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Development, characterization and Evaluation of Doxycycline containing novel In-situ Gel for effective Ocular Drug Delivery

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ABSTRACT: Aim of the present study was to select the suitable pharmaceutical ingredients which provides suitable aqueous based system for designing and developing the *in-situ* gel-forming formulation containing Doxycycline Hyclate (DOX). Pharmaceutical ingredients specially used in ophthalmic formulation like Polymers (Gellan gum, Xanthan gum, Hypromellose, Povidone and etc.), Tonicity modifiers (Sodium chloride, Mannitol and etc.), Preservatives (Benzalkonium chloride, Oxychlorocomplex, Benzododecinium bromide and etc.), Buffers (Tromethamine, Sodium citrate, disodium hydrogen phosphate and etc.) stabilizing agents or Antioxidants (Sodium metabisulfite, Citric acid and Sodium thiosulfate and etc.). The pre-formulation studies were performed with selected pharmaceutical ingredients. Drug-Excipients compatibility studies were performed at 25°C and 40°C. Stability of Doxycycline Hyclate at different pH 5.5-7.4 were checked to finalize the pH of final formulation. The obtained results indicated that, the selected ingredients were suitable and provide stable system for in-situ gel formulation containing Doxycycline Hyclate. Results showed that a suitable combination of polymers (Gellan gum, Povidone K30 & Hypromellose), Preservative (Benzalkonium chloride), Buffer (Tromethamine), Tonicity modifier (Mannitol) and Antioxidant (Sodium metabisulfite) were found compatible. This could be the better and stable hydrogel system for Doxycycline Hyclate for *in situ* gel formulation.

Keywords: Dry eye disease, *In-situ* gel, doxycycline Hyclate, drug delivery, polymer, Gellan gum, Benzalkonium chloride, Antioxidant.

INTRODUCTION

The aim of the present study was to develop a stable aqueous based *in situ* gel forming Ophthalmic formulation containing tetracycline i.e., Doxycycline Hyclate. The ophthalmic preparations describe herein are designed for local administration in the treatment of eye surface inflammation and related dry eye disease. Primary goal of ocular drug delivery for the researchers is to achieve an acceptable therapeutic drug concentration at the target area, whether it be the anterior or posterior eye segment. The anatomy, physiology, and biochemistry of human eye (barrier role of corneal epithelium, tear turnover, reflex blinking), as well as the intrinsic characteristics of the most frequently used ophthalmic dosage forms. e.g., eye drops-low viscosity, relatively large volume of applied drop led to short precorneal contact time, rapid elimination and determined the poor bioavailability in ocular therapeutics (Ahmed *et al.,* 2023). The poor bioavailability of medication from conventional delivery system is resulted from a great extent of precorneal drug loss by nasolachrymal drainage. The rapid clearance of the topically applied drug into the eye often results in a short duration of pharmacological activity, and therefore, the need for a frequent dosing regimen. Moreover, 50% - 100% of an instilled dose could undergo systemic absorption through drainage via the nasolachrymal duct. This could lead to a possible increased risk of unwanted systemic toxic effects (Kolawole and Cook 2023). The administered effective dose may be altered by increasing the retention time of medication into the eye by using *in-situ* gel forming system (Kilbinger *et al.,* 2022; Mandal *et al.,* 2012). In order to overcome these limitations, the present study was to formulate the *in-situ* gel formulation using novel gum system. *In-situ* forming hydrogels are liquid upon instillation and undergo phase transition in the ocular clu-de-sac to form viscoelastic gel and this provides a response to environment changes (Kumar *et al.,* 2013).

MATERIALS AND METHODS

Materials: Doxycycline Hyclate received as a gift sample from Jagannath Chemical and Pharmaceutical works Pvt. Ltd., Odissa, India. Gellan gum from CP Kelco (California, United state), Povidone 30 from Dow, Hypromellose from Colorcon, Benzalkonium chloride from Novo Nordisk Pharmatech A/S (supplied by signet chemical), Mannitol from Merck, Tromethamine from Merck, Citric acid, Sodium metabisulfite &Sodium thiosulfate from Avantor performance material LLC, Edetate disodium from

Merck. Other reagents and solvents used in the study were of an analytical grade.

Methods. The working examples illustrate the screening process by which researcher developed ophthalmic products comprising the Active pharmaceutical Ingredients (API) of Doxycycline Hyclate. Doxycycline Hyclate 0.5mg/mL concentration in the formulation was described in the working examples.

A) In the first set of experiments, the stability of Doxycycline Hyclate were performed with different antioxidants at different pH and temperature.

B) In another set of experiment compatibility with selected pharmaceutical ingredients were performed by including each ingredient and the stability of the resulting formulation were evaluated.

Screening of ingredients (antioxidants). Few selected Antioxidants (Table 1) were tested in Doxycycline Hyclate solutions. Samples were analyzed for its stability through physical appearance and assay of Doxycycline Hyclate over the time period for 1week, 2 week & 4 weeks at pH 5.5 & 7.4 and at temperature 25°C and 40°C.

The Doxycycline could be degraded by the so-called Fenton process (Borghi *et al.*, 2015) i.e. Oxidation in the presence of Fe^{2+}. Physical appearance of Doxycycline solution turns pale yellow to yellow brown as it degraded (Gilbard *et al.*, 2012).

Compatibility studies. After finalization of suitable Antioxidant from above screening process. Other suitable pharmaceutical ingredients were also tested for its compatibility with Doxycycline Hyclate (Drug-Excipients) to select the final composition for formulation and development of *in situ* gel. Doxycycline Hyclate was mix with each ingredient in their optimum concentration under the allowable limit as per Inactive Ingredient Guide (IIG) data base (Table 2). Aqueous solution was prepared for each sample and keptfor the time period of 1 week, 2week & 4 weeks at a temperature 25°C & 40°C.

Table 1: List of antioxidants.

Tr. No.	Ingredients	IIG limit (Ophthalmic)	Concentration (mg/mL)
1	Sodium metabisulfite	0.2%	2.0
2	Citric acid	0.09%	0.9
3	Sodium thiosulfate	0.5%	5.0
4	Sodium bisulfite	0.1%	1.0
5	Edetate disodium	0.13%	1.3

Samples were analyzed for their physical appearance Doxycycline Hyclate: Excipients mixture solution. The Pharmaceutical ingredients selected for the *in-situ* gel formulation is shown in the below Table 2.

Evaluation of Screening Experiments. All the five formulation trials of Doxycycline Hyclate mentioned in Table 1 was analyzed for its stability through physical appearance at pH 5.5 and temperature 25°C kept for 1week, 2week & 4weeks. All the formulation trials were found colorless to pale yellow colour at pH 5.5, temperature 25°C for a time period of 1week, 2week, & 4 weeks. However, over the same time period at pH 7.4, colour of the samples was varied from pale yellow to Dark Brown colour. The physical appearance of the above five formulation trials at pH 5.5 and pH 7.4 at a temperature 25°C and 40°C mentioned in Table 3 and 4 respectively.

Table 2: Pharmaceutical ingredients selected for in-situ formulation.

Sr. No.	Ingredients	IIG limit (Ophthalmic)	Concentration (mg/mL)	Function
1.	Doxycycline Hyclate	Not applicable	0.5	Active Pharmaceutical ingredient (API)
2.	Benzalkonium chloride	2%	0.05	Preservative
3.	Gellan gum	Not available	6.0	Viscolizer
4.	Povidone K30	0.6%	4.0	Polymer/stabilizer
5.	Hypromellose	0.5%	4.0	Polymer
6.	Tromethamine	0.8%	1.75	Buffer
7.	Mannitol	4.7%	45.0	Tonicity modifier
8.	Sodium thiosulfate	0.5%w/w	5.0	Antioxidant
9.	De-ionized water	Quantity sufficient	Quantity sufficient	Solvent

Table 3: Physical appearance at pH 5.5.

Trial	0 day	At 25°C			At 40°C		
		1 week	2 weeks	4 weeks	1 week	2 weeks	4 weeks
1	Colorless	Colorless to pale yellow	Colorless to pale yellow	pale yellow	pale yellow	Yellow	Brown
2	Colorless to pale yellow	Colorless to pale yellow	pale yellow	pale yellow	Dark yellow	Brown	Brown
3	Colorless	Colorless to pale yellow	Colorless to pale yellow	Colorless to pale yellow	Colorless to pale yellow	pale yellow	Light brown
4	Colorless to pale yellow	Colorless to pale yellow	Colorless to pale yellow	yellow	Yellow	Light brown	Brown
5	Colorless to pale yellow	Colorless to pale yellow	pale yellow	Colorless to pale yellow	Yellow	Light brown	Brown

Table 4: Physical appearance at pH 7.4.

Trial	0 day	At 25°C			At 40°C		
		1 week	2 weeks	4 weeks	1 week	2 weeks	4 weeks
1	Colorless to pale yellow	Colorless to pale yellow	pale yellow	Yellow	pale yellow	Yellow	Brown
2	Colorless to pale yellow	Pale yellow	pale yellow	Light Brown	Dark yellow	Light Brown	Dark Brown
3	Colorless to pale yellow	Colorless to pale yellow	pale yellow	Yellow	Pale yellow	Pale yellow	Brown
4	Colorless to pale yellow	Pale yellow	Yellow	Light Brown	Yellow	Light brown	Dark Brown
5	Colorless to pale yellow	Pale yellow	Yellow	Light Brown	Yellow	Light Brown	Dark Brown

Table 5: Assay of Doxycycline Hyclate.

Trial No.	Ingredients	% of Doxycycline Hyclate At T0	25°C, 4week		40°C, 4week	
			% of Doxycycline Hyclate at pH 5.5	% of Doxycycline Hyclate at pH 7.4	% of Doxycycline Hyclate at pH 5.5	% of Doxycycline Hyclate at pH 7.4
1	Sodium metabisulfite	99.7	93.1	95.2	91.1	87.8
2	Citric acid	100.1	89.0	93.3	87	76.3
3	Sodium thiosulfate	98.2	95.4	96.0	93.4	90.1
4	Sodium bisulfite	98.6	87.4	75.7	82.3	78.7
5	Edetate disodium	100.0	88.2	91.6	89.2	87.1

Analysis of the Assay of Doxycycline Hyclate was also performed to check the stability of Doxycycline Hyclate in above five trials4-weeksamples. The assay of Doxycycline Hyclate in above five formulation trial samples at pH 5.5 and pH 7.4 at a temperature 25°C and 40°C mentioned in Table 5.

Drug-excipients compatibility stuydies evaluation. Drug-Excipient compatibility study were performed with each selected pharmaceutical ingredient. Physical appearance of Doxycycline solution turns pale yellow to yellow brown as it degraded (Gilbard *et al.*, 2012). Physical appearance was evaluated as an indicating parameter to check the any incompatibility between the drug and excipient. Antioxidant was common for all the mixture. Data presented in below Table 6 for physical appearance.

Table 6: Compatibility study data.

Trial	Drug - Excipients Mixture	0 day	At 25°C			At 40°C		
			1 week	2 weeks	4 weeks	1 week	2 weeks	4 weeks
T1	DOX: BKC: Sodium thiosulfate	Colorless	Colorless to pale yellow	Colorless to pale yellow	Colorless to pale yellow	Pale yellow	Pale yellow	Yellow
T2	DOX: Gellan gum: Sodium thiosulfate	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Yellow	Yellow
T3	DOX: Povidone K30: Sodium thiosulfate	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Yellow	Yellow
T4	DOX: Hypromellose: Sodium thiosulfate	Colorless	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Yellow
T5	DOX: Tromethamine: Sodium thiosulfate	Colorless	Colorless to pale yellow	pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
T6	DOX: Mannitol: Sodium thiosulfate	Colorless	Colorless to pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Yellow

DISCUSSION

Five different antioxidants were selected to stabilize the Doxycycline Hyclate and provide better stable aqueous media to formulate *In-situ* gels forming solution. Physical appearance and Assay of Doxycycline Hyclate were selected as an indicating parameter. Samples were analyzed to check its stability over the time period of 1week, 2 week and 4 weeks at pH 5.5 & 7.4 and at a temperature 25°C & 40°C. Out of five antioxidant the results with sodium thiosulfate and sodium metabisulfite were found satisfactory. Doxycycline Hyclate was found stable with sodium thiosulfate and sodium metabisulfite. Drug-Excipient compatibility studies were performed with selected pharmaceutical ingredients. Samples were analyzed for physical parameter as an indicating parameter. Mixtures were kept for a time period of 1week, 2week and 4 weeks at a temperature 25°C & 40°C. From the above tabulated data (Table 6) it was observed that, the selected excipients are compatible with each other and with Doxycycline Hyclate.

CONCLUSIONS

In the present study, stability study of Doxycycline with five different antioxidants were studied and found that, the Doxycycline Hyclate was more stable with sodium thiosulfate at a concentration of 5mg/mL at selected temperature 25°C & 40°C, pH -5.5 & 7.4. However, Doxycycline Hyclate was found more stable at a temperature 25 °C as compared to 40°C. Doxycycline Hyclate was found compatible with all selected excipients mentioned in Table 2. Further same qualitative composition shall be used to develop *in situ* gel forming solution. However, Quantitative composition shall be further optimized as per requirement.

FUTURE SCOPE

This study will be further carried forward to develop the *in-situ* gel forming solution containing Doxycycline Hyclate for effective ocular drug delivery.

Conflict of interest. None.

REFERENCES

Ahmed, S., Amin, M. M. and Sayed, S. (2023). Ocular Drug Delivery: A Comprehensive Review. *AAPS Pharm Sci Tech., 242. 24*, 1–29.

Borghi, A. A., Silva, M. F., Al Arni, S., Converti, A. and Palma, M. S. (2015). Doxycycline degradation by the oxidative Fenton process. *Journal of Chemistry, 2015*.

Gilbard, J. P., Gilbard, E., Fieldson, G. and Huson, R. B. (2012). Therapeutic eye drop comprising doxycycline and a stabilizer. United state Patent application (US 2012/0190653 A1).

Kolawole, O. M. and Cook, M. T. (2023). In situ gelling drug delivery systems for topical drug delivery. *Eur. J. Pharm. Biopharm., 184*, 36–49.

Kumar, D., Nagaich, U., Gulati, N. and Jain, N. (2013). Nanoparticles laden in situ gelling system for ocular drug targeting. *J. Adv. Pharm. Technol. Res*. 4.

Mandal, I., A.F.M. (2022). Kilbinger, Practical Route for Catalytic Ring-Opening Metathesis Polymerization. *JACS Au. 2* (12), 2800–2808.

Mandal, S., Prabhushankar, G., Thimmasetty, M. and Geetha, M. (2012). Formulation and evaluation of an in-situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. *Int. J. Pharm. Investig*., *2*, 78.

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