaker shake vigorously and the suspension was filtered by Whatman filter paper. The obtained solution was analyzed by UV spectrophotometer at 271 nm [(12)].

3. Drug content

20 mg of drug loaded nanosponge was diluted up to 10 ml with ethanol and kept overnight. The stock solution was filtered by 0.2µ membrane filter. Dilute 1 ml filtered stock solution up to 100 ml with buffer ph 7.4. Analyzed by UV spectroscopy 271nm[(6)].

Drug content = Concentration × dilution factor/1000

4. *In-vitro* release study of drug and drug-loaded nanosponge

In-vitro release of trimethoprim loaded nanosponge formulations and pure drug were performed on dissolution USP Type II apparatus II at 100 rpm at 37 ˚C. The accurately weighed quantity of trimethoprim loaded nanosponge and drug in a small tea bag of muslin cloth and tied to the paddle of dissolution test apparatus. The medium used was 900 ml of water. At regular time intervals, aliquot samples were withdrawn and replaced with the same sample .drug content was determined by using UV visible spectroscopy at 271 nm.

EVALUATION OF PREPARED TRIMETHOPRIM LOADED NANOSPONGE OCULAR *IN-SITU* GEL

Clarity and visual appearance [9]

Appearance and clarity were determined by visual examination of the formulations before and after gelling under light alternatively against white and black backgrounds. The formulations were also observed for any unwanted particle or turbidity dispersed in a solution

Determination of gelling temperature [9]

Gelling temperature is measured by using a thermometer. The cool liquid formulation was allowed to warm-up slowly and once attained the room temperature it was further warmed at a slow rate in a water bath. The temperature and phase change was monitored throughout the process. Similarly, the temperature at the phase change from gel to sol was also recorded. The average was taken as the sol to gel transition temperature.

Drug content [14]

For estimation of drug content, 1 ml of *in-situ* gel was diluted to 100 ml of STF. Again 1 ml of this sample was diluted with 10 ml STF. The drug content was measured by using UV visible spectrometer at 271 nm.

Determination of pH[15]

The pHof the gel was determined using calibrated pH meter.

An antimicrobial study [16]

The sustained release *in situ* gel of trimethoprim was subjected to antibacterial studies by agar cup plate method. This study was performed on *Staphylococcus aureus*. Trimethoprim (pure drug), drug-loaded nanosponge and the *in situ* gel formulation was tested for antimicrobial study.

The nutrient agar media was prepared and sterilized by autoclaving. It was immediately poured into sterile Petri plate. After solidification of the media, the seeded test organism spread on the solid media using a spreader. Three cups of 5 mm diameter were made using sterile borer. The aliquot test samples were poured to cup aseptically and labeled accordingly.

After allowing diffusion of the solution for 2 h, the plate was incubated at 37 °C for 24 h. The diameters for *Staphylococcus aureus* were measured after 24 h respectively.

***In-vitro* drug release study** [4]

In-vitro drug release study was performed by using Franz diffusion cell. It was placed on a magnetic stirrer and temperature was adjusted to 37 ± 0.5°C. Accurately measured 1 ml of the preparation *in-situ* gel was spread uniformly in a cellophane membrane which was in contact with receptor medium. The receptor medium was stirred continuously using magnetic stirrer. Samples were withdrawn at periodic intervals and dilution was done with 10 ml of pH 7.4 buffer. The content of the drug was analyzed using UV spectrophotometer at 271 nm.

RESULTS AND DISCUSSION:

Preformulation study:

Physical properties:

Table 3: Physical properties of drug

Appearance	White crystalline powder
Solubility	Very slightly soluble in water, soluble in ethanol, chloroform, DMSO
Melting point	199-203°c

Calibration curve:

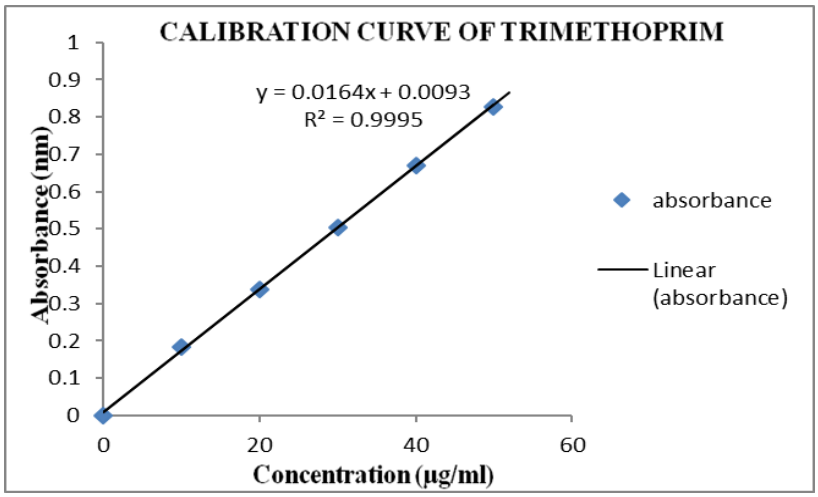


Figure 1: calibration curve of trimethoprim

Drug content of 15 nanosponge formulation

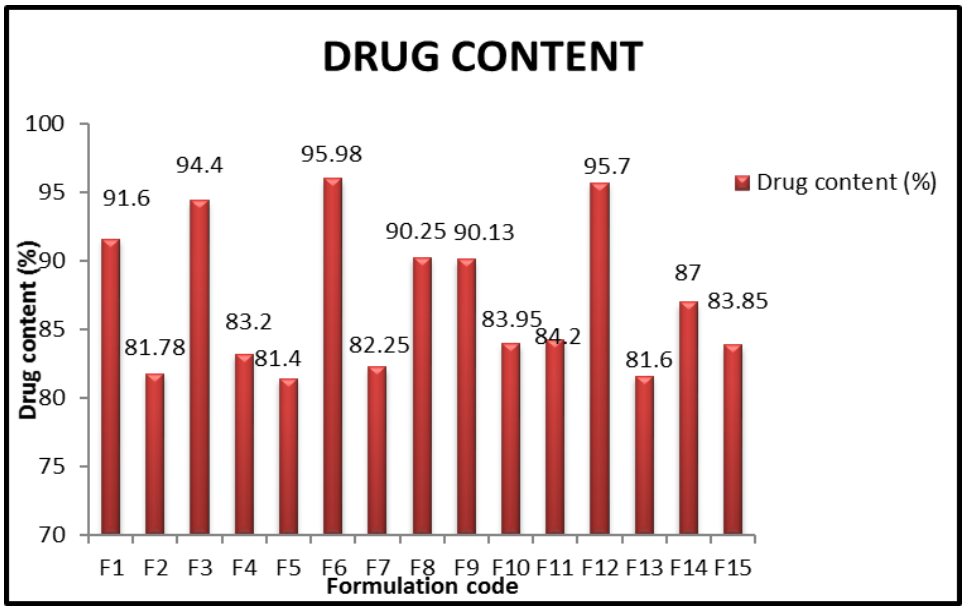
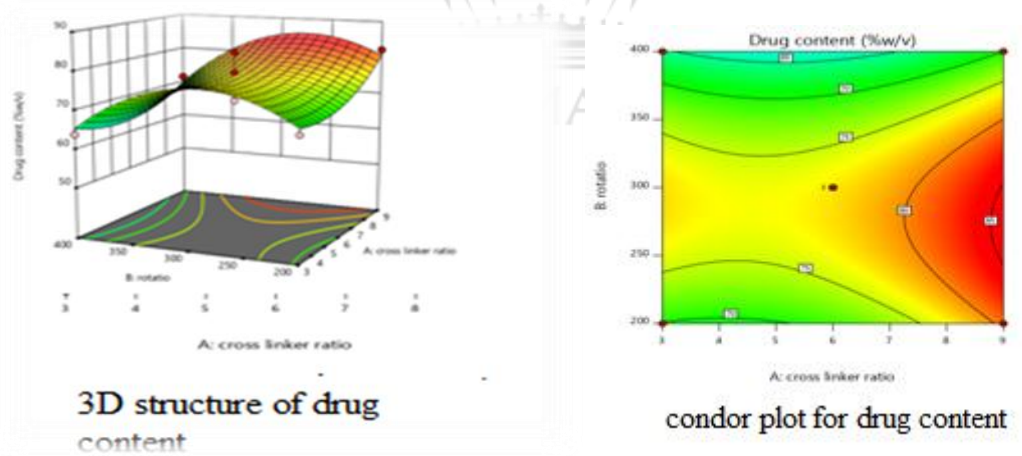


Figure 2: Drug content of different formulation of nanosponge

Optimized by Box Behnken design:



3D structure of drug content

condor plot for drug content

Figure 3: Condor plot and 3D structure of drug content given bellow

Solubility of 15 nanosponge formulation:

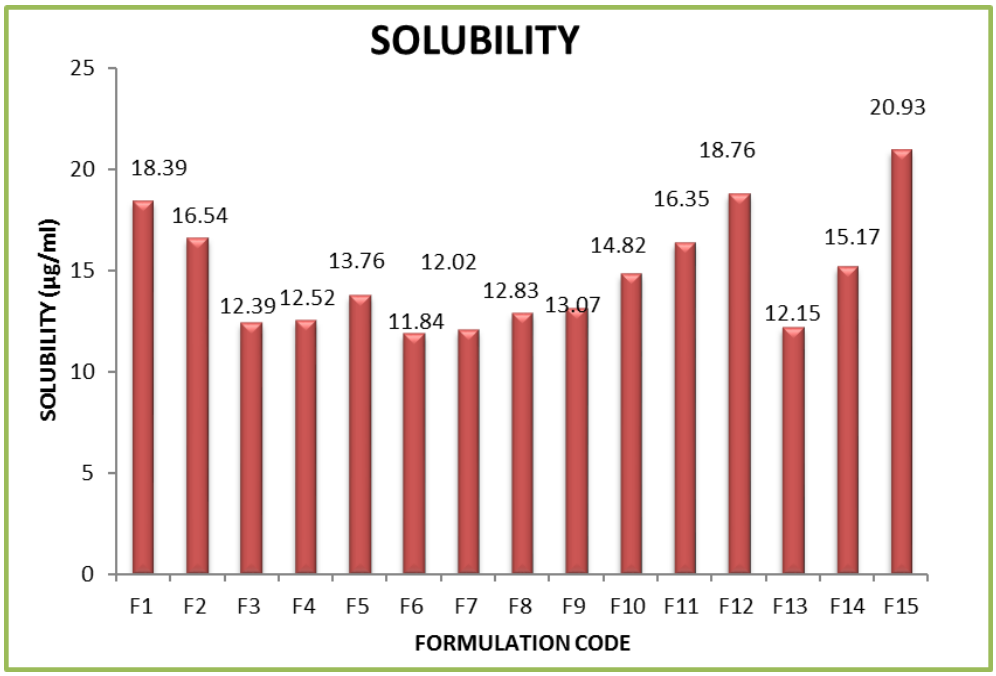


Figure 4: Solubility of a different formulation of nanosponge

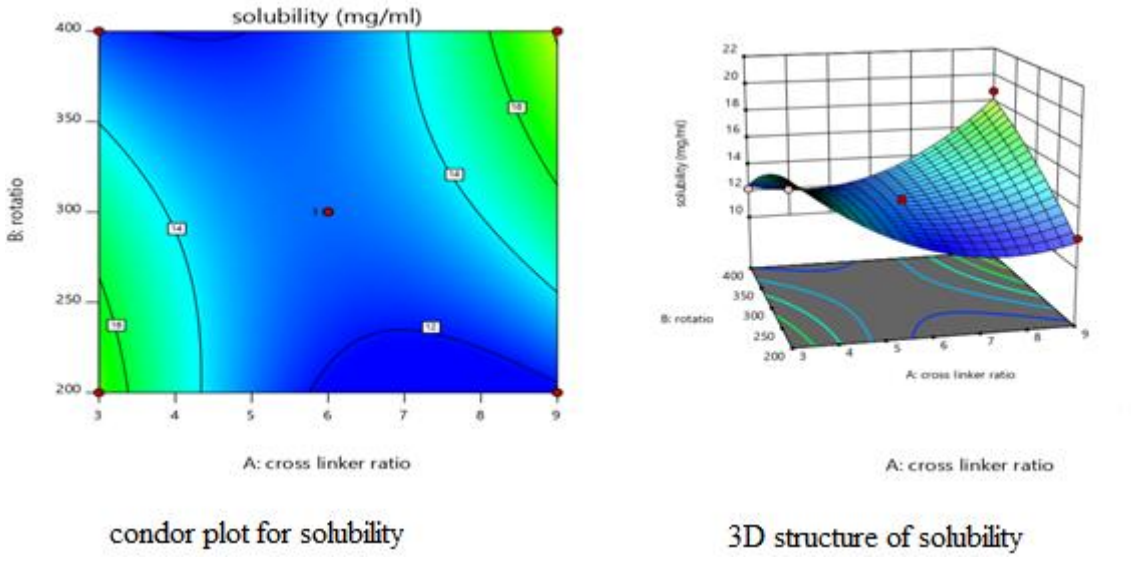


Figure 5: Condor plot 3D structure and cubic form of solubility given bellow

Scanning electron microscopy:

Trimethoprim loaded nanosponge was observed crystal shaped particle.

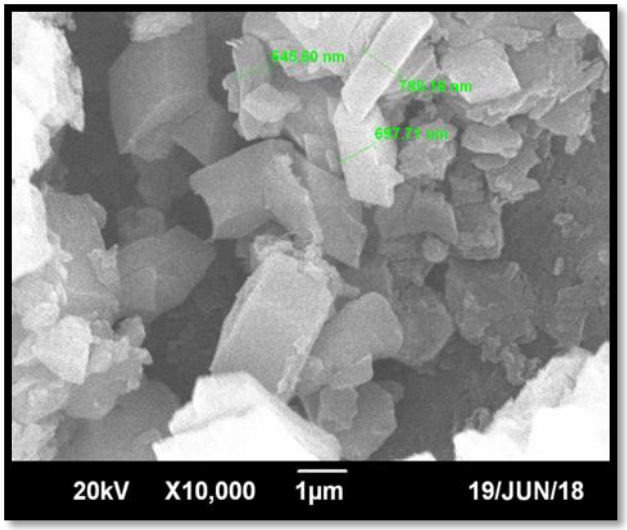


Figure 6: Scanning electron microscopic image of nanosponge

Solubility study:

Solubility of trimethoprim loaded nanosponge was improved as compared to pure drug. Solubility of trimethoprim is 10.729 µg/ml.

Solubility of trimethoprim loaded nanosponge was found to be 14.3 µg/ml.

Drug content:

Drug content of trimethoprim loaded nanosponge is 59.15 %w/w.

Comparison of the *in-vitro* study of trimethoprim (pure) and trimethoprim loaded nanosponge

***In-vitro* study of trimethoprim**

Table 4: *In-vitro* study of trimethoprim

Time	Absorbance (nm)	Concentration (µg/ml)	con×dilution factor×900/1000(mg)	%release
0	0	0	0	0
15	0.019	0.590	5.31	10.62
30	0.026	1.016	9.14	18.28
45	0.032	1.080	9.72	19.44
60	0.045	2.171	19.53	39.07
120	0.056	2.84	25.56	51.12
240	0.060	3.083	27.74	55.48
360	0.068	3.567	32.12	64.24

Table 5: *In-vitro* study of trimethoprim loaded nanosponge

Time	Absorbance (nm)	Concentration (µg/ml)	con×dilution factor×900/1000(mg)	%release
0	0	0	0	0
15	0.035	1.563	14.06	28.30
30	0.048	2.353	21.17	42.62
45	0.054	2.718	24.46	49.23
60	0.062	3.204	28.83	58.03
120	0.078	4.177	37.59	75.67
240	0.084	4.542	40.87	82.28
360	0.088	4.785	43.06	86.67

Comparison of *in-vitro* study of trimethoprim (pure) and trimethoprim loaded nanosponge

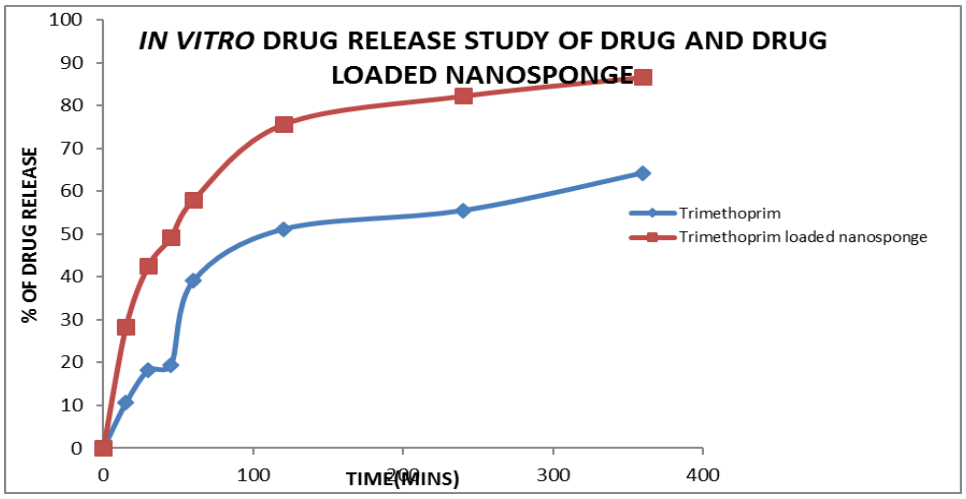


Figure 7: *In-vitro* drug release of trimethoprim loaded nanosponges and pure drug

EVALUATIONS OF THE *IN SITU* GEL

Clarity and visual appearance

The *in-situ* gel was a clear and transparent solution.

Determination of gelling temperature

Different % of Poloxamer 407 solutions were evaluated for the phase transition and the temperature was recorded at the phase change from sol to gel. The average was taken as the transition temperature.

Table 6: gelling temperature of Poloxamer 407 solution

Poloxamer 407 solution (%W/V)	Sols to gel transition temp range (ºC)	Mean temperature (ºC)
20%	30-32	31
19%	28-30	29
18%	35-36	35.5

From this study, it was shown that a 20% solution transforms to gel at 31 ºC. The ocular temperature is 32^0 C. Therefore 20% solution was taken for the formulation of in-situ gel.

Determination of drug content

Drug content of trimethoprim loaded *in-situ* gel was found to be 84.7%

Determination of pH

The pH of the *in-situ* gel was 6.2

Antimicrobial studies

The anti-microbial activity of pure Trimethoprim, drug-loaded nanosponge and the *in-situ* gel formulations were carried out by the cup-plate method. All showed antimicrobial activity.

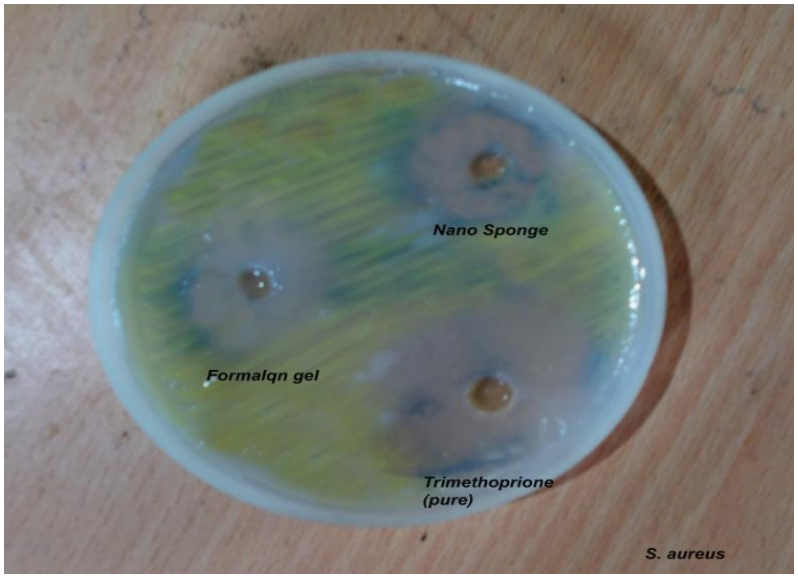


Figure 8: Zone of inhibition of Trimethoprim, drug containing nanosponge and *in situ* gel formulations

6.8.6 *In-vitro* drug release

Table 7: *In- vitro* drug release study of formulated solution

Time	Absorbance (nm)	Concentration (µg/ml)	con×dilution factor × 21/1000(mg)	%release
0	0	0	0	0
15	0.013	0.225	0.0472	4.7
30	0.019	0.590	0.123	12.3
45	0.023	0.833	0.174	17.4
60	0.029	1.198	0.251	25.1
120	0.042	1.98	0.458	41.58
240	0.048	2.35	0.493	49.3
360	0.056	2.84	0.596	59.64

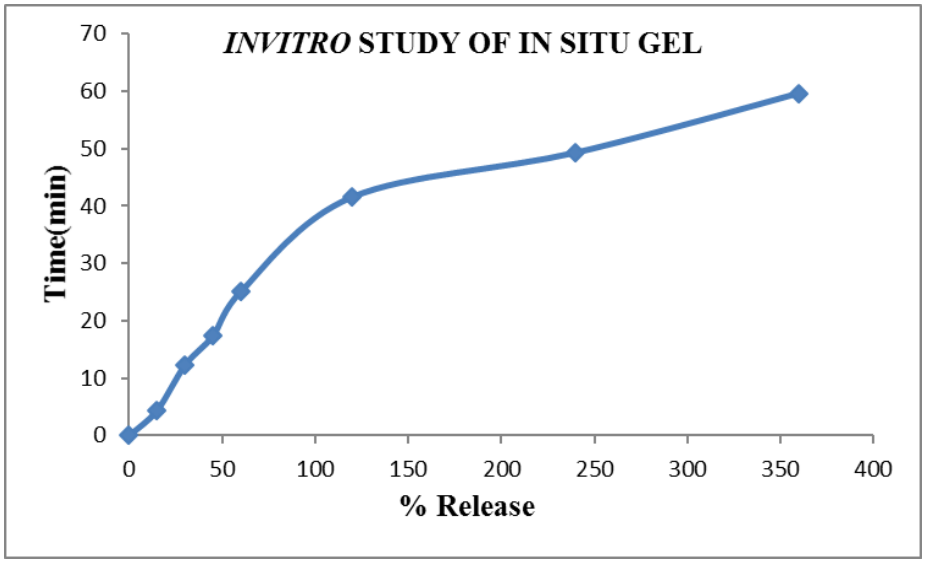


Figure 9: Cumulative in vitro drug release of the formulated solution

CONCLUSION

Cyclodextrin-based nanosponges carry a verity of the drug. Nanosponge was prepared by melt procedure. Nanosponges are optimized by Box Behnken design hence obtain the best formulation of nanosponge. The prepared nanosponges are solid (crystal) shaped. They are biocompatible. Nanosponge was prepared by using only two ingredients; the method is simple and easy. Trimethoprim loaded nanosponges improve the solubility of the drug. Optimized nanosponge carrying drug was incorporated into the gel and analyzed and confirmed their characters such as color, gelling temperature, antimicrobial study, *in-vitro* drug release.

REFERENCES

1. Gursalkartejashri et al Cyclodextrin based nanosponges for pharmaceutical use: A review Acta Pharm. January 29, 2013, 335–358
2. Divya Singh et al Recent advances in nanosponges as drug delivery system: a review article European Journal of Pharmaceutical and Medical Research August 2016, volume 3(10), 364-371.
3. Baburao A. Bachkaret alNanosponges: a potential nanocarrier for targeted drug delivery World Journal of Pharmaceutical Research Dec 2014, Volume 4 (3) 751-768.
4. Kadam et al Design and evaluation of modified chitosan-based in situ gel for ocular drug delivery International journal of pharmacy and pharmaceutical sciences volume 9 (11) 87-91 2017
5. Ashish Y. Pawaret alNanosponges: A Novel Drug Delivery System Asian Journal OF Pharmaceutics • Oct-Dec 2016 10(4) 456-462
6. GautamSeemaet al Development and evaluation of curcumin loaded nanosponges for colon drug delivery World Journal of Pharmaceutical Research Volume 4 (5) April 2015 1650-1665
7. Khateraet al*In-Situ* Gelling Ophthalmic Formulations for Sustained Release and Enhanced Ocular Delivery of Fluconazole IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) Volume 11(2) (Mar.- Apr.2016), 43-51
8. Eagaet alPreparation and Evaluation of In-Situ-Gels for Ocular Drug Delivery Journal of Pharmacy Research 2009, 2(6),1089-1094
9. Bachhavet al development of poloxamer-based temperature sensitive in situ ocular gel of betaxolol hydrochloride international journal of pharmacy and pharmaceutical science volume 7 (6) 2015 287 -291
10. Ramanjitet alInsitu gels a new trend I ophthalmic drug delivery systems international journal of pharma sciences and research 2015, volume 6(5) 886-890
11. Uday B bolmalet al Recent advances in nanosponges as drug delivery system, International Journal Pharmaceutical Science And Nanotechnology April- June 2013 volume 6 (1) 1934-1943
12. Honey Tiwari et alA Review On NanospongesWorld Journal Of Pharmacy And Pharmaceutical Sciences Volume 3 (11), 219-233
13. HemalataDolet al, Formulation and evaluation of in situ ophthalmic gel of moxifloxacin hydrochloride The Pharma Innovation Journal vol 3, issue 5, 2014, 60-66
14. HemalataDolet al Formulation and evaluation of *in situ* ophthalmic gel of moxifloxacin hydrochlorideThe Pharma Innovation Journal volume 3(5) 2014 60-66
15. Amita H. Patel et alFormulation and evaluation of sustained release *in situ* ophthalmic gel of neomycin sulfateBulletin of Pharmaceutical Research 2015;5(1):1-5
16. Sheikh et al Development and Characterization of Novel *In Situ* Gel of Moxifloxacin HydrochlorideAsian Journal of Pharmaceutics volume 11 (3) Jul-Sep 2017 (Suppl) S616 –S624