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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

FORMULATION DEVELOPMENT AND EVALUATION OF SITE SPECIFIC PERIODONTAL GELS

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ABSTRACT

Periodontal pockets act as a natural reservoir filled with gingival crevicular fluid for the controlled release of antimicrobials directly. This work reflects the present status of nonsurgical controlled local intrapocket delivery of antimicrobials in the treatment of periodontitis. These sites have specialty in terms of anatomy, permeability and their ability to retain a delivery system for a desired length of time. A number of antimicrobial products and the composition of the delivery systems, its use, clinical results, and their release are summarized. In the present study development of site specific periodontal drug delivery system was developed for Metronidazole and Minocycline HCl. Metronidazole is an important active substance that has been widely used in the treatment of some protozoal and anaerobic bacterial infections. Minocycline hydrochloride is a semi synthetic tetracycline, which has been primarily indicated for the treatment of Acne vulgaris. Polarizing photographs of gel lacking CMC, PVP and a gel encircling 10% CMC and 5% PVP are naked. Photographs demonstrated a dark background in the instance of plain gel, though some fan like formations were supposed in the polarizing photograph of the P5C10 formulation. The consequence of drug(s) concentration on gelation and gel melting is done. The gelation temperature was miserable in the company of drug(s) and conical linearly with its growing concentration, while the melting temperature amended with the concentration of drug(s). The mechanical properties of the augmented formulations for the supervision of periodontal disease were resolute.

Keywords: Periodontal pockets, Periodontitis, Metronidazole, Minocycline HCl, gel.

1. INTRODUCTION

Periodontal ailment is a cooperative term designated to numerous pathological circumstances categorized by relapse and inflammation of gums, periodontal sinews, alveolar bone and dental cementum. It is a confined inflammatory rejoinder origin by bacterial infection of a periodontal pocket allied with sub gingival plaque. Even though bacteria are the prime origin of periodontal disease, the appearance of microbial pathogenic aspects unaided may not be necessary to origin periodontitis. Periodontal pathogens yield unsafe byproducts and enzymes that interruption extracellular matrices as well as host cell membranes to create nutrients for their advance. In exploit so, they pledgee impairment directly or indirectly by triggering host facilitated retort that lead to self-injury [1, 2].

Mucoadhesive, Metronidazole (MTZ) comprehending gel systems centered on hydroxyethyl cellulose, corbopol 974 and polycarbophil have been made. Gel is pragmatic sublingually with the help of blunt cannula and syringe [3]. The gel is only marginally operative in shrinking the anaerobic bacterial count. This may be due to low number of bacteria susceptible to MTZ or due to manifestation of bacterial biofilms. Locally reasonable controlled release Minocycline hydrochloride (MHCl) gel may moderately counteract the negative effect of smoking on periodontal healing, ensuing no surgical therapy [4]. The first was tetracycline base overloaded into the microtubular excepient, which was coated with chitosan to assisting impede drug release. The syringeability of this formulation at countless temperatures was reviewed to indorse ease of conveyance to periodontal pocket. A permanence study was accomplished to analyze deviations in the thermoresponsivity over time. In totaling, lidocaine release from gels was probable expending a release apparatus encouraging buccal ailment. The results postulates that an increase in carbopol concentration

vividly increases gel compressibility, hardness and adhesiveness traits that distress ease of gel exclusion from ampoule, ease of gel presentation onto mucosal membrane and gel bioadhesion. Characterization of tetracycline encircling bioadhesive polymer network premeditated for the controlling of periodontal disease and upshot spectacles that upshot of increasing drug concentrations on the rheological and textural belongings was reliant on PVP concentration. Nearby realistic controlled release MHCl gel may moderately counteract the negative conclusion of smoking on periodontal healing. The safety contour, long term retention, antimicrobial commotion instructs that tetracycline encompassing copolymer gels denotes a safe and effective bioerodible therapy for periodontitis [5]. Tetracycline burdened bioadhesive semisolid, polymeric system based upon hydroxyethyl cellulose and polyvinylpyrrolidone and mettronidazole loaded systems based upon Carbopol 974P, hydroxyethyl cellulose and polycarbophil are reported. Alternative such system unruffled of Poloxamer 407 and Carbopol 934P and comprehending propolis abstract were premeditated for the management of periodontal disease. The release of the propolis was organized by the relaxation of polymer chains and the paramount mucoadhesion was noted for the formulation encompassing 60:1 ratio of Poloxamer 407: Carbopol 934P. Another injectable biodegradable gel based on poly (DL-lactide) thawed in a biocompatible solvent N-methly-2-pyrrolidone (NMP) (Atrigel) was widely studied.

The system is based on the ability of glycerides to form liquid crystals, that is, reverse hexagonals on contact with water. The reverse hexagonal form has more favorable sustained release properties, compared with the initial cubic form. The matrix is degraded by neutrophils and bacterial lipase in the GCF. Biodegradable gels are other useful prospects for the delivery of therapeutic agents into periodontal pockets. Bioerodible lactic glycolic acid gels were found to be safe and tetracycline levels observed at days three and eight probably represent significant antimicrobial efficacy [6].

2. MATERIAL AND METHODS

Metronidazole was obtained as a gift sample from Sanctus Drugs & Pharmaceuticals Pvt. Ltd. Hyderabad, Minocycline hydrochloride gift sample from SR Finechem India Pvt Ltd Hyderabad, Hydroxy ethyl cellulose (HEC), Methylcellulose (MC) and poloxamer 407 were obtained from SD Fine- Chemicals Ltd; Mumbai.

2.1. Drug(s) Identification Tests [7]

2.1.1. Melting Point Determination

Melting point of Metronidazole and Minocycline HCl was determined by capillary fusion method.

2.1.2. UV Spectrophotometric Study

MTZ powder 10 mg was accurately weighed and transferred to 100 ml volumetric flask. It was dissolved and diluted to 100 ml with 0.1 ml HCl solution to obtain a final concentration of 100 ug/ml. 3.5ml of the above solution was pipetted out and diluted to 10 ml to obtain a final concentration of 35 ug/ml and scanned in the range 200-400nm in basic spectrum mode148. Similarly standard solution of MTZ was scanned in phosphate buffer.

MHCl powder (10 mg) was accurately weighed and transferred to 100 ml volumetric flask. It was dissolved and diluted to 100 ml with phosphate buffer pH 7.4 solution to obtain a final concentration of l00 µg/ml. 3.5 ml of above solution was pipetted out and diluted to 10ml to obtain a final concentration of 35µg/ml and scanned in the range 200-400 nm in basic spectrum mode150. In a similar manner the scan was made in double distilled water.

2.2. Drug(s) Excipient Compatibility Studies [8]

2.2.1. Physical State

For this study, drug(s) and excipients were taken in ratio similar to that to be taken in formulation. Water was added in a quantity of 0.45%w/v as a worst case. The mixtures prepared were placed in vials, sealed and stored in an oven at a temperature of 50°C±l°C for two weeks. At the end of two weeks the mixtures were observed for their physical state i.e. (discoloration, caking and liquefaction) and analyzed by TLC158.

2.2.2. FT-IR Spectroscopy

The desired quantities of drug with specified excipient(s) (1:1 and 1:5) were granulated. Sieved with # 22 (mesh), dried in air, filled and sealed in dried glass vials and stored at 55°C for 2 weeks. Examined periodically each day for discoloration, caking, liquefaction. Drug excipient compatibility studies were carried out using diffuse reflectance spectroscopy (DRS). In this technique solid drug, excipient(s) and their physical mixtures were diluted with KBr (IR grade) to get the samples for measurement in the transmittance mode (%T). The diffuse reflectance spectrum of the samples against the diluting material was measured by setting the accumulation times to approximate 50. The spectra obtained were evaluated for any incompatibility [9].

2.3. Preparation of Bioadhesive Gels containing MTZ and MHCl

HEC, MC (5%, 10%, 20%, 30% w/w) and poloxamer 407 (10% w/w) were thawed in the applicable weight of phosphate buffered saline (PBS, pH 6.8, 0.03M) using an automatic stirrer. This gel was borne onto an ointment slab and into this, PC (1, 5% w/w), and MTZ laterally with MHCl (5%, w/w; particle size, 63 mm) were steadily mixed (Table 1). PVP, CMC (5%, 10%, 20%, 30% w/w) and poloxamer 407 (10% w/w) were dissolved in phosphate buffered saline (PBS, pH 6.8, 0.03 M). To this gel, PC (1, 5% w/w) and MTZ laterally with MHCl (5%, w/w; particle size, 63 mm) were sundry (Table 1). Ensuing amputation of air under vacuum, formulations were each characterized as entitled beneath or, on some occasions, were stored at 48°C in grade 2 amber glass ointment jars overnight prior to analysis [10].

Table 1: Formulation design of MTZ and MHCl bioadhesive gels

Formulation code	MHC (%w/w)	MC (5%w/w)	MC (10%w/w)	MC (20%w/w)	MC (30%w/w)	Poloxamer (%w/w)	MTZ and MHCl (%w/w)
$M_{10}H_5$	10	5	-	-	5	10	5
$M_{10}H_{20}$	10	20	-	-	1	10	5
$M_{10}H_{30}$	10	30	-	-	5	10	5
M_5H_{10}	5	10	-	-	1	10	5
$M_{20}H_{10}$	20	10	-	-	5	10	5
$M_{30}H_{10}$	30	10	-	-	1	10	5
$P_{10}C_5$	-	-	10	5	5	10	5
$P_{10}C_{10}$	-	-	10	10	1	10	5
$P_{10}C_{20}$	-	-	10	20	5	10	5
$P_{10}C_{30}$	-	-	10	30	1	10	5
P_5C_{10}	-	-	5	10	5	10	5
$P_{20}C_{10}$	-	-	20	10	1	10	5
$P_{30}C_{10}$	-	-	30	10	5	10	5

2.4. Evaluation of Periodontal Gel

2.4.1. Polarizing Light Microscopy

Gel samples were observed beneath a polarizing light microscope (Nikon, Melville, NY) expending a λ¼ compensator to alteration the actuality of birefringence beneath crossed polarized light, fetching a magnification of 100x. The lamellar, cubic and hexagonal phases were acknowledged convening to the sorting reputable by Rosevear [11].

2.4.2. Gelation and Gel Melting

Gelation and gel melting were gauged expending a discrepancy of the Miller and Donavan technique. A 5 ml aliquot of gel was redistributed to test tubes, submerged in a water bath at 4°C, and airtight with aluminum foil. The temperature of the water bath (Haake Phoenix c25P, Karlsruhe, Germany) was enlarged in augmentations of 0.5°C and left to equilibrate for 1 minute at each innovative setting. The sections were then examined for gelation, which was said to have supervened when the meniscus would no extensive move upon slanting through 90°. The gel melting temperature, the temperature at which a gel starts sophisticated upon tilting through 90°, was exhaustive [12].

2.5. Mechanical Characterization of Bio-adhesive Formulations

The automatic properties of all formulations beneath scrutiny were surveyed using texture contour analysis. Formulations were transferred into McCartney (30 ml volume, grade 2 clear glass) bottles to a static height, enchanting care to dodge the hasty of air into the samples. Texture profile scrutiny was accomplished using a Stable Micro Systems Texture Analyzer (Haslemere, Surrey, UK), in texture profile inquiry mode in which the analytical probe (10 mm diameter) was twice crushed into each trial at a distinct rate (2mm s^{-1}) to a depth of 15 mm. A delay period (15s) was permitted among the end of the first and the creation of the second compression and all scrutinizes were achieved at least in quadruplicate [13]. From the ensuing force-time plots, some mechanical constraints may be consequent. These are as follows.

2.5.1. Product Hardness

Force prerequisite to conquer a given deformation was restrained by a Rotovisco (R'V3) cone and plate viscometer.

2.5.2. Compressibility or Spreadability

This is the force criterion to warp the sample during the compression. 24 hrs old gels (1 g) was constrained amid two horizontal plates of 20 cm^2 of which the greater one weighed 46.36 g and a 200 g weight was positioned over it at ambient temperature. A circle of 5 mm in diameter was through and the diameter of the gel was reserved after 5 min.

2.5.3. Cohesiveness

The ratio of the area beneath the force-time curve formed on the second compression cycle to that on the first compression cycle, where progressive compressions are detached by a demarcated rescue period.

2.6. Mucoadhesion of MTZ MHCl Formulations

The mucoadhesion of the formulations beneath consideration was unwavering expending the texture analyzer in tension approach as tracks. Mucin discs were organized by compression of a recognized weight of crude pig gastric mucin (250 mg) in a Carver press for 30 s using a compression force of 10 tones. These were then intricated to a cylindrical probe (length 5 cm, diameter 1 cm) using double-sided adhesive tape. MTZ-MHCl covering formulations were filled into McCartney bottles and centrifuged (3000 rpm for 5 min.) to impound any captured air. The mucin discs were then positioned in contact with the gel formulations and a plunging force was realistic (0.1 N) for a range of times (0.5, 1, 2, 3 and 4 min.). The probe was impassive steeply at a persistent upward speed of 1 mm/sec. and the force criterion to disengage the mucin disc from the gels was reserved as the peak value in the force-time plot [14].

2.7. Rheological Studies

Rheological magnitudes had been agreed out by using two divergent instruments, contingent on the sample viscosity. Oscillatory scopes were carried out at low amplitude (within the linear viscoelastic region) with an angular velocity (w) of between 0.1 and 100 rad/s. Scopes were conducted at four sundry temperatures, namely 10, 20, 30 and 37°C. Pay for to the Bohlin theory that deliberates flow as a supportive spectacle, the coordination coefficient z was cautious from the slope of the curve assimilated by plotting the elastic modulus (G') vs 0) in a log-log plot. The sol-gel transition temperature (T_c) was designed by 'time cure tests' achieved by plotting elastic (G') and loss (G") moduli as function of temperature. Purposes were realised at 1 Hz and at low amplitude, the temperature range was 4-40°C and the temperature ramp was 1°C/ min. The viscosity has been restful at a low shear rate ($0.1\text{-}10^{-1}$) in order to evade slipping assets at the wall surface, perchance instigated by high shear rates [11].

2.8. *In-Vitro* release of MTZ-MHCl

In vitro release of MTZ-MHCl from the bioadhesive gel formulations was executed (in triplicate) using a 37 ml Franz diffusion cell. The diameter of the donor cell was 26 mm and the dissolution standard was PBS. The diffusion cell was water jacketed at 37±2°C. 1.5 g of the gel was displaced to the Durapore HVLP membrane (0.45 um) of the vessel. At unwavering time intervals, 2 ml illustrations of the receptor fluid were taken and analyzed for MTZ and MHCl spectrophotometrically at 318 nm and 273.8 nm individually by synchronised equation routine. The standard was bartered after each specimen to endure sink ailment [15].

2.9. Antibacterial Activity Test

Bacterial strains and advance situations *P. gingivalis*, *S. aureus* and *Escherichia coli* were used in this learning. *S. aureus* and *E. coli* represented Gram-positive and Gram negative bacteria exclusively, and were used as locality strains for antibacterial bustle testing. *P. gingivalis* was subcultured tabloid on augmented blood agar [SBA; trypticase soya agar, supplemented with yeast extract 1 mg/ml, vitamin K_1 5 ug/ml, hemin 5mg/ml and 5% (v/v) human blood]. The other bacteria were cultured on Mueller-Hinton agar (MHA; Merck, Germany) slant at 37°C. To standardize the cells, the allusion strains were advanced to reach log phase and then the suspension was adjusted to 25% transmittance at an OD_{560} compatible to bumpily 10^8 colony-forming units/ml, and this was used assisting for antibacterial bustle testing [16].

3. RESULTS AND DISCUSSION

3.1. Drug(s) Identification Tests

3.1.1. Melting Point Determination

The results of the pharmacopoeial drug(s) identification tests for MTZ and MHCl are tabulated in table 2. The results confirm the identity and purity of both the API'S.

3.1.2. UV Spectrophotometric Study

First process schedules the formation and solving of simultaneous equation using 319 nm and 273.8 nm as two analytical wavelengths for both drugs in phosphate buffer of pH 7.4. The second process is Q value exploration based on extent of absorptivity at 319 and 291.6 nm (as an iso-absorptive point). The overlay spectra of MTZ and MHCl exhibit λ max of 319 nm and 273.8 nm for MTZ and MHCl individually which are rather alienated from each other. Furthermore two isoabsorptive points were pragmatic; one at 291.6 nm and other at 346 nm. 291.6 nm was nominated as the wavelength for synchronized equation of MTZ and MHCl as it lies among the absorption maxima of both the drugs and hereafter it is adopt to be sensitive wavelength. Standard calibration curves for MTZ (Fig.1) and MHCl (Fig. 2) were linear with correlation coefficients (r2) values in range of 0.9995-0.9999 at all the certain wavelengths and the values were average three impressions with standard deviation in the range of 0.001-0.014.

Table 2: Identification Test

Identification Tests	Drug	Experimental Value	Literature Value	Remark
Melting Point	**Metronidazole**	160°C	151-163°C	Purity indicated
	Minocycline HCL	200°C	200°C	Purity indicated
UV Spectrophotometric Study	**Metronidazole** (0.01 M hydrochloric acid 0.001% w/v drug solution)	277 nm	277 nm	indicated
	Minocycline Hcl (0.01 M hydrochloric acid 0.001% w/v drug solution)	275.5 nm	-	indicated
	Metronidazole (Phosphate buffer (pH=7.4))	319 nm	-	-
	Minocycline Hcl (Phosphate buffer (pH=7.4))	273.8 nm	-	-

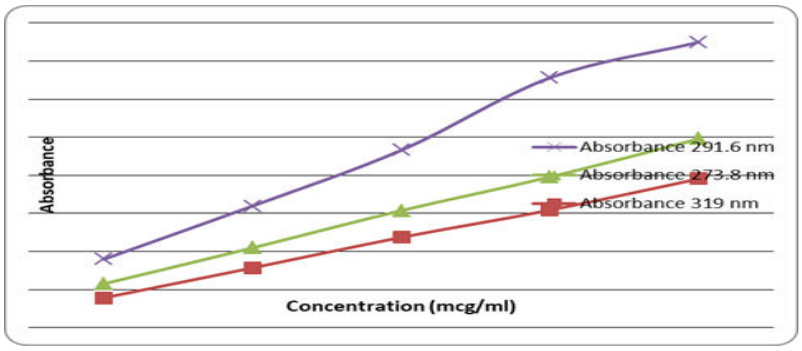


Fig. 1: calibration curve of MTZ at 319nm, 273.8 nm 191.6 nm in phosphate buffer (pH 7.40)

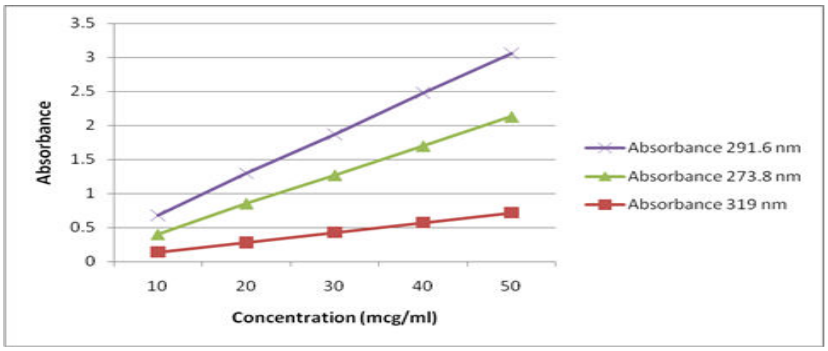


Fig. 2: calibration of MHCl AT 319nm, 273.8 nm AND 191.6 nm in phosphate buffer pH 7.40

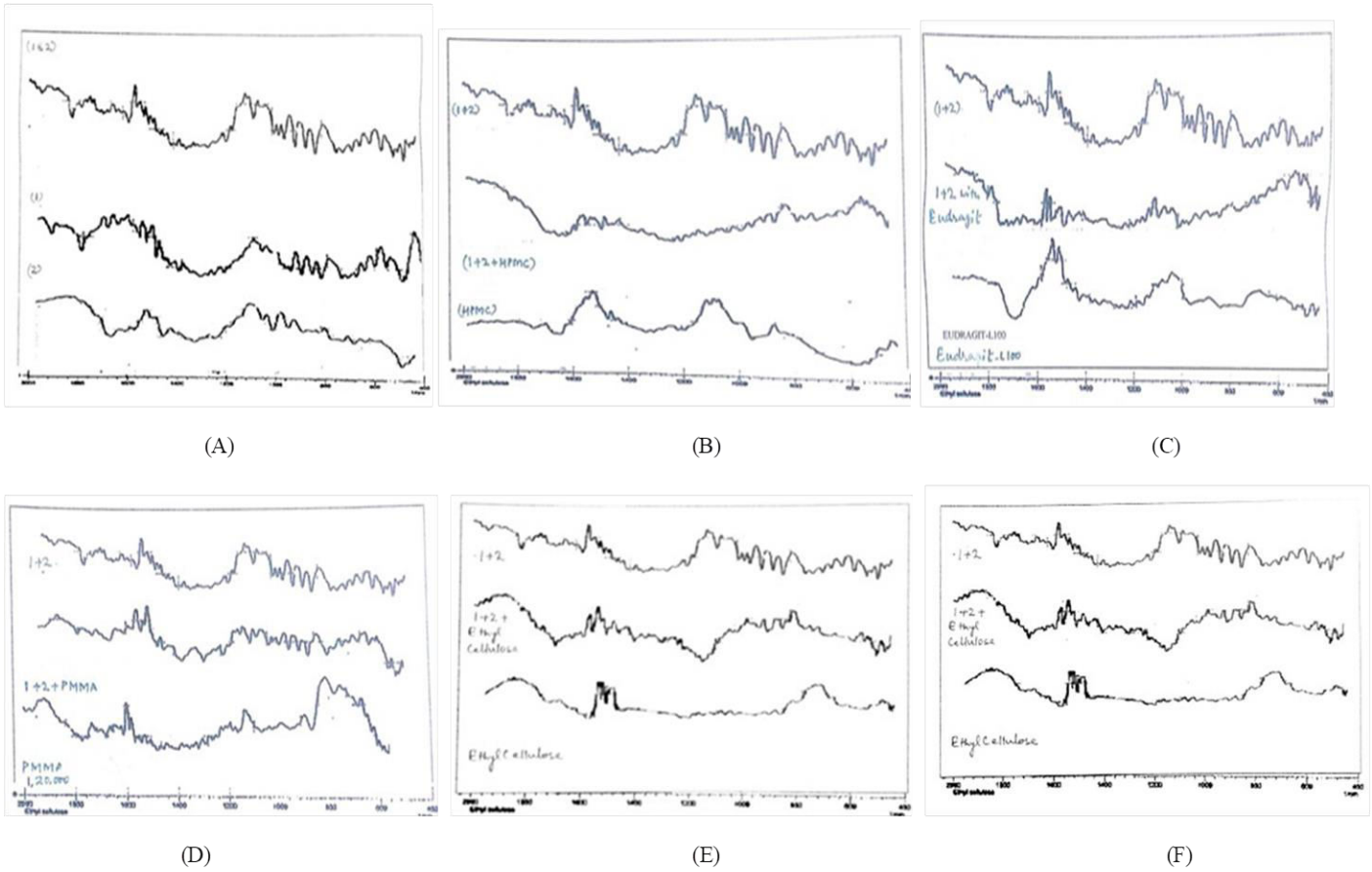
3.2. Drug(s) Excipient Compatibility Studies

3.2.1. Physical State

The physical mixtures of drug and excipient(s) did not show any physical incompatibility in terms of discoloration, caking and liquefaction.

3.2.2. FT-IR Spectroscopy

The presence of excipient(s) did not result in any shift in the DRS of the drug(s) nor did it show the appearance of new peak (Fig. 3). DRS Spectra of mixture of MTZ and MHCl along with polymers retained all the characteristic peaks of MTZ and MHCl and showed no incompatibility. Hence, it can be concluded that periodontal films prepared by polymers ethyl cellulose, hydroxyl propyl cellulose, hydroxyl propyl methyl-cellulose K4M (HPMC K4M), Eudragit L- 100 and polymethyl methacrylate (PMMA 1, 20,000) are stable in terms of physical and chemical stability.



(A) (B) (C)

(D) (E) (F)

Fig. 3: DRS spectra of (A) MHCl , MTZ and mixture, (B) HPMC, MTZ AND MHCl in combination, (C) Eudragit L-100, MTZ and MHCl in combination, (D) PMMA1, 20,000, MTZ and MHCl in combination, (E) HPC, MTZ AND MHCl in combination, (F) EC, MTZ and MHCl in combination

3.3. Preparation of Bioadhesive Gels containing MTZ and MHCl

HEC and MC polymers had been used in the formulation design because of the presence of poloxamer, is reported to diffuse drug in a controlled manner with a linear (zero order) release profile. Such a release profile is desirable in controlled release applications, as it allows for constant dose per time delivery. As polymers exhibits good bioadhesive properties which will prolong its contribution to retention of drug in periodontal pocket as well as release in prolonged fashion so as to enhance clinical effect. Additionally polymers exhibits good bioadhesive property that can prolong its contribution to retention of drug in periodontal pocket so as to enhance clinical effect.

Poloxamer had been selected as the gel prepared from HEC and MC swell in water (responsible for bio-adhesion), is soluble in water and give rise to pH inde-pendent drug release because of presence of ammonium

groups as salts and mechanism of drug release would probably be by dissolution of partially embedded drug allowed by diffusion of embedded drug via matrix pore.
High speed mechanical stirrer had been nominated phosphate buffered saline (PBS, pH 6.8, 0.03 M). Subsequent confiscation of air beneath vacuum, formulations were each categorised as designated below or, on some occasions, were deposited at 48°C in grade 2 amber glass ointment jars overnight prior to analysis and the technique is unassuming, economical and less time unbearable.
Bioadhesive gels containing MTZ and MHCl were prepared by using an automatic stirrer with varied proportions of excipients as mentioned in table 1.

3.4. Evaluation of Periodontal Gel

3.4.1. Polarizing Light Microscopy

Polarizing photographs of gel destitute of CMC, PVP and a gel encircling 10% CMC and 5% PVP are shown in fig. 4. Photographs presented a dark background in the case of plain gel; however some fan like structures were superficial in the polarizing photograph of the P5C10 formulation. Amalgamation of drug(s) did not distress the liquid crystalline phase of gel; it underwent in the cubic phase. Incorporation of CMC and PVP did disrupt the phase structure where it acclimatizes from the cubic phase into the hexagonal phase. However, an improvement in the concentration of PVP did not craft any amendment in the phase edifice. It was uncovered that plain gel is in the cubic phase, which refurbishes to the hexagonal phase after the scheming of CMC and PVP. For PVP, the type of structures accomplished in the presence of discriminatory solvent looks to be a function of the volume fraction of the polar/apolar module. This is endorsed to the aptitude of the macromolecule lumps to swell to a diverse magnitude (created on the expanse of solvent unfilled) with the particular solvents and thus to annoyance the interfacial curvature and ensuing structure. The interfacial curvature is divergent as positive when the interface curvatures near the apolar provinces that is, the micelles are encircled by the polar sticks restricting the apolar provinces inside them, and vice versa. In the cubic phase, interfacial curvature is remarkably positive because of the spherically shaped micelles. The normal hexagonal phase has been assimilated at a high content of CMC (10%) and at 5% PVP because of decreased solvation of the PVP blocks. PVP engrosses water from the system; as a result, less water was presented for CMC, which begun the restoration of the cubic phase to the hexagonal phase.

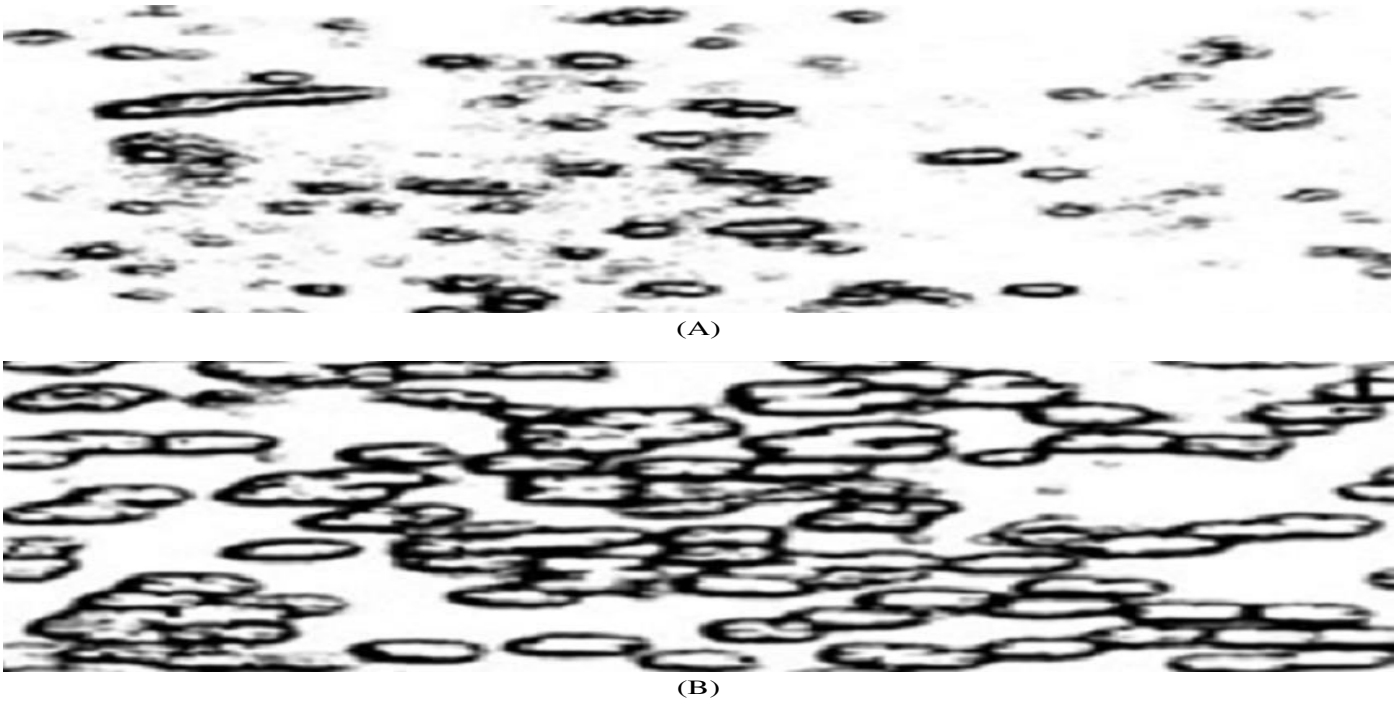


Fig. 4: polarizing light microphotographs of poloxamer gel showing different phases: (A) cubic phase (plain gel), (B) hexagonal phase (PVP-containing gel)

3.4.2. Gelation and Gel Melting

The effect of drug(s) concentration on gelation and gel melting is publicized in fig. 5. The gelation temperature was dropped in the prevalence of drug(s) and waned linearly with its swelling concentration; nevertheless the melting temperature improved with the concentration of drug(s). Substantially, gel formation is concomitant to micellar packing and volume fraction. Scholars have endorsed gelation to the dehydration in the micelle core, a tuning in the micellar volume, or a dwindling in the critical micelle concentration and an escalation in the aggregation number. The finding that drug(s) depressed the gelation temperature was parallel to the result conversant by Esposito. This piece was timidly progressive by an empowering of the collaboration among the hydrophobic portion of the polymer molecules, which might dislocate the micellar edifice and escalation the entanglement of micelles. At established concentration of drug(s), depressing of the critical micellar concentration streamlines closer packing of micelles, which possessions that more energy is prerequisite to interruption the gel structure. The gel structure was rumoured to sustain infrangible with temperature up until an arbitrarily high temperature triggered the wreckage of the gel structure. At higher temperatures, the gel tolerated dehydration, but undue hydrogen bonding and closely packed micelles embarrassed the destruction of the gel structure. As the concentration of the HEC engorged and concentration of MC waned, the gel structure became more strictly packed, with the organization in a lattice decoration. In turn, the interruption of the lattice melting of the gel arises at higher temperatures.

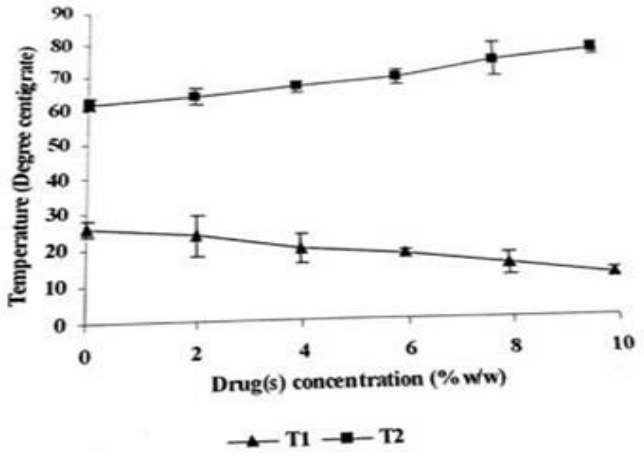


Fig. 5: effect of drug(s) concentration on gelation point (T_1) and gel melting point (T_2)

3.5. Mechanical characterization of bioadhesive formulations

In this study, the mechanical properties of the entrant formulations for the management of periodontal disease were resolute. Texture profile analysis (TPA) outlines the mechanical constraints in empathies of hardness, compressibility and adhesiveness, properties that will anguish the ease of product solicitation into, and preservation within, the periodontal pocket, unconventionally. TPA also accords an assessment of the scope of organizational reformation subsequent product administration (cohesiveness), and facet which will impact product recital. Therefore, in this character, TPA is a pertinent observes for the depiction of formulations deliberated for solicitation to the periodontal pocket. Increased product hardness, compressibility and syringebility were allied with enlarged concentrations of HEC or CMC and PC in apiece formulation. Each of these parameters elected the fighting of each formulation to compression and, consequently, reflects regulations in product viscosity, as previously recognized. Statistical exchanges seemed among the possessions of the polymeric constituents on these mechanical properties and were due to the unexpectedly large firmness, work of compression and syringeability escorting with formulations encircling 30% (w/w) HEC or CMC and 5% PC. These formulations encircled the greatest extents of suspended, unswollen particles and the larger ensuing semisolid properties accounted for the unexpected solidity properties.

Product cohesiveness has been carried to designate spatial traits of structural reorganization resulting product compression. As the PC content was amplified, the mass of adjourned solids enlarged. Subsequently, the semisolid fauna of the product enlarged, which, in turn, decreases formulation cohesiveness. Decreased product cohesiveness akin with increased concentrations of HEC or CMC is a meaning of increased product viscosity, as the viscoelastic properties of these formulations will be expensive by this parameter. The statistical collaboration term imitates the unexpectedly outstanding decline in cohesiveness of formulations encircling the higher concentrations of respectively polymer and is once more due to the comparably superior semisolid character of these formulations.

The mechanical parameters of apiece formulation are handy in table 3. Aggregate concentrations of HEC or CMC and/or PC alluringly increase formulation hardness, compressibility and adhesiveness, yet, they

diminutions cohesiveness. Archetypally extreme and tiniest hardness, compressibility and adhesiveness were akin with formulations encompassing 30% (w/w) HEC or CMC and 5% (w/w) PC, and 5% (w/w) HEC or CMC and 1% (w/w) PC, respectively. In the case of cohesiveness, the inverse was witnessed, i.e. extreme and tiniest values being complementary with formulations encompassing 5% HEC or CMC and 1% PC, and 30% HEC or CMC and 5%, PC, individually. With the omission of formulations surrounding 5% (w/w) HEC or CMC and 1% PC, HEC encircling formulations divulged expressively grander hardness, adhesiveness and compressibility than their equivalents encircling CMC.

Table 3: mechanical properties of the bioadhesive gel formulations of MTZ and MHCl

Formulation code	Hardness Mean (±S.D.)	Adhesiveness Mean (± S.D.)	Compressibility Mean (± S.D.)	Cohesiveness Mean (± S.D.)
$M_{10}H_5$	2.05±0.03	4.11± 0.01	16.87±0.32	0.81±0.01
$M_{10}H_{20}$	2.11±0.01	4.32± 0.25	17.25±0.65	0.76±0.01
$M_{10}H_{30}$	2.55±0.52	4.45± 0.15	17.46±0.85	0.74±0.02
M_5H_{10}	2.01±0.06	3.89± 0.11	16.81±0.11	0.82±0.03
$M_{20}H_{10}$	1.87±0.05	3.77± 0.02	16.74±0.02	0.86±0.01
$M_{30}H_{10}$	1.82±0.07	3.75± 0.18	16.71±0.07	0.87±0.02
$P_{10}C_5$	0.70±0.08	2.58± 0.09	6.75±0.11	0.88±0.03
$P_{10}C_{10}$	0.87±0.04	2.71± 0.11	7.24±0.05	0.86±0.01
$P_{10}C_{20}$	0.93±0.10	2.79± 0.23	7.96±0.45	0.85±0.10
$P_{10}C_{30}$	1.26±0.12	2.97± 0.56	8.42±0.52	0.83±0.02
P_5C_{10}	0.68±0.05	3.81± 0.48	11.41±0.23	0.79±0.01
$P_{20}C_{10}$	0.64±0.09	1.88± 0.20	6.67±0.33	0.89±0.03
$P_{30}C_{10}$	0.61±0.14	1.29± 0.53	6.25±0.25	0.94±0.02

Each value is the mean ± SD (n=3)

3.6. Mucoadhesion of MTZ MHCl Formulations

The syringeability of each formulation is prevailing in table 4. Once more, aggregate concentrations of each polymeric element (HEC/CMC and/PC) vividly escalations the force essential to exorcise apiece formulation from a periodontal syringe over a fixed aloofness. Formulations encircling (5% w/w) HEC or CMC presented statistically analogous values of work of syringeability (P.0.05), nevertheless the work of syringeability of formulations encircling 30% HEC encouragingly outdone those formulations encircling (30% w/w) CMC.

In this investigation, the adhesive properties of the entrant formulations were dissected using two methods, unambiguously texture profile analysis, which entitles the work criterion to sequester a polymeric probe from the test formulation, and also by estimation of the detachment force prerequisite to daze the adhesive bond between each formulation and a compressed mucin disc. In tallying, for all formulations, time of exchange with the mucin disc sensitively predisposed the strength of the mucoadhesive bond. This may be progressive by hydration of the mucin, due to the promise of moisture from each formulation, which in turn approvals interpenetration of the polymeric chains in mucin and those in each formulation. The absolutions from these annotations were additional with formulations encircling (5% w/w) CMC in which cohesive bond disaster befallen.

The consequence of HEC on the bioadhesive properties of formulations encircling PC was vividly greater than that of CMC. The probable mechanisms originating this inconsistency were emphasized within the statistical interaction term amid polymeric constituents with admiration to adhesiveness and impartiality force. Once more, in these interactions, formulations encircling 5% (w/w) PC and either 30% (w/w) HEC or CMC unveiled surprisingly large numerical values of adhesiveness and impartiality forces. It has been recounted that the bioadhesive properties of formulations including PC increases as the number of uncharged carboxylic acid groups increases. Therefore, formulations encircling the higher concentration of HEC or CMC and 5% (w/w) PC haunted the greatest multitudes of unswollen, uncharged particles and, subsequently, these formulations definite the greatest adhesion to both mucin and the polymeric probe. The preservation of the product in the periodontal pocket was the foremost distress, as it was prerequisite to guarantee that the product would sustain there for the

planned period of drug release. The results of the detachment force revision provisions the hypothesis that the probable mechanism of the mucoadhesion uncovered by the dehydration of the mucosa (i.e. water uptake by the mucoadhesive material). The extent of water taken up by the liquid crystalline gels directed the mucoadhesive force; the gel having grander water uptake capacity revealed greater mucoadhesion.

Table 4: Mucoadhesive strength of formulations containing MTZ and MHCl

Formulation code	Force required to break the mucoadhesive bond following contact between formulations and mucin for a range of times(s)			Work of syringe-ability (N mm)
	60	180	240	
$M_{10}H_5$	0.37±0.02	0.42± 0.01	0.57±0.01	77.43±3.24
$M_{10}H_{20}$	0.40±0.01	0.47± 0.02	0.58±0.05	81.23±2.31
$M_{10}H_{30}$	0.48±0.01	0.56± 0.01	0.61±0.04	89.49±1.02
M_5H_{10}	0.34±0.02	0.37± 0.01	0.54±0.02	74.69±4.20
$M_{20}H_{10}$	0.32±0.03	0.38± 0.02	0.51±0.01	71.23±1.22
$M_{30}H_{10}$	0.30±0.02	0.36± 0.03	0.49±0.02	69.45±2.41
$P_{10}C_5$	0.15±0.02	0.21± 0.02	0.26±0.05	52.11±3.25
$P_{10}C_{10}$	0.18±0.01	0.22± 0.01	0.29±0.03	53.98±1.23
$P_{10}C_{20}$	0.21±0.02	0.28± 0.03	0.35±0.01	55.06±3.62
$P_{10}C_{30}$	0.27±0.03	0.36± 0.05	0.30±0.01	56.71±2.55
P_5C_{10}	0.14±0.01	0.20± 0.0	0.24±0.02	49.36±1.02
$P_{20}C_{10}$	0.12±0.02	0.18± 0.01	0.22±0.01	47.89±1.85
$P_{30}C_{10}$	0.11±0.03	0.16± 0.02	0.20±0.03	44.33±2.32

Each value is the mean ± SD (n=3)

3.7. Rheological Studies

In this alteration, a sequence of preliminary possessions on the rheological portrayal of poloxamer based gels is attainable (Table 5). This reading was accomplished in order to delineate the universal rheological behavior of these judiciously novel constituents and to pay for information on their edifice, as a function of temperature and of the survival of solubilized guest molecules (i.e. MTZ and MHCl). In convinced, we indomitable the sol-gel transition temperature (To) by 'time cure tests', the regularity obsession of the elastic modulus G' by regularity swish tests, and the temperature reliance of G' and the z coefficient. From the analysis of the effects attained by this series of experimentations, the ensuing overall contemplations can be drawn.

(a) The poloxamer gels are categorised by a piercing transition from a liquid (sol) to a structured (gel) behavior at a well demarcated temperature (Tc) unwavering by the analysis of T Vs. G' curves. The utmost value of Tc (21oC) was institute in the case of 25% poloxamer gel while 20% and 30% gels confirmation a lower Tc value.

(b) In all sections, both elastic modulus G' and coefficient enlarged as temperature increases. In this affection, it should be eminent that G' and z give suggestions about the edifice strength and the structure synchronization, separately.

(c) At temperature above 15°C, samples illustration pseudo plastic behavior pigeonholed by a typical shear thinning behavior.

(d) The manifestation of drug(s) causes a transferal T_c to a lower value (ΔT_c=6.8°C) and parallel increase of both G' and z value.

Taken organized, these upshots specify that MTZ and MHCl can have a positive influence on both gel structure and strength. This piece was tentatively explicated by a facilitation of the interfaces among the hydrophobic portions of the polymer molecules accountable for the gelation practice. This facilitation might be due to the enclosure of the planar MTZ and MHCl molecule within the polyoxypropilenic fractions.

The biocompatibility of poloxamer 407 and tranquil riddance from the body and individualities such as low viscosity at the twinkling of organization were the aims to use this polymer to articulate a gel, constructed, for the handling of periodontitis. It was revealed, in a maiden revision, that this poloxamer gel proclamations MTZ and MHCl for the epoch of seven days *in vitro*.

Table 5: Rheological characterization of MTZ and MHCl containing gels

Gel	Composition	Guest molecule	Tc [°C]	G'[Pa]			G"[Pa]			z [Pa. s]			η [x10s^{-1}]
				10°C	30°C	37°C	10°C	30°C	37°C	10°C	30°C	37°C	37°C
Poloxamer	20	No	15.8	0.0004	26.240	30.060	0.6	1703	1053	-	73.8	185.4	68.6
	25	No	21.01	0.0007	12.440	15.390	0.5	1149	1443	4.1	11.2	23.6	46.6
	25	Yes	15.81	0.0063	24.090	24.120	1.1	1869	1040	0.7	61.5	106.6	67.5
	30	No	12.20	0.992	24.120	24.770	177	1992	2316	8.8	40.0	37	63.1

3.8. *In-Vitro* release of MTZ-MHCl

HEC and/or CMC encompassing gels ensuing dissolution in PBC or water correspondingly deliberate primary gels, the viscosity of which was aquiline on of the concentration of polymers specified the upshot of dissimilar types of bioadhesive polymers and their emergent concentrations on the release of drug(s) from gel formulations. PC is a cross linked imitative of polyacrylic acid which does not liquefy but disclosures swelling, the latitude of which is contingent on the extent of presented water extant in the formulation i.e. the water does not escorting with the dissolved polymer. Consequently, in the formulations encircling 30% (w/w) HEC or CMC, the amount of free water is decayed and the magnitude of swelling of PC in these formulations is wilted in evaluation to the formulations incorporating 5% (w/w) HEC or CMC. In formulations encircling 5% (w/w) HEC or CMC, PC ensued mostly in swollen state. In all formulations MTZ and MHCl was existent in a suspended form. The state of PC in every formulation was predisposed, at least in part for voluminous of the annotations of this adjustment. Decreased drug(s) release from formulations comprehendding enlarged concentrations (30% w/w) of either HEC or CMC may be nominated to the assenting increase in product viscosities that are related with enlarged polymer concentrations. Decreased release affiliated with increased concentrations of PC in formulations grasping 5% (w/w) HEC or CMC may also be illuminated by the conglomerated increased product viscosities consequent swelling of this polymer within the formulation.

Drug(s) release from CMC systems was grander than from their HEC parallel item and was due to loftier viscosities of HEC/PC formulations. An improved PC concentration predominantly declines the release of drugs. The release of drug(s) from formulations restricted 30% (w/w) HEC and 5% (w/w) PC was sensitively countless than those encompassing 30% (w/w) HEC and 1% (w/w) PC. These interpretations may be liberal by the practiced degrees of swelling of PC in each formulation. Product swelling was countless for formulations comprehending HEC, in appraisal to those comprehending CMC, due to the grander masses of unswollen PC. Incontestably as an upshot of unnecessary swelling of this polymer concluded dissolution testing, partial product disintegration arisen for formulations comprehending 30% (w/w) HEC and 5% (w/w) PC. Therefore, surface areas of these formulation amplified, which in turn growths the rate of drug(s) release.

Table 6: model independent parameters anad release data analysis of bioadhesive gels

Formulation code	DE_{24}%±S.D.		K±S.D.		$t_{50\%}$±S.D.		n	
	MTZ	MHCl	MTZ	MHCl	MTZ	MHCl	MTZ	MHCl
$M_{10}H_5$	60.11±0.11	64.36±0.41	18.20±0.11	23.44±0.11	32.84±0.94	33.65±0.94	0.564	0.554
$M_{10}H_{20}$	62.78±0.23	67.36±0.24	17.98±0.20	17.98±0.20	34.21±0.25	36.36±0.56	0.636	0.654
$M_{10}H_{30}$	68.20±0.56	72.36±0.55	18.95±0.02	18.95±0.02	38.76±0.89	39.25±0.44	0.587	0.557
M_5H_{10}	58.77±0.54	66.36±0.25	17.57±0.55	18.34±0.54	29.87±0.98	30.21±0.11	0.649	0.657
$M_{20}H_{10}$	56.29±0.89	60.36±0.25	17.38±0.002	19.34±0.36	27.86±0.78	28.25±0.81	0.548	0.557
$M_{30}H_{10}$	55.25±0.54	56.25±0.54	17.01±0.50	19.36±0.55	24.72±0.46	25.36±0.44	0.570	0.573
$P_{10}C_5$	39.21±0.23	43.56±0.58	1.57±0.006	3.65±0.56	18.27±0.82	18.55±0.88	0.51	0.514
$P_{10}C_{10}$	41.01±0.25	42.58±0.58	1.62±0.85	2.36±0.55	19.11±0.45	19.21±0.45	0.500	0.543
$P_{10}C_{20}$	44.60±0.45	47.65±0.54	1.78±0.12	4.25±0.52	21.89±0.72	21.89±0.72	0.500	0.515
$P_{10}C_{30}$	46.91±0.56	49.81±0.60	16.11±0.002	18.23±0.02	22.77±0.48	22.77±0.48	0.539	0.554
P_5C_{10}	25.61±0.89	25.61±0.89	1.51±0.006	5.36±0.08	17.28±0.25	17.28±0.25	0.516	0.551
$P_{20}C_{10}$	22.13±0.78	22.13±0.78	1.47±0.01	1.55±0.05	15.78±0.28	15.78±0.28	0.532	0.523
$P_{30}C_{10}$	21.45±0.80	25.36±0.88	1.41±0.23	2.73±0.25	14.60±0.26	14.60±0.26	0.521	0.524

Each value is the mean ± SD (n=3)

The time criterion for the 50% release of drug(s) i.e. $t_{50\%}$ from respectively formulation is revealed in table 6. The times critical for 50% drug(s) release from formulations comprehending HEC were encouragingly greater than those enfolding equivalent concentrations (%w/w) of CMC and PVP. DE after 24 hrs (DE_{24}%) and $t_{50\%}$ were used to comrade the drug release characteristics of sundry formulations.

3.9. Antibacterial Activity Test

Antibacterial deeds are concised in table 7. Briefly, Sample 1 had a growth inhibition zone on agar with all three strains. Remarkably, MHCl pay for related zones of inhibition with reverence to E. coli and S. aureus [16.4 and 23.7 mm for MHCl, 18.2 and 18.4 mm for Sample 1]. However, with P. gingivalis, MHCl was substantially more persuasive [45 mm for MHCl , 24.7 mm for Sample 1]. The E. coli and P. gingivalis used were inclined to MHCl with MICs of 0.2-2 uM and MBCs of 1-8 uM. P. gingivalis was inclined to MTZ with an MIC of 0.7 uM and an MBC of 1.4 uM. At the critical concentration (0.14 uM) through incubation times of 10 mm, 1 h and 2 h, the numbers of P. gingivalis declined vividly with no practicable counts after 2 h.

Table 7: Antibacterial activity of $M_{10}H_{30}$ formulations

Agent	pH (±S.D.)	Bacterial Strains	Amount/inhibition zone		MIC	MBC
			n = 3	mm (±S.D.)		
Sample 1 ($M_{10}H_{30}$)	3.52±0.5	*E.coli*	20mg	18.2 (0.5)	ND	ND
		S. aureus	20mg	18.4 (0.6)	ND	ND
		P. gingivalis	20mg	24.7 (0.8)	ND	ND
MHCl	2.1-2.3*	*E.coli*	30µg	16.4(0.4)	2 µM	8 µM
		S. aureus	30µg	23.7(0.3)	ND	ND
		P. gingivalis	30µg	45.0(2.4)	0.2 µM	1 µM
Metronidazole	5.8*	*E.coli*	30µg	(-)	ND	ND
		S. aureus	30µg	(-)	ND	ND
		P. gingivalis	30µg	75.0(1.6)	0.7 µM	1.4 µM

*ND: Not determined; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration *Values from Merck Index*

4. CONCLUSION

The management of periodontitis customarily involves a systemic schedule with antibiotics to alter the doubtless pathogenic flora. Additionally, some tetracyclines, by inhibiting collagenase, appear to contract bone demolition. Alternative methodology is to surgically disregard the pocket and raconteur the bone to reassure alveolar bone progress. Local application into periodontal pocket could be very advantageous, both in terms of increasing drug concentration directly in the action site, and in preventing systemic side effects such as gastrointestinal complaints, depression and tachycardia. Gels were categorized by an atypical rheological comportment, as a function of polymer concentration, temperature and existence of drug(s) and retain apt properties as intrapocket drug(s) delivery system for periodontal healing. In the present study, a substantial lessening in mean plaque index, gingival index, sulcus bleeding index, and probing pocket depth, and a substantial gain in clinical affection were perceived.

In conclusion, metronidazole sustained-release film combined minocycline hydrochloride is an effective drug to treat chronic periodontitis, for it can apparently reduce gingival index, plaque index, sulcus bleeding index and pocket depth so as to improve periodontal condition. Moreover, its therapeutic effect is evident and with less adverse reactions and low relapse rate. Therefore, it has good clinical effect and is worthy of promotion.

Conflict of interest

There is no conflict of interest and disclosures associated with the manuscript.

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