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Original Article

Augmented Optical Retention and Improved Antibacterial Activity against Conjunctivitis of Moxifloxacin Hydrochloride Entrapped in Microsponges *in situ* Gel: Formulation Evaluation and *in vivo* Remedial Value in Rabbits

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ABSTRACT

Objectives: Moxifloxacin hydrochloride is an antibiotic related to flouroquinolones class. It possessed reasonable to exceptional efficacy against gram-negative ocular pathogens. The prime goal of this study was to formulate and estimate moxifloxacin hydrochloride entrapped microsponge *in situ* gel to treat conjunctivitis. **Materials and Methods:** Moxifloxacin hydrochloride loaded microsponges was manufactured using modified emulsion-solvent dispersion technique. Optimized microsponge group was integrated into Carbopol base after *in vitro* characterization to formulate 1% moxifloxacin hydrochloride entrapped microsponge gel. Then, the prepared gel was examined for ocular irritancy, isotonicity, sterility testing, antimicrobial activity. Drug deposition ability and *in vivo* efficacy of above optimized batch was compared with marketed preparation in rabbit eye with conjunctivitis. **Results:** The mean particle size and *ex vivo* release of drug from optimized microsponge formulation was depicted to be 4.83±0.26 µm and 75.80±1.5 at the ending of 24 hr and entrapment was found to be 85.51±0.74%. SEM study evidenced the porous, spherical morphology of microsponges whereasFT-IR confirmed the microsponges' formation. No irritation in eye was observed with moxifloxacin hydrochloride microsponge in animals. **Conclusion:** It was concluded that moxifloxacin hydrochloride entrapped microsponge have shown augmented penetration of drug and *in situ* gel enhance the exposure time of drug loaded microsponges and conjunctiva of eye. Thus, moxifloxacin hydrochloride encapsulated microsponge gel can be investigated further as a medication to cure conjunctivitis.

Keywords: Microsponges, Moxifloxacin hydrochloride, Occular, Conjunctiva, Emulsion.

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INTRODUCTION

Microsponges are sponge-like, exceptionally cross-linked, non-pliable, polymeric system that can encapsulate a wide variety of active moieties in range of 5 to 300 µm and liberate them in sustain manner for extended duration.[1,2] They reduced irritation, allergenicity, mutagenicity and side effects for the topical applications. These products are available in unadventurous forms like gels, creams, or lotions containing high concentration of active ingredients to the customer. Different methods like polymerization methods, liquid-liquid suspension, emulsion systems and quasi–Emulsion Solvent Diffusion (ESD) techniques are used to prepare microsponges.[3,4] Moxifloxacin is an antibiotic which belongs to the fluoroquinolones class. It inhibited the normal functioning of bacteria by tying with DNA gyrase and topoisomerase IV, the essential bacterial enzymes required for imitation, transformation, renovate and recombination of deoxyribonucleic acid. It increased penetration of drug into ocular tissues and produced reasonable to exceptional efficacy against gram-negative ocular pathogens, streptococci and staphylococci species.[5] Thus, it works by killing bacteria

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that cause conjunctivitis. Conjunctivitis is the condition of eye diseases in which eye become pink or red with little inflammation. Moxifloxacin hydrochloride is available as eye drops in the market. The eye drops has main disadvantage that they get spilled out rapidly upon administration. Almost, 90% of ophthalmic preparations are introduced as ocular drops. In the meantime, the contact time with corneal surface is extremely minor approx. 1 to 2 min and just little amount of medication really enters the cornea and conjunctiva of eyes. The majority of the medicines are quickly lost because of unconstrained impact of tearing, spillage during blinking of eyes. Moreover the actual drug amount can vary by application strategy, the type of eye drop transporter and consistence of the patient.[6] To overpower these issues, the advancement in the opthalmic drug delivery system is of great importance because of its different useful properties like non-obtrusiveness, enhanced contact duration between corneal surface and drug and improved control on few eye contaminations.[7] *In situ* gelling systems have exhibited thick consistency from sol to gel after application on human body due to variation in pH, temperature, or ionic quality. These frameworks authorized specific and reproducible organization of medications and are equipped for delaying the habitation time to the mucosal surfaces.[8,9] These systems prolonged the exposure duration of formulation on mucosal surfaces and improved the contact time among formulation and corneal surface. Thus, permeable structure of microsponge makes a decent carrier for moxifloxacin like medications. Literature demonstrated the different visual medication conveyance frameworks like nanoemulsion,[10] nanoliposomes,[11] niosomes,[12] microparticles,[13] and microsponges have been scrutinized to enhance the precorneal arrangement time and corneal penetration of medication. These delivery structures are concocted FDA endorsed characteristic and blended with different polymers like eudragit,14 hyaluronate,15 cyclodextrins,16 Polylactic Corrosive (PLA),17 chitosan,18 poly (vinyl liquor),19 poly (vinyl-pyrrolidone),20 poly(lactic corrosive co-glycolic corrosive) PLGA and PCL and so forth for visual course. Another methodology for the enhancement of medication viability is to improve the home time of ophthalmic plans by utilizing heat sensitive in situ gel. These frameworks are manufactured by using sodium alginate, gellan gum, poloxamer 407 and HPMC[21] and so on, with modified physio-chemical properties to cure eye contamination. These preparations have shown steadiness and sol-to-gel consistency because of modified physicochemical characteristics at ecological conditions. Consequently, the primary point of the current examination is to build up the advanced plan of *in situ* gel moxifloxacin hydrochloride loaded microsponges for continued release of medication and to defeat the issues related to eye preparations. In the given research we formulated moxifloxacin hydrochloride encumbered microsponges and poured them into *in situ* gel for eye application. The purpose behind this study was to expose the impact of gel base on % drug release from microsponge to treat conjunctivitis. ICH guidelines were followed to conduct the stability studies of optimized batch.

MATERIALS AND METHODS

Materials

Moxifloxacin hydrochloride was procured from Godavari drug Ltd., India. Carbopol 940 was purchased from Loba Chemie, Mumbai, India. Poloxamer 407 was available as a bequest sample from BASF Corporation, Mumbai, India. Moxeza® was bought from drugstore. All other compounds used in this work were of pure analytical level. Proper feed and water were provided to all rabbit and all were retained at temperature of 22±2°C and relative humidity of 55±5% for 12 hr with day-night cycle, according to the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocol for this study was permitted by Hindu College of Pharmacy's animal ethics committee.

Induction of Conjunctivitis in rabbits by Sulfur mustard

Proper care and attention was given to animals according the organization's rules, as mentioned in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. Approximately 0.4 µL pure Sulfur mustard was inserted into the central cornea of rabbits to weigh between 2.5 and 4.0 kg with age not less than 3 months. Sulfur mustard has been documented to induce conjunctivitis including inflammation, burning, edema and pain.[22] Opthalmic exposure of sulfur mustard enhanced the goblet cell production of neutral mucins in rabbits. Recognition of morphological and biochemical alterations in the conjunctiva persuaded by sulfur mustard will help to identify the targeted receptors that will be beneficial to invent the effective remedy against ocular toxicity associated with mustard molecules.

Preparation of microsponges

The technique used for the manufacture of microsponges was modified emulsion solvent dispersion technique (Table 1). Bhatia and Saini were conducted studies to augment the release of curcumin from microsponges formulated by using PVA and ethylcellulose as carriers with quasi-emulsion solvent diffusion technique. Triethyl citrate and ethyl cellulose polymer were dissolved in 10 mL of dichloromethane. Triethyl citrate (1% w/v) was utilized to increase plasticity of formulation. The polymeric solution containing measured quantity of moxifloxacin hydrochloride (0.05% w/v) was then dropped into the aqueous mixture (prepared by dissolving Poloxamer 407 in 100 mL distilled water at 70°C) with continuous stirring at 3000 rpm for 2 hr to completely evaporate the carbon-based solvent and to develop the microsponges. Then, the mixture was transferred in fridge for one day that leads to precipitation of microsponges.

Table 1: Different formulation code and experiential value of particle size, % drug entrapment, % drug loading % yield and micrometrics parameters of different prepared formulations.

Formulation code	Drug polymer Ratio	Poloxamer 188	Stirring rpm	Particle size (µm)	% Drug Entrapment	% Drug Loading	% Yield	Carr's index (%)	Hausner's ratio	Angle of repose (°)
M1	1:1	0.5	3000	5.77±0.46	45.16±0.52	16.93±0.19	48.46±1.095	15.38±0.023	1.18±0.054	22.26±0.034
M2	1:2	0.5	3000	12.22±1.25	66.10±0.78	18.02±0.21	62.35±1.647	12.38±0.036	1.14±0.035	25.64±0.021
M3	1:3	0.5	3000	19.51±0.79	70.659±1.06	15.14±0.22	79.36±1.306	19.04±0.027	1.23±0.055	28.96±0.078
M4	1:4	0.5	3000	23.95±1.55	42.33±1.17	6.04±0.16	81.61±1.288	25.16±0.054	1.33±0.066	33.67±0.022
M5	1:2	0.25	3000	10.11±1.01	54.28±0.72	19.16±0.25	68.33±1.963	24±0.068	1.31±0.028	30.56±0.056
M6	1:2	0.75	3000	9.61±1.56	83.78±0.52	18.61±0.11	91.49±1.221	9.89±0.074	1.10±0.099	21.04±0.038
M7	1:2	1	3000	7.47±0.95	84.06±0.43	15.76±0.08	94.31±1.149	11.72±0.021	1.13±0.074	20.36±0.026
M8	1:2	0.75	2000	9.31±1.16	82.260.66	18.28±0.14	72.22±1.568	23.38±0.033	1.30±0.082	28.64±0.033
M9	1:2	0.75	3500	4.83±0.26	85.51±0.74	19.00±0.16	95.36±0.805	6.06±0.086	1.06±0.054	18.45±0.08
M10	1:2	0.75	4000	5.11±0.86	82.74±1.14	18.38±0.25	96.17±1.093	8.40±0.014	1.09±0.062	20.44±0.034

Dilute sodium hydroxide solution was used to wash and remove any free drug from microsponges. Repeat washing with double distilled water and to dry the sample transferred it in hot air oven at 40°C for 2 days. After that, the samples were used for further examinations.[23]

Evaluation of microsponges

Appearance of microsponges

All prepared formulations were visually observed under microscope for any defect and shape of the microsponges.

Particle size examination

An optical microscope was utilized to assess the mean particle range of microsponge of all prepared batches with the help of pre-standardized ocular micrometer and stage micrometer. Approximate 100 particles were examined from every sample.[24]

Drug entrapment efficiency, drug loading and percentage yield

Accurately weighed drug encumbered microsponges identical to 50 mg of drug were suspended in 20 mL distilled water. Then, the mixture was placed on magnetic stirrer to break the microsponges. Then, this preparation was passed through 0.45 µm membrane filter and the filtrate was examined spectrophotometrically at 291 nm after dilution with distilled water.[24]

The Entrapment Efficiency (EE %), drug loading (DL %) and percentage yield (%)[25] were calculated with following equations:

$$\% \text{ Entrapment efficiency} = \frac{\text{Total drug} - \text{Amount of unentraped drug}}{\text{Total drug}} \times 100$$

$$\% \text{ Drug loading} = \frac{\text{Total entraped drug}}{\text{Weight of microsponge}} \times 100$$

$$\% \text{ Percentage yield} = \frac{\text{Weight of total microsponge}}{\text{Weight of drug and polymer}} \times 100$$

Infrared spectroscopic analysis

The FT-IR spectras of moxifloxacin hydrochloride was recorded by potassium bromide pellet method in FT-IR Spectrophotometer (Prestige-21, Shimadzu and Tokyo, Japan) between the ranges of 400 to 4000 cm^{-1}. The IR Spectra of the drug was documented and compared with reference.[25]

Flow property

The flow properties of powder were estimated by calculating the Carr's index, angle of repose and Hausner's ratio. Flow property of microsponges was analyzed by estimating the angle of repose by fixed funnel technique. A funnel was preset at a height that the tip of the funnel was 6 cm from the surface. The microsponges of 30/40-work size were gone through the funnel, with the goal that they structure a tapered stack on a superficial level. The height

(h) and radius (r) of load were estimated and the edge of rest was determined.[26]

Morphological Characterization

Scanning electron microscopy (SEM, Environmental Scanning Electron Microscope model FEI Quanta 200F with Oxford-EDS system IE 250×Max 80) was used to determine the morphological uniqueness of the microsponges. Under vacuum, a drop of microsponges from uniformly suspended formulation was combined with gold-palladium. Then, the covered samples were examined under SEM.[26]

Amalgam of moxifloxacin hydrochloride loaded microsponge *in situ* ocular gel

The carbopol solution was prepared by mixing with double distilled water with persistent mixing and the volume is made by double distilled water. The poloxamer preparations were formulated by the cold method. A specific volume of double distilled water was chilled upto 4°C. Then P407 were added gradually with continuous stirring in water. The preparations were placed at 4°C till we get clear preparations and final volume was made by double distilled water. Poloxamer analogs/carbopol arrangements were prepared by adding required quantity of P407 and P188 in chilled (4°C) carbopol preparations. The pH of all sample solutions was attuned according to requirement by 0.5 M sodium hydroxide preparation and then transferred in fridge. Then, the compositions were distinguished reasonably for use as *in situ* gelling and mucoadhesive frameworks. The fluid preparations of different compositions (plan codes G1M9 to G5M9) were arranged and assessed for gelling limit at different physiological condition.[27]

The microsponge formulation containing drug was slowly added in gel base of poloxamer 407-carbopol 940 and mixed by using a mechanical stirrer for 5 min.

Physical Evaluation of *in situ* Microsponge Gel

Determination of the pH

The calibrated pH meter was used to determine pH of all formulations in triplicate. Then, the average of three reading was recorded as final value.

Estimation of gelation time

The gelation time was measured by tube reversal technique. 2 mL sample was poured in a test tube at 4°C, then, the test tube was shifted at gelation temperature approx. 35±1°C on water bath. The samples were observed at regular interval by reversing the test tube to study the gelation time. The gelation time evidenced that there is no progression of the gel on tube reversion.[28]

Estimation of gelling capacity

The gelling capacity of sample was calculated as follow. A drop of sample was placed in 2 mL of freshly prepared simulated tear fluid at 35±1°C. Then, the time-span of this solution to convert into gel was recorded and the dispersion of gel was perceived physically. The gelling capacity was estimated as follows:[29]

(#) No gel form.

(*) The formation of gel is delayed by some duration and deceased promptly.

(**) Instant gelation and remains stable for a couple of hours.

(***) Instant firm gelation that was stable for long duration.

Estimation of viscosity of prepared gels

Consistency of all batches was analyzed at different shear rates by utilizing Brookfield Programmable Rheometer (Model RVDV-III U). Different shear rates were used in ascending and descending manner at preset intervals at every rpm to analyze the consistency. The samples were equanimited before each examination at 35±1°C. Different shaft from T-F was fitted in viscometer to explore the consistency of preparations. All analyses were performed in thrice.[30]

Percentage drug content of microsponge gel

1 mL of sample preparation was attenuated with Simulated Tear Fluid (STF) upto 50 mL to estimate the drug content. 5 mL sample was pipette out and further diluted up to 50 mL with STF. Then, this sample was analyzed at 291 nm utilizing an UV-visible spectrophotometer.[31]

Table 2: pH, Gelation time, gelling capacity and % drug content of all prepared moxifloxacin hydrochloride loaded microsponge gel.

Sl. No.	Formulation code	pH	Gelation time (Seconds)	Gelling capacity	Percentage drug content
1	G1M9	6.86±0.25	26.01±1.73	*	93.75±0.31
2	G2M9	6.96±0.20	18.33±1.52	**	98.29±0.72
3	G3M9	6.7±0.10	16.04±1.21	**	96.98±0.51
4	G4M9	6.93±0.25	15.22±2.01	**	97.70±0.35
5	G5M9	7.1±0.17	14.33±2.51	**	98.78±0.23

* = Gel was getting solubilized after some time.**= Gel remains in gel form after 24 hr.

In vitro release

In vitro release was determined by utilizing the dialysis strategy in simulated tear fluid (pH 7.4 at 35±1°C) at 50 rpm. STF is prepared by using calcium chloride dihydrate (0.008%), sodium bicarbonate (0.2%) and sodium chloride (0.67%). A standard semi-permeable cellophane layer was extended over the dialysis tube, a precise weight of the microsponges or prepared microsponges gel (equivalent to 5 mg of medication) was kept on the film in the dialysis tube with the objective that the film supported the dialysis arrangement. The samples were elated at 50 rpm at 35±1°C. The aliquots were pipette out at preset time spans from the release medium on measuring glass (0.5, 1, 2, 3, 4, 5, 6, 7 and 12 hr) and were examined spectrophotometrically at 291 nm. The dissolution medium was supplanted by equivalent volumes of STF under similar conditions. The analyses were performed in thrice.[31-33]

Ocular Irritancy

Three rabbits (obtained from the institutional animal house) of either sex with weight about 2.5-4 kg were used for ocular irritancy test.27, 34 Investigational animals were adapted for 4 days before initiating the testing. 0.1 mL optimized formulation was introduced into the cul-de-sac and examined at regular interval upto 72 hr. The similar procedure is recurring for 7 days. The subjects were examined for their eye's redness, mucosal discharge, watering of eyes and puffiness.[35,36]

Isotonicity Estimation

Isotonicity is an imperative attribute of eye formulations. The sample was examined under the magnifying instrument after amalgamation with small amount of blood. The veracity and structure of red blood cells were compared with the cells placed in isotonic solution.[37]

Table 3: Anti-bacterial potency of optimized and marketed formulation (Moxeza).

Formulation	Zone of inhibition (diameter (mm))	
	Staphylococcus aureus	*Pseudomonas aueroginosa*
Optimized preparation	32.1±0.7	32.4±0.8
Moxeza	30.2±1.2	31.2±1.4

*All values were depicted as mean±SD (n=3).

Sterility Testing

Sterility testing is a significant factor for eye formulations. Direct inoculation strategy was utilized to assess the sterility of primed batches. 2 mL of sample was transferred to thioglycolate medium and in Soybean-casein digest medium independently by using sterile pipette. The samples were incubated separately in both mediums for least of 14 days at 30-35°C and 20-25°C.

Antimicrobial Activity

The antimicrobial efficacy of the optimized batch was compared with marketed formulation by using Kirby-Bauer disk diffusion method. In this method, the optimal weight of nutrient media was transferred into sterile petri plates under laminar air flow. Sterile cotton swabs were used to inoculate the microbial culture of*Staphylococcus aureus* and *Pseudomonas aueroginosa* in them. Drug loaded disk of dimension about 10 mm was nurtured in nutrient agar media. After 2 hr of initial diffusion, the petri dishes were incubated for 1 day at 37±0.5°C. The Vernier caliper was used to calculate the Zone of Inhibition (ZOI).[38]

Accelerated Stability Testing

Optimized formulations were tested according to ICH guidelines for accelerated stability study. In this, the optimized formulations were stored in stability chamber at temp 40±2°C and 75±5% RH for 6 months. Aliquots were taken out at regular interval of times and assessed for alteration in EE (%), mean particle size (µm), pH and drug content (%).[39]

Statistical Analysis

Statistical investigation was carried out using Graph Pad Prism software version 6. ANOVA (one-way analysis of variance) was used to analyze variations in experimental groups. Newman-Keuls method was used as a *posthoc* test. The value of probability $p<0.05$ was measured statistically significant. All experiments were performed in thrice and the outcomes were depicted as mean±SD.

Table 4: The entrapment efficiencies (%) and mean particle size of the microsponges devoid of the gel base during accelerated stability study.

Parameters	Code	Sampling Time					
		Primary	1 Month	2 Months	3 Months	4 Months	6 Months
EE (%)	M7	70.23±3.9	69.02±2.3	68.82±1.5	68.75±2.8	67.55±1.3	66.97±1.4
	M8	50.02±2.6	49.12±3.9	49.10±1.2	48.82±2.32	48.02±3.43	47.59±2.3
	M9	85.51±2.74	84.60±3.32	84.51±4.24	83.35±5.29	83.21±3.13	82.35±3.5
Mean particle size (µm)	M7	5.77±0.46	5.76±0.34	5.76±0.24	5.75±0.22	5.74±0.20	5.73±0.42
	M8	5.11±0.86	5.10±0.23	5.10±0.12	5.09±0.78	5.08±0.18	5.07±0.81
	M9	4.83±0.26	4.82±0.32	4.80±0.22	4.80±0.26	4.79±0.21	4.79±0.12

RESULTS AND DISCUSSION

Preparation of moxifloxacin encumbered microsponge

The modified emulsion solvent diffusion method was selected for the manufacturing of moxifloxacin hydrochloride loaded microsponges because it is simple, reproducible and free from solvent toxicity. Poloxamer was used as matrix forming material because it is safe, non-mutagenic, hypo-allergenic and non-irritating.

Characterization of moxifloxacin loaded microsponge

Visual observance

All prepared formulations were visually observed to detect any aggregation, clump formation. Observations demonstrated that all prepared formulations were non-aggregated and uniformly powdered.

Particle Size

Particle size of a formulation play vital role in ocular drug delivery. The average size of the developed microsponges was observed in range of 4.83±0.26 µm to 23.95±1.55 µm (Table 1). The shape of particles was found to be enlarged pointed after enhancing the concentration of polymer. This happens due to high viscosity of dispersed phase brought about arrangement of enormous globules that were difficult to be isolated into minute particles. Thus, bigger particles were framed. Further rise in quantity of poloxamer 407 from 0.25% w/v to 0.75% w/v leads to decrease in size of particles. The reason behind this is that the escalating concentration of stabilizer leads to less coalescence between globules and produced formulation with smaller particles. The impact of stirring speed on size of microsponges was depicted in Table 1. The average size of particle was found to be declined considerably from (12.22±1.25) to (4.83±0.26) with the increase in stirring speed. High mixing speed prompted separation of droplets and diminished the tendency to coalescence little microsponges and maintain the molecule size about 4.83±0.26 µm which is appropriate size for ocular application.[39]

Percentage entrapment efficiency, Drug loading and % yield

The effect of amount of surfactant, drug polymer ratio and stirring speed on drug entrapment efficiency, loading capacity and % yield were displayed in Table 1. Percentage drug entrapment, loading and % yield of all formulations were observed in the range of 42.33±1.17 to 85.51±0.74%, 6.04±0.16 to 19.16±0.25% and 48.46±1.095 to 96.17±1.093%. Upon augmenting the drug polymer proportion from 1:1 to 1:2% drug entrapment, loading and percentage yield were increased but they decreased on further enhancement of drug/polymer ratio. This outcome could be credited to the augmenting in viscosity as a result of escalating the polymer fixation which brings about preparations of all the more firm polymer coat and trouble of drug release with increasingly thick medium brought about low EE%.

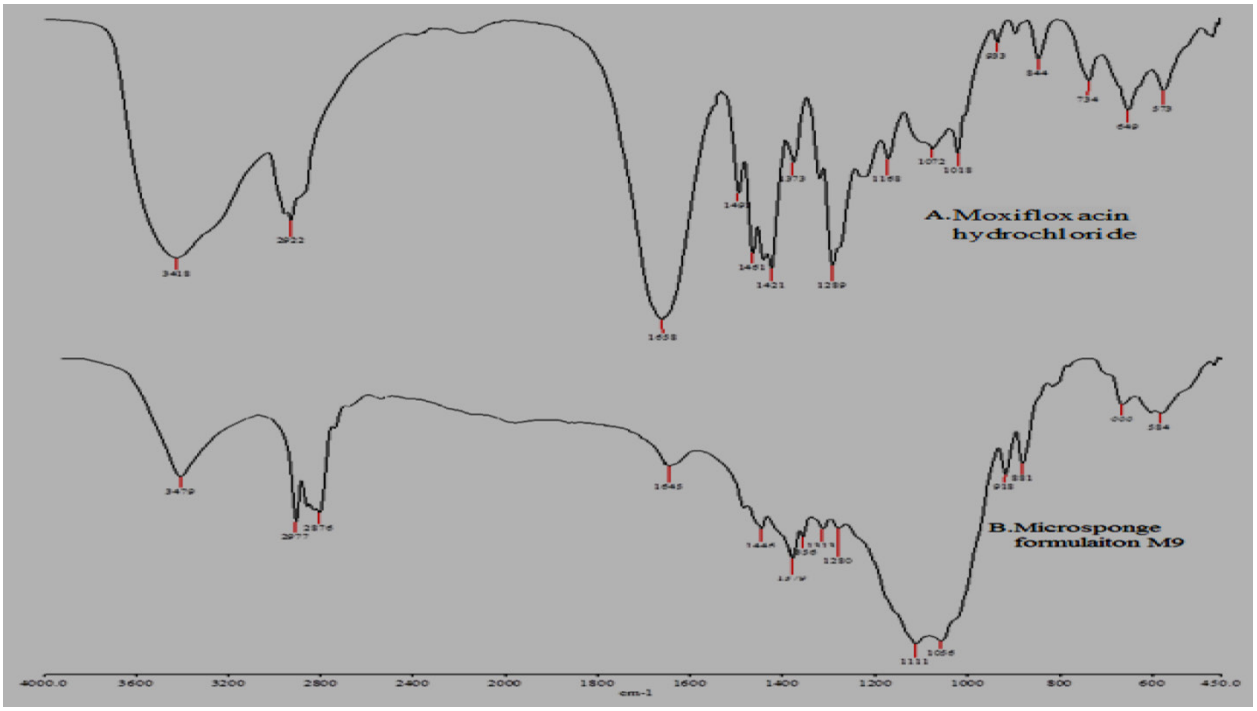

Figure 1: Overlay FTIR spectrum of moxifloxacin hydrochloride and optimized microsponge formulation M9.

Micrometric properties

Micrometric properties play a vital role in physical stability of formulation. Micrometric parameters of all prepared formulations were shown in Table 1.

Values of Hausner's ratio, cars index and angle of repose were observed between 1.06±0.054 to 1.33±0.066, 6.06±0.086 to 25.16±0.054 and 18.45±0.08 to 33.67±0.022. Based on the above results M9 formulation was chosen as optimum formulation having high entrapment efficiency and minimum particle size along with optimum flow properties.

FT-IR spectrum of Moxifloxacin hydrochloride and optimized formulation

In FT-IR spectra of Moxifloxacin hydrochloride (Figure 1), one observable peak at top was found at 3418 cm^{-1} indicated OH extending vibration. Another band at 2922 cm^{-1} spoke to alkenes and aromatic C-H extending, essentially extending vibration of fragranted enes. The peak at 1645 cm^{-1} was appointed to quinolones. The peaks between 1300 cm^{-1} to 1250 cm^{-1} proposed twisting vibration of O-H bunch which showed the nearness of carboxylic acid (-COOH). A solid retention peak between 1018 cm^{-1} was due to C-F gathering. FT-IR spectra of optimized formulation depicted fewer peaks of active moiety and demonstrated the encapsulation of drug into microsponge's matrix.

Morphological Characterizations

Figure 2 has shown the scanning electron micrographs of optimum formulation M9. The micrograph visibly depicted the morphology of particles. It evidenced that particles are hollow spherical in shape with porous surface and within the size range. Small drug units were seen on the outer of microsponges.

Evaluation of prepared moxifloxacin hydrochloride loaded micro sponge gel

Measurement of pH

The pH is an essential parameter to be determined for those formulations administered by ocular route. Table 2 depicted the pH of all primed formulations. The pH values of the primed formulations were calculated by pH meter and were found in range of 6.7±0.10 to 7.1±0.17. The tears have ability to regulate the pH of a formulation to the physiological pH if the formulations have pH range 3.5 to 8.5.[39] Therefore, the prepared moxifloxacin hydrochloride loaded microsponges gels are satisfactory for ocular appliance because their observed pH value lies close to ocular pH that could be attuned to the physiologic pH by tears.

Gelation time and gelling capacity

The gelation time of gelling agent is based on structure and concentration of gelling agent. Gelation time of all prepared microsponges formulations was shown in Table 2. The idyllic *in situ* gelling framework is gelled quickly at human body temp to repel the brisk disposal by tear fluid. All prepared formulations have shown quick gelation time. Among all formulation G4M9 and G5M9 have shown rapid gelation time of about 15±2.01 and14.33±2.51 sec respectively. It was observed that all prepared formulation form gel immediately but in case of G1M9 gel was getting solubilized after some time of interval. But other formulations G2M9, G3M9, G4M9 and G5M9 remain in gel form after 24 hr.

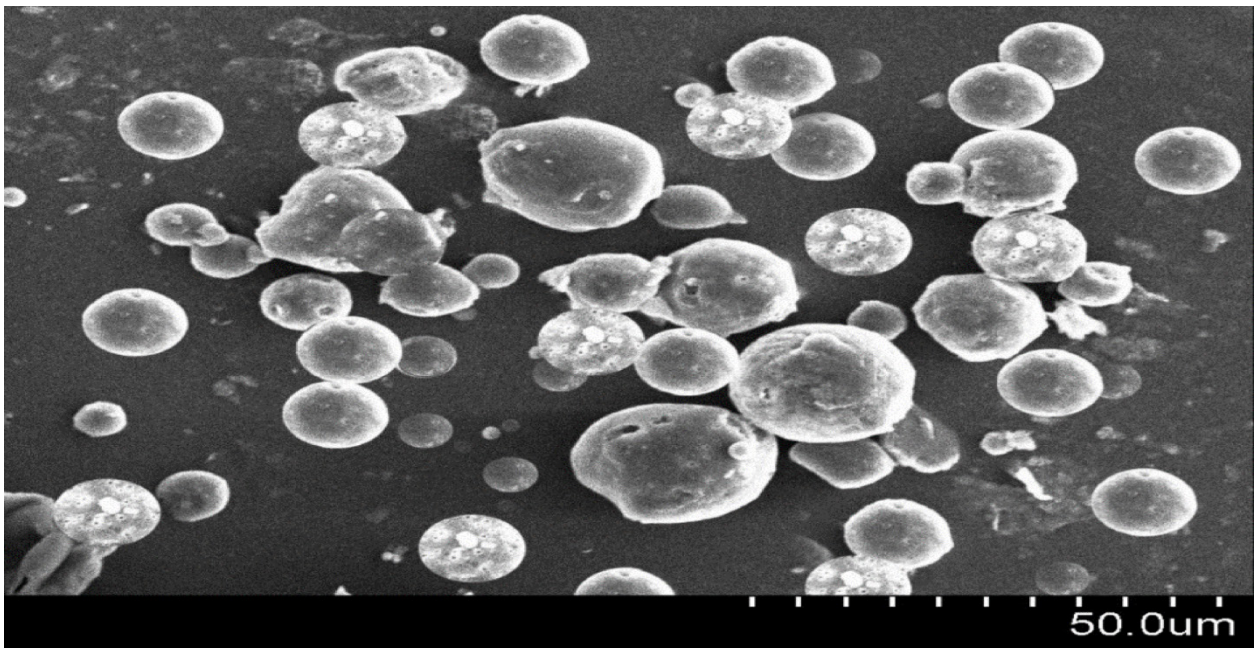

Figure 2: SEM image of M9 formulation.

Percentage drug content

Percentage drug content of prepared batches were revealed in Table 2. The results evidenced the % drug content of given formulations in range of 93.75±0.31 to 98.78±0.23%.

Determination of viscosity

Viscosity has played a significant role in physical stability and drug release from gel. Therefore, measurement of viscosity is very significant in case of gel. Viscosities of different formulations were determined at different rpm and data of viscosity of all prepared formulations were depicted in Figure 3a.

The results demonstrated that the formulations have revealed shear thinning behavior. The consistency was decreased as the rpm increases. An advantage of this behavior is that the viscosity is augmented in open eye and alleviated the tear film. Thinning of polymer film during blinking avoided irritation due to high consistency of Newtonian fluid[39] and thus allowed a smooth allocation of the preparations on eye surface.

In vitro drug release study

In vitro drug release study determined the absorption of drug across corneal membrane. *In vitro* drug release was also depended on concentration of gelling agent as shown in Figure 3b. Fusion of the drug loaded microsponges in pluronic gels increased the drug discharge, as gelling specialist diminished the hydrophobic attributes of the microsponges. Pluronic is non-ionic polymeric surfactant that increased the wettability of microsponges. G4M9 and G3M9 formulations depicted better medication discharge than other preparations. G2M9 AND G5M9 formulations depicted the less medication release as the increased concentration of gelling and viscosity modifying agent, enhanced the consistency of system that reduced the consequent diffusion of drug through polymeric chain network. Among all formulation G3M9 showed maximum release up to 75.80±1.5 at 12 hr.

Drug release kinetic study

The results from release kinetics demonstrated that the medication followed Higuchi dispersion model to discharge from the microsponges loaded *in situ* gels having value of relapse coefficient (0.991). The values of correlation coefficient for zero order, first order, Higuchi's release model and Korsemeyer-Peppas's model were recorded as 0.983, 0.989, 0.991 and 0.967, respectively. The value of diffusion exponent 'n' of the Korsmeyer-Peppas equation was 0.88 (0.5≤n≤0.8) which demonstrated incoherent diffusion or Non-Fickian diffusion mechanism for drug liberation.

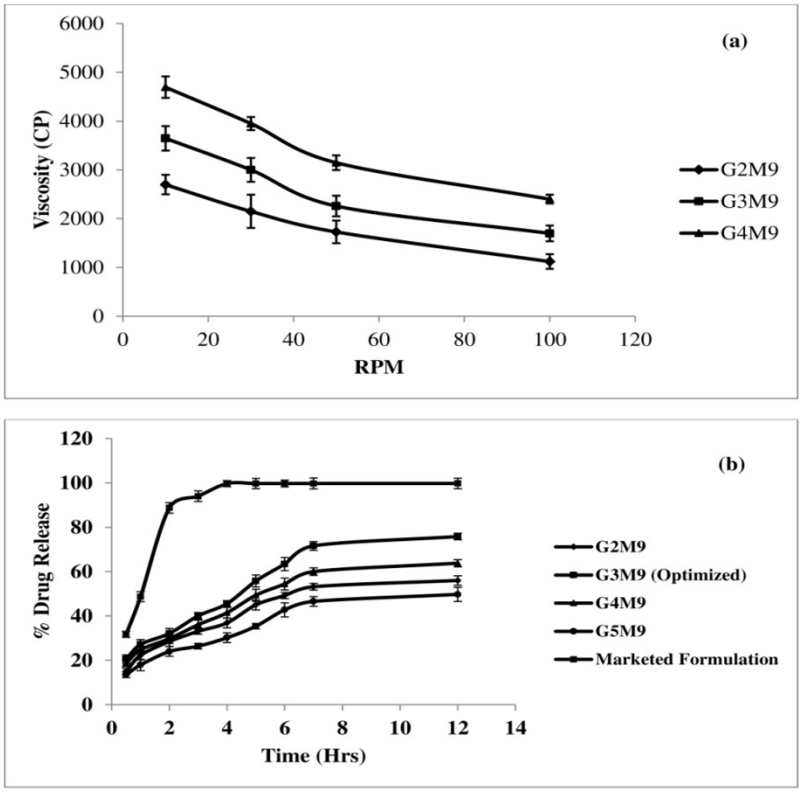

Figure 3: (a) Viscosity of different formulation at different rpm (b) *In vitro* drug release of different moxifloxacin loaded microsponge gels and marketed formulations.

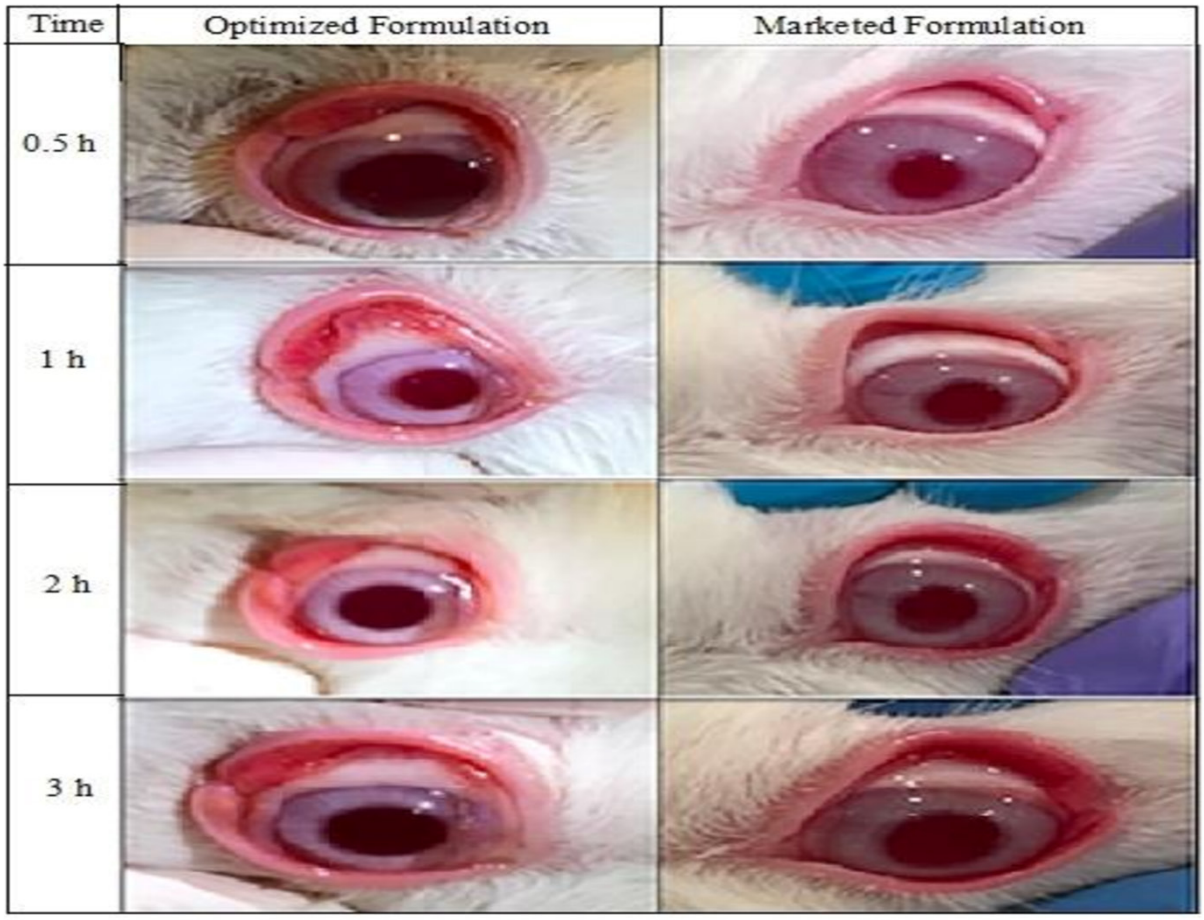

Figure 4: Rabbit's eye exposed with marketed formulation (Moxeza) and microsponge *in situ* gel.

Comparison of *in vitro* drug discharge between optimum formulation and marketable formulation

The results demonstrated that the *in vitro* drug discharge of marketed formulation (Moxeza) was quicker than the optimum microsponges *in situ* gel formulation (G3M9) (Figure 3b).

Ocular irritancy test

The Ocular irritancy study was initiated after inserting *in situ* gel in the left eye of rabbit and assessed for succeeding 3 days. No water and mucosal discharge from eyes and swelling were found during the application period. Hence, the upgraded formulation amalgamated into *in situ* gel was observed to be harmless, nonirritant and non-sarcastic.

Antimicrobial activity

Antibacterial activity of optimized batch was judge adjacent to the marketed formulation (Moxeza) (Table 3). The results evidenced the higher potency of optimized formulations than the marketed formulations. The higher potency is correlated with the higher consistency of optimized batch that leads to slow and prolonged liberation of active moiety from the preparation.

In vivo studies on rabbit eye

In vivo studies on rabbit eye with induced conjunctivitis were performed which shows that the outcomes of optimized microsponges *in situ* gel formulation were more effective than marketed formulation. Marketed formulation get spilled out from the eye rapidly and hence there is very less amount of drug that penetrates through conjunctiva whereas moxifloxacin hydrochloride loaded microsponge *in situ* gel has increased contact time with conjunctiva and better permeability as the drug liberate slowly from microsponges *in situ* gel which cure conjunctivitis to a great extent as compared to Moxeza (Figure 4).

Isotonicity Measurement

The results evidenced the isotonicity of optimized formulation with tears. The results confirmed no change in shape of RBCs with optimized formulation as with isotonic solution (Figure 5).

Sterility Testing

Medias inoculated with the sterile preparations for more than 14 days were found to be cleared, depicting no turbidity and no proliferation of microbes in the samples. This evidenced that the prepared batches were passed the test of sterility.

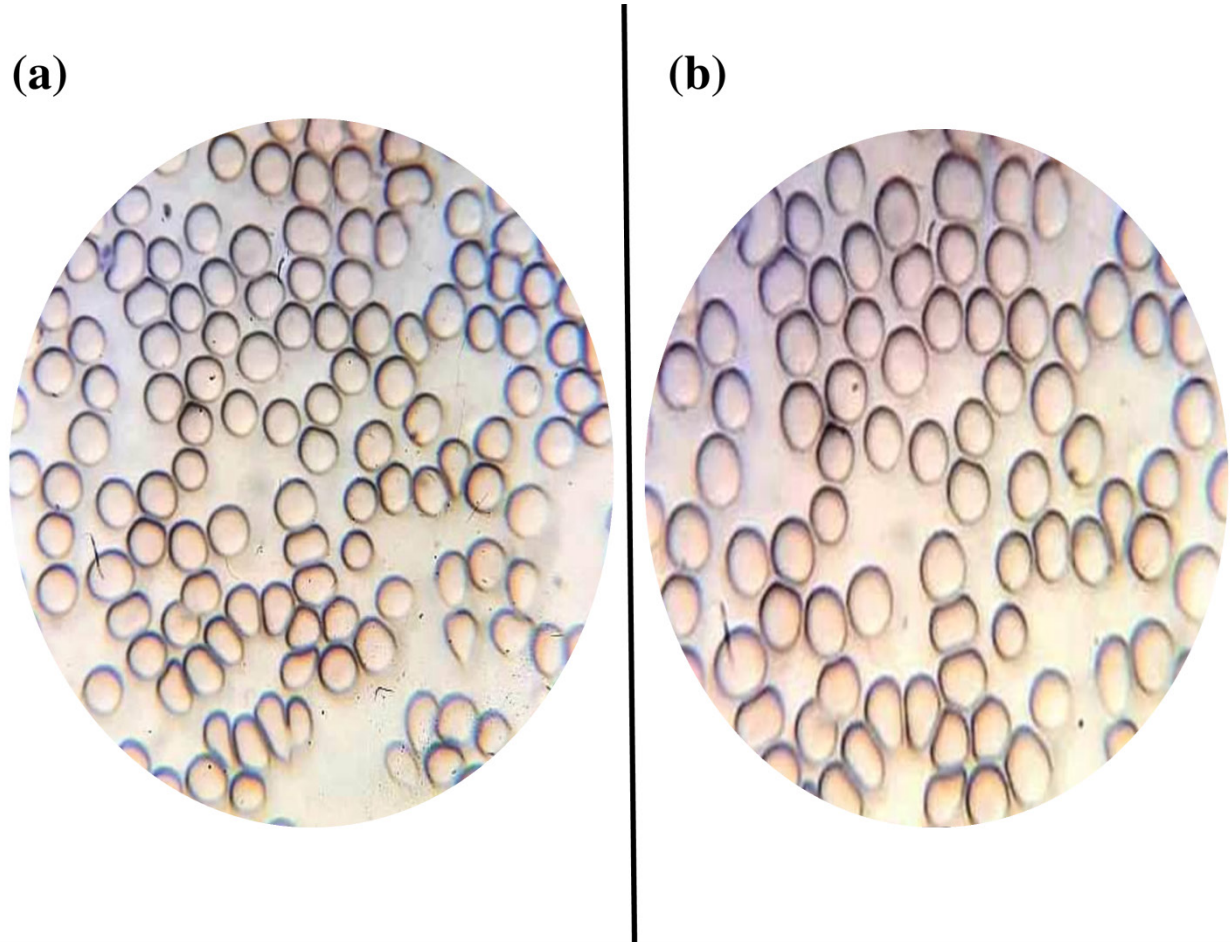

Figure 5: Blood cells exposed with (a) 0.9% NaCl (b) with optimized formulation.

Accelerated Stability Study

The results were not evidenced any noteworthy alterations in the EE (%) and the dimensions of the Microsponges (M7-M9) over the entire duration of storage (Table 4). The outcomes evidenced the stability of microsponges' formulation alone for 6 months at room temperature (25°C). The outcomes of stability studies of optimum batch (G3M9) and free drug amalgamated in gel at 4°C evidenced the decline in drug content of free drug in gel base by 8.4% and 19.24% after 2 weeks and 8 weeks of storage respectively. Thus, it was concluded that the stability of the drug in gel was enhanced due to incorporation of drug in microsponges. The results evidenced the pH stability of the formulations studied during the entire duration of storage.

CONCLUSION

The modified emulsion solvent diffusion method was successfully used to prepare stable moxifloxacin hydrochloride microsponges. SEM evidenced the spherical morphology of porous particles of microsponges. The optimized formulation has shown a mean particle size of about 4.83±0.26 µm that is appropriate for opthalmic application. The microsponges were fused into pluronic F-127-Carbapol *in situ* gels that shown moderate and continued drug discharge. The prepared gels have shown shear thinning properties that is more suitable for eyes. It was investigated that *in situ* gel may have good capacity to carry microsponges encapsulated drug and made the formulation remedially effective. It was observed that the drug observed to follow Higuchi model in release kinetics with most appropriate value of R^2 esteem 0.991. The after effects of *in vitro* and transcorneal penetration have evidenced the drug discharge from developed *in situ* gel up to 24 hrs. Optimized gel formulation has shown 40.05±1.45% at 4 hrs and monitored by sustained release 75.80±1.5% up to 12 hr while marketed formulation has shown 88.74±2.19% at 2 hr. This indicated that improved *in situ* gel demonstrated better conjunctivitis bringing down limit as compared to marketed Moxeza eye drops. No ocular irritancy has been observed with prepared gel in eyes. The *ex vivo* penetration study demonstrated that the optimum formulation releases appropriate quantity of drug with time than other formulations which depicted that the microsponges has a more noteworthy effect in conjunctivitis treatment. The prepared microsponges' loaded *in situ* gel may

affirm to be a decent option in contrast to current marketed formulations and could be used in the treatment of different eye diseases. The developed moxifloxacin loaded microsponge *in situ* gel shows better penetration of drug across the conjunctiva and sustained release from the gel by increasing the contact time with conjunctiva without obscuring the vision in conjunctivitis, hence enhancing the compliance of patient.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

"All applicable international, national and/or institutional guidelines for the care and use of animals were followed." The experimental protocol for this study was permitted by animal ethical committee of Hindu College of Pharmacy, Sonipat.

ABBREVIATIONS

PLGA: Poly(lactic corrosive co-glycolic corrosive); **PCL:** Polycaprolactone; **HPMC:** Hydroxypropyl Methylcellulose; **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **STF:** Simulated tear fluid; **ZOI:** Zone of inhibition; **ANOVA:** One-way analysis of variance: **RBCs:** Red blood cells.

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