Azhar Int J Pharm Med Sci 2024; Vol 4 (2):88-100
(Research Article)

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Fabrication and *In Vitro* Assessment of Mucoadhesive Gels for Inner Ear Drug Delivery

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Article history: Received 2023-08-08 **Revised 2023-09-26** **Accepted 2024-01-08**

Abstract: The purpose of the current study was to fabricate and assess thermoreversible mucoadhesive in-situ otic gels of ofloxacin. This drug delivery technique may alleviate the drawbacks of conventional otic formulations like short residence time, leakage of medicaments from ear and repeated use. A variety of in situ otic gel formulations were fabricated using various mucoadhesive polymeric ratios of HPMC 100, HEC, carrageenan, chitosan and Carbopol 934. There was no drug-polymer interaction, according to the results of the FTIR and DSC analyses. In the solid state, all formulations appeared to be clear, with pH between (5.1 ± 0.08 and 7.1± 0.04) i.e., no irritation is expected after administration. The gelling temperature was ranged between (28.17± 0.56- 32.11±0.15°C). Mucoadhesive strength was ranged between (512.11±6.35- 3716±14.2 dyne/cm2) that insure prolonged adhesion. The drug content was complied with pharmacopeial range. Sol-gel transition was confirmed by the rheological study at physiological ear temperature. The in-situ gel that was created released the OFX up to 8 hours compared to the ear drops in the market which release the drug within 2 hours. Zero and Higuchi order kinetics were shown by the Kinetic evaluation of the release data. According to the greatest dissolution efficiency % (DE%), F1, F2, F4, F7 and F10 were chosen for short term accelerated test of stability at two different temperatures of 4 ± 2 and 25 ± 2 ºC/60 ± 5% relative humidity (RH). F1 containing poloxamer 407 (18% w/v) and hydroxypropyl methyl cellulose K100 (0.5% w/v) displayed the highest t90 value.

Keywords: Ofloxacin; In- situ; Otic gel; Poloxamer 407; Thermoreversible; Dissolution efficiency.

1. INTRODUCTION

Acute otitis media (AOM) is an infection-related illness that results in inflammation of the middle ear mucosa. This abnormal fluid collection in the middle ear creates a resistance to the transfer of acoustic energy and conductive hearing loss because the tympanic membrane cannot vibrate as easily. Additionally, Chronic Suppurative Otitis Media (CSOM) is a chronically inflamed condition of the middle ear mucosa that may also be accompanied with tympanic membrane perforation and dysplasia of the middle ear mucosa[1]**.** Both (AOM) and (CSOM) are acute bacterial infections of the ear canal's skin that are typically caused by the bacteria Escherichia coli, Pseudomonas aeruginosa, or Staphylococcus aureus[2]. This illness typically gets better completely in 7 days. But if the infection isn't treated, it can spread to deeper nearby structures and cause consequences like tympanic membrane (TM) perforation, mastoiditis, labyrinthitis, petrositis, meningitis, brain abscess, hearing loss, lateral and cavernous sinus thrombosis, and meningitis[3]. Several antibiotics are available for treatment of such cases and the most common one is Ofloxacin as its otic solution is the only topical medication approved by the US Food and Drug Administration (FDA) for treatment in perforation of tympanic membranes[4]. OFX is a synthetic fluoroquinolone that has a bactericidal action. It acts by attaching to and blocking the bacterial enzymes topoisomerase II (DNA gyrase), which relaxes supercoiled DNA, and topoisomerase IV, which separates connected daughter chromosomes after replication. These inhibiting effects restrict DNA transcription, replication, and repair, which prevent bacterial cells from dividing[5,6]. OFX is available in market in the form of otic solution but because of rapid leakage of

Cite this article: Ammar AA., Shawky SM., Selim RA. Fabrication and In Vitro Assessment of Mucoadhesive Gels for Inner Ear Drug Delivery. Azhar International Journal of Pharmaceutical and Medical Sciences, 2024; 4(2): 88-100. doi: 10.21608/AIJPMS.2024.228098.1229

DOI: 10.21608/AIJPMS.2024.228098.1229

solution out of the ear it leads to short residence time and require frequent administration that may not meet the patient compliance.

Otic drug delivery has gained increasing interest and has advanced rapidly over the past few decades. The formulation of thermosensitive reversed gel provides a unique approach to drug delivery, specifically targeting the middle ear. This innovative gel system undergoes a transformation from a liquid to a gel state upon contact with body temperature[7,8], allowing for prolonged drug release, reduce systemic drug absorption, extend the drug effect and reduces the frequent administration so, improves patient compliance[9-11].

In the current study, an effort was made to establish an alternative and new dosage form of in-situ otic gelling system of ofloxacin utilizing different mucoadhesive polymer at different concentration and was assessed for clarity, pH, gelation temperature, mucoadhesive strength, rheological study, in vitro release and dissolution efficiency % (DE%). Finally, the formulation exhibited the highest DE% were chosen for stability test. The selected formulation at each temperature were examined for concentration remained of ofloxacin and the change in pH and gelation temperature before and after storage period.

2. METHODS

2.1. Materials

Ofloxacin was provided as a gift sample from Al Kahira Pharmaceutical & Chemical Industries Company, Egypt. The AUG Pharm for Pharmaceutical Industries, Egypt provided the hydroxypropyl methyl cellulose (HPMC K100). Poloxamer 407 and hydroxy ethyl cellulose (HEC) were graciously provided by the EPICO (10th of Egypt's Ramadan City) Egyptian International Pharmaceutical Industries Co. The kind donations of carrageenan and Carbopol 934 (CP934) came from Delta pharm (Egypt). Chitosan was purchased from Sigma pharmaceutical company, Egypt. Al Hekma pharm, Egypt provided the benzalkonium chloride. From the Egyptian pharmaceutical company El-Nasr, sodium hydrogen phosphate and potassium dihydrogen phosphate were purchased.

2.2. Investigation of ofloxacin's compatibility with proposed polymers

Both fourier transform infrared (FTIR) and differential scanning calorimetry (DSC) were employed to study the compatibility of ofloxacin with the recommended polymers.

2.2.1. Fourier transform infrared

Fourier transform infrared is a form of vibrational spectroscopy which is incredibly helpful method for identifying functional groups in pure molecules to establish their identity. The process involved spreading a sample (ofloxacin and physical mixes of medication with each polymer 1:1 w/w) compressed into disc utilizing KBr. In order to record the bands of IR, the prepared disc was stored in the instrument by being placed in the sample holder. Wavelengths between 4000 and 400 cm^{-1} were employed to record the entire spectrum[12].

2.2.2. Differential scanning calorimetry

DSC was employed to create thermograms of the pure substance as well as physicochemical combinations of the medication and suggested polymers in a 1:1 (w/w) ratio. About 3 mg of the samples were hermetically sealed in an aluminium crucible and heated between 25 and 400 °C at a rate of 10 °C/min while being scanned at a flow rate of 25 ml/min in a dynamic N2 environment. Compatibility of both was determined by observing any changes occurred in melting point of the drug[13].

2.3. Formulation of in-situ thermoreversible mucoadhesive otic gels

The gel-forming solutions were made using the cold process[14]. Poloxamer (18%) was added to half of the distilled water with constant stirring, then left at 4°C for 24 hours until a transparent solution was produced. This polymer base received the required amount of benzalkonium chloride (0.01% w/v) as a preservative. Mucoadhesive polymers (HPMC 100, HEC, carrageenan, chitosan and Carbopol 934) in different ratios were dissolved in distilled water before being added to the poloxamer solution[15-17]. The drug (0.3%) which was separately solubilized in PBS 7.4 was added to the previously prepared polymer solution[18]. 0.1 N acetic acid was needed to dissolve chitosan[19]. Triethanolamine was added to Carbopol 934 solution for adjusting the pH needed for gel formation[20]. The component of ofloxacin formulations is presented in Table 1.

2.4. Evaluation of ofloxacin in situ otic gel formulations

2.4.1. Physical investigation of thermoreversible mucoadhesive otic gel

2.4.1.1. Clarity

On a black and white background, all formulas were observed visually. The clarity of formulations received a grade of: opaque +, clear ++, and extremely clear (glassy) +++[7,21].

2.4.1.2. Assessment of pH

In 10 ml volumetric flask 1 ml gel sample was transferred and complete the volume with distilled

water. pH meter which was calibrated before the measurement used to determine the pH of resulting solution[22,23].

Table 1. Composition of thermoreversible mucoadhesive otic gel formulations using different concentrations of different mucoadhesive polymers.

Formulae	HPMC K100 (%)	HEC (%)	Carrageenan (%)	Chitosan (%)	Carbopol 934 (%)
F 1	0.5				
F 2	1				
F 3	1.5				
F 4		0.5			
F 5		1			
F 6		1.5			
F 7			0.5		
F 8			1		
F 9			1.5		
F 10				0.1	
F 11				0.3	
F 12				0.5	
F 13					0.2
F 14					0.4
F 15					0.6

2.4.1.3. Measurement of the gelation temperature

Ten millilitres aliquots from each formulation were put in a water bath after being placed into a test tube and parafilm-sealed. The water bath's temperature was gradually raised by 1 ºC every 2 minutes until the gel was created, and then it was allowed to acclimatize for 5 minutes at each subsequent setting. After that, the sample's gelation was checked[24].

2.4.1.4. Mucoadhesive strength

Mucoadhesive potential of each formulation was identified by measuring the force needed to separate each formulation from the membrane. It was measured utilizing a modified physical balancing (mucoadhesion test device) containing a central lever, an arm holding a pan, and an additional arm holding a glass vial. Using double-sided sticky tape, a second glass vial was attached to the balance's hardwood base in an inverted orientation. The balance was equilibrated on both sides. Then The membrane was inserted into the space between the fixed vial's upper and lower surfaces. A double-sided adhesive tape was used to attach (0.5 g) of otic gel to the base of an inverted glass vial. To ensure that the gel sample stayed adherent to the mucosal membrane, the distance between the two vials was adjusted. For ten seconds, enough pressure was applied to both vials to ensure that the gel adhered to the mucosa properly. A constant weight was applied to the pan until separation occurs. The detachment stress in dyne/cm2 was used to express the mucoadhesive force. as demonstrated by the following equation:

$$\text{Mucoadhesive strength } \left(\frac{dyne}{cm2}\right) = \text{m } \frac{g}{A}$$

Where, m represents the weight in grammes needed to separate the object, g or gravity's acceleration, is 980 cm/s^2, and A is the mucosa exposed area in cm^2 [25].

2.4.1.5. Determination of drug content

Exactly measured one milliliter of each compound was shaken with 100 milliliter PBS with a pH 7.4 and vigorously shaken until all of the drug has dissolved. The aforementioned solution was filtered using Whatman filter paper, With PBS pH 7.4, 1 ml was obtained and diluted to 10 ml. Using a standard

calibration curve and plain PBS with a pH of 7.4 as a blank, the drug content was determined spectrophotometrically at 288 nm. An average of three readings was used to determine the mean percent drug content[5].

2.4.1.6. Viscosity and rheological study

By means of Brookfield equipment (PVSTM Rheometer, Brookfield, WI, BOB/STATOR: PVSB1-D-HC, Cup Type: Hastelloy C) The viscosity of each formulation's sol phase (at temperatures below 10°) and preformed gels (at temperatures between 35 and 37°) of thermoreversible mucoadhesive otic gel was measured. The measurement was carried out at various RPMs between 5 and 250. The rheological data such as viscosity in centipoises (cps), shear stress (dyne/cm^2) and shear rate (sec^{-1}) were employed to create a comprehensive rheogram[26].

2.4.1.7. In vitro release testing of ofloxacin from formulations of thermoreversible mucoadhesive otic gel

The dialysis bag technique was used to conduct ofloxacin's in vitro release from formulations of thermoreversible mucoadhesive otic gel. The cellulose dialysis bag was presoaked in distilled water for 12 hours. Otic gels equivalent to 0.03 g of ofloxacin were precisely weighed and transferred into the dialysis bag, and both ends of the bags were sealed tightly. The bags were submerged in 20 ml PBS of pH 7.4 which served as the receptor cell, placed in a shaker water bath set at 37± 0.5°C and shaked at 100 rpm. At regular intervals, aliquots were taken out of each sample's receptor cell and replaced with comparable volumes of the fresh release media[27]. Samples were analysed spectrophotometrically at λ_{max} 288 nm using PBS as a blank. The average concentration was determined after measuring three samples. A number of drug release kinetic models were applied to the in vitro release data in order to better understand the pattern of ofloxacin release from thermoreversible mucoadhesive otic gels (zero-order, first order, Higuchi and Korsmeyer-Peppas models). The correlation coefficient was obtained.

2.4.1.8. Dissolution efficiency calculation (DE%)

The dissolution profile of all formulations can be compared using dissolution efficiency. It was estimated using the ratio between the area under each dissolution curve (AUC) generated from the profile dissolution (as described by the trapezoidal rule) at time (t) and the complete area of the rectangle considered to represent 100% dissolving for the same time period. The following equation represent DE%

$$DE\% = \frac{\int_0^t y\ x\ dt}{y100\ x\ t} \text{ x } 100$$

Where $\int_0^t y\ x\ dt$ x100 is the area under the curve at time intervals t, represented as a percentage value; and (Y_{100}) is the amount of drug release in 100% at the same time[28,29].

2.4.1.9. Stability studies

Formulations exhibited the highest DE% were further studied for its stability. Glass vials with tight lids were used to store a portion of each composition and kept in a thermostatically controlled oven at 25 ± 2 °C/60 ± 5% RH and refrigerator at a temperature of 4± 2 ºC. At the start of the experiment and at intervals of 7, 14, 30, 45, 60, 75, and 90 days, samples of these formulations at each temperature were taken. The gelation temperature and pH before and after storage was measured[24]. After varying times, calculations were made to determine how much ofloxacin was still present in the gel formulation. The content of ofloxacin was determined using UV spectrophotometry after carrying out forced degradation study (see supplementary material). The correlation coefficient was calculated using zero, first, and second order equations. The more appropriate correlation coefficient was used to compute the decomposition rate constant, and t_{90} was also determined.

3. RESULTS & DISCUSSION

3.1. Investigation of ofloxacin's compatibility with proposed polymers

3.1.1. Fourier transform infrared FTIR

The spectrum of OFX (Fig. 1) shows its bands at 3423 cm^{-1} for O-H stretching, 3041 cm^{-1} for C-H stretching that is aliphatic, 2929 cm^{-1} for C-H that is aromatic and 1718 cm^{-1} for C = O stretching. NH3 at 1621 cm-1, CF at 1550 cm-1, CH3 at 1459 cm-1, CH at 1086 cm-1 and O-H deflection at 707 cm^{-1}. The spectral studies revealed a comparison between the absorption peaks of OFX alone and the peaks of the mixture. The findings showed that ofloxacin and the proposed polymers were compatible because the mixture showed all of the drug's distinctive spectral bands without significant spectral change[30-32].

3.1.2. Differential scanning calorimetry (DSC)

The thermal behaviors of OFX alone as well as its physical mixtures with the suggested polymers were illustrated in figure (2). A distinct endothermic peak was visible in the DSC thermogram of pure OFX at 276.92 ^{0}C as a result of melting of the drug. The endothermic peak of ofloxacin was seen in all of the acquired thermal profiles of the drug-polymer combinations, demonstrating the drug's compatibility with the employed polymers[31].

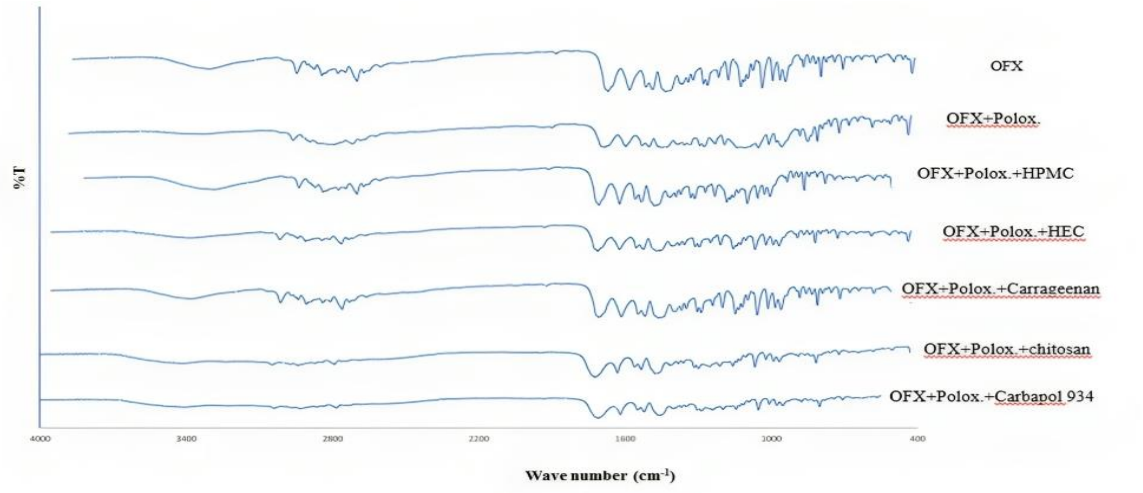

Figure 1. FTIR spectrum of OFX and its physical mixture with suggested polymers.

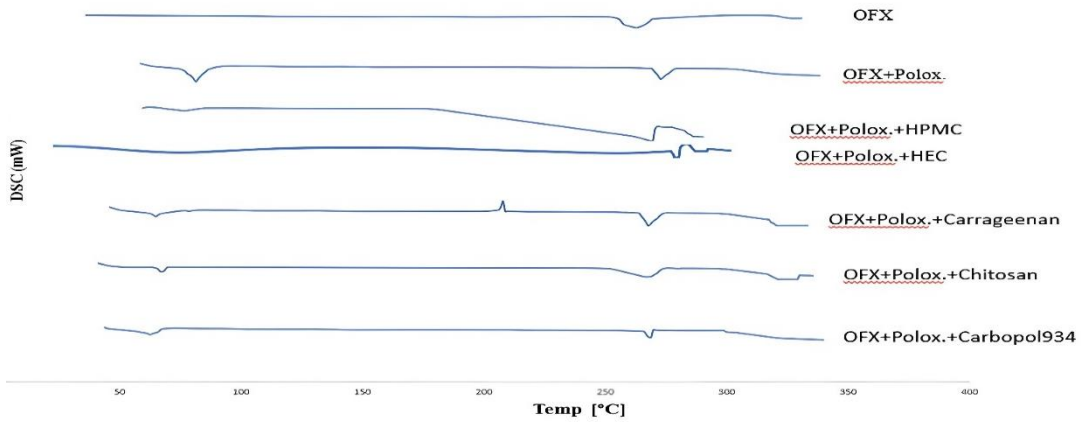

Figure 2. DSC thermogram of OFX and its physical mixture with suggested polymer.

3.2. Physical investigation of thermoreversible mucoadhesive otic gel

The fabricated thermoreversible mucoadhesive otic gel formulations should include a polymer concentration at which the gel will form once it reaches ear temperature which lies between 35.5^{0}C-37.5^{0}C[33]. Normally, the thermoreversible gel should be gelled between 25^{0}C-34^{0}C. A difficulty will arise during production, handling, and administration if the formulation gels at temperatures below 25 ^{0}C. Conversely, it will remain liquid at body temperature if it doesn't gel at temperatures below 34 ^{o}C. and simply wash out of the ear after being administrated in it[34].

An increase in the number of micellar aggregates and a decrease in the critical micelle concentration will occur when the temperature of the poloxamer solution is raised, enabling the production of a more tightly packed and viscous gel. Moreover, the gelation process is caused by the expulsion of hydrating water from the micelles and conformational changes in the orientation of methyl groups in the side chains of poly (oxy-propylene) polymer chains, which make up the micelle's core[35], also (Kolsure and Rajkapoor) explain the thermoreversibility of poloxamer solutions may attributed to that Poloxamers have more solvation and hydrogen bonding at low temperatures, making them more soluble in cold water than in hot water. Poloxamer solutions' temperature-dependent gelation could be explained by the fact that they are natural surfactants, which cause a shift in micellar characteristics as temperature rises[36].

Thermo-gelation is reversible phenomenon and considered to be concentration dependent. The gelation temperatures of all P407 solutions decreased by increasing the concentration of P407. The larger volume and number of micelles occupied at low temperature may be the cause of the decrease in gelation temperature with increase in P407 concentration. As P407 concentration rises, the lattice pattern is packed more tightly into the gel

structure, which causes it to gel quickly at low temperatures[37,38]. Based on preliminary trials, it was found that 18% of P407 gel of the drug, showed optimum gelation temperature (32.15±0.14), We can safely suppose that the formulations were gelled at 37.5°C, body temperature and thus was used for further study.

3.2.1 Clarity

As indicated in table (2), the created sets of formulations were assessed to be clear and extremely clear (glassy). This test confirmed that the drug preparation is in a completely mixed state and no foreign particles are present in the preparation.

3.2.2. Assessment of pH

The pH values for all formulations were between (5.1 ± 0.08 and 7.1± 0.04). This pH values indicate that it was close to otic pH and would not cause any irritation upon administration[39].

3.2.3. Gelation temperature

Optimization of the gelation temperature is a preparatory step in the creation of thermoreversible gel. As shown in table (2), the results of gelation study revealed that the formulation batches (F1 - F15) shown the formation of gel at temperatures between (28.17-32.11). Regarding the impact of different mucoadhesive polymers on the gelation temperature of the in-situ gel composed of 18% P407, results demonstrated that adding the mucoadhesive polymers HPMC K100, HEC, carrageenan and Carbopol 934 lowered the temperature of gelation to the gel. The ability of mucoadhesive polymers to attach to PEO chains that present in molecules of P407, which make it easier to dehydrate, Also the cause of their ability to lower gelation temperature was the increase in the entanglement of nearby molecules with more intense intermolecular hydrogen bonding, leading to decrease in gelation temperatures[40,41]. Despite the fact that chitosan is a large molecule, it was anticipated that it would stop poloxamer from gelling, it was found that it had a slight effect on gelation temperature at all concentration ranges used[38,42].

3.2.4. Mucoadhesive strength

The mucoadhesion force is a crucial factor for in situ gelling otic formulations because it extends the duration that gels stay in the ear and prolong their clearance. It also keeps the gelled solution from leaking out of the ear. According to results in Tab. 2, the mucoadhesive strength of all formulations of thermoreversible mucoadhesive otic gel increased proportionally with an increase in mucoadhesive polymer concentration. The cause of adhesion may be attributed to the hydrogen bonding between the polymers and oligosaccharide chain of the mucus that lines the mucus membrane. The strength of the mucoadhesive polymer depends on the ability of polymer to bind with the membrane, which varies from polymer to polymer. Consequently, it was discovered that the mucoadhesive strength of various polymers varied[41,43].

3.2.5. Drug content uniformity

To achieve dosage uniformity, the active component must be distributed uniformly. Values of all formulations were ranged from 98.5%- 100.1% as shown in table (2). All the investigated gels met the pharmacopeial standards for their content uniformity which was found to lie between ± 15%. It confirms that the medicine is evenly dispersed throughout the gel composition. As a result, it appears that the procedure utilized to prepare the gels in this work is reproducible[44].

3.2.6. Viscosity and rheological study

The viscosity value in (cp) for all formulations measured at speed 5 rpm at two temperature (25°C and 35°C respectively) were arranged in descending order according to different concentration of each mucoadhesive polymer to HPMC K100: F3(780.2, 11512.1)> F2 (622.12, 9482.5)> F1 (511.3, 6812.5)- HEC: F6 (652.4, 12504.1)> F5 (539.2, 8220.1)> F4 (403.5, 7312.2)- carrageenan: F9 (550.8, 10121.6)> F8 (510.2, 9272.5)> F7 (446.5, 7740.5)- chitosan: F12 (694.1, 4321.3)> F11 (402.1, 3401.5)> F10 (397.5, 3327.8)- Carbopol 934: F15 (751.6, 2522.1)> F14 (583.4, 1783.1)> F13 (414.2,994.5) as illustrated in figure (3). The formulation must have an appropriate viscosity to enable simple instillation into the ear as liquid droplets and to undergo a rapid sol-gel transition upon administration. This rapid gelation is essential to overcome the clearance of the gel[45].

It was clear that the consistency (viscosity) was dramatically increased by increasing the mucoadhesive polymer concentration. Increased consistency was due to enhance polymeric entanglements which increase the resistance to deformation[46].

In addition, this change in viscosity may be caused by variations in the size, shape, and ordering of the crystallites of various polymers inside the resulting three-dimensional network, where the

liquid phase is retained by capillary adsorption and molecular contact mechanisms[47].

By comparing the viscosity of all formulations at speed 5 rpm and two temperature (25°C and 35°C) It was seen that viscosity increased dramatically as the temperature increased, which confirm the gelation at otic temperature.

All formulations displayed pseudoplastic behavior in the rheological investigation, which means that their viscosity decreased as the shear rate increased. Because of this shear thinning behavior, the gel will be easier to spread and the medicine will be distributed evenly on the ear. The high viscosity at reduced shear rate is desirable to maintain the formulation within the treated area for prolonged pharmacological activity[11].

Table 2. Physical examination of the formulated OFX thermoreversible mucoadhesive otic gels.

Formulae	Clarity*	pH ± SD	Gelation temp. ±SD (^{0}C)	Mucoadhesive strength (dyne/cm^2)	Drug content ± SD (%)
F1	+++	5.34±0.02	32.11±0.15	963.71±9.71	100±0.58
F2	+++	5.64±0.01	31.41±0.57	1886±13.2	99.7±0.42
F3	+++	5.41±0.06	30.67±0.28	2713±12.1	100.1±0.22
F4	+++	6.51±0.03	31.5±0.5	1102±7.11	99.5±0.15
F5	+++	5.86±0.04	30.12±0.25	2251±13.4	99.2±0.13
F6	+++	6.02±0.15	29.16±0.16	3716±14.2	99.8±0.22
F7	++	5.1±0.08	29.71±0.12	713.22±12	99.6±0.25
F8	++	6.5±0.01	29.15±0.51	1425±10.13	100±0.24
F9	++	5.72±0.12	28.33±0.24	2210±13.1	99.4±0.12
F10	+++	5.63±0.03	31.6±0.31	512.11±6.35	99.6±0.31
F11	+++	5.51±0.08	31.32±0.12	990.12±8.57	98.5±0.11
F12	+++	5.13±0.02	30.9±0.17	1324±9.05	100±0.13
F13	++	7.1±0.13	30.12±0.14	810±9.48	99.3±0.2
F14	++	6.94±0.02	29.5±0.29	1925±15.12	99.5±0.22
F15	++	7.02±0.07	28.17±0.56	2312±10.20	100.01±0.3

*+++ (glassy or very clear), ++ (clear)

3.2.7. In vitro release testing of ofloxacin from formulations of thermoreversible mucoadhesive otic gel

Figure (4) represent the in vitro release of OFX alone and the fabricated thermoreversible mucoadhesive otic gel formulations. It is clear that the marketed product completed its release at 2 hours while otic gel exhibited sustained release up to 8 hours. The type or concentration of mucoadhesive polymer may be responsible for the variation in release behavior. According to the study's findings, the formulations with the lowest concentrations of each mucoadhesive polymer—HPMC K100, HEC, carrageenan, chitosan, and Carbopol 934 showed the greatest release of the drug in correlation to formulations that contain the highest concentrations of the same polymer. The delay in the dissolution may be due to the capability of the polymer to raise the total viscosity of the product also their capability to squeeze and distort an additional aqueous channel in micelle of poloxamer through which the medication diffuses[48,49].

3.2.8. Kinetic assessment of the in vitro release data

Kinetic parameters for the in vitro dissolution of OFX from various formulations of the otic gels was calculated also tabulated in tables (3). According to the values of correlation coefficient of determination (r), it was observed that the invitro release of OFX from various formulations follow zero-order except F10, F11 and F13 follow Higuchi diffusion order. The in vitro release of OFX from the

investigated formulations was managed using the Korsmeyer-Peppas equation. For all formulations, it was discovered that the values of (n) exceeded 0.5. The result with highest correlation coefficient (r) represent the most reliable kinetic model for drug release.

According to Korsmeyer-Peppas equation. For all formulations, it was discovered that the values of (n) exceeded 0.5, indicating a non-fickian mechanism of drug release that involved polymer relaxation initially and then drug diffusion[49,50].

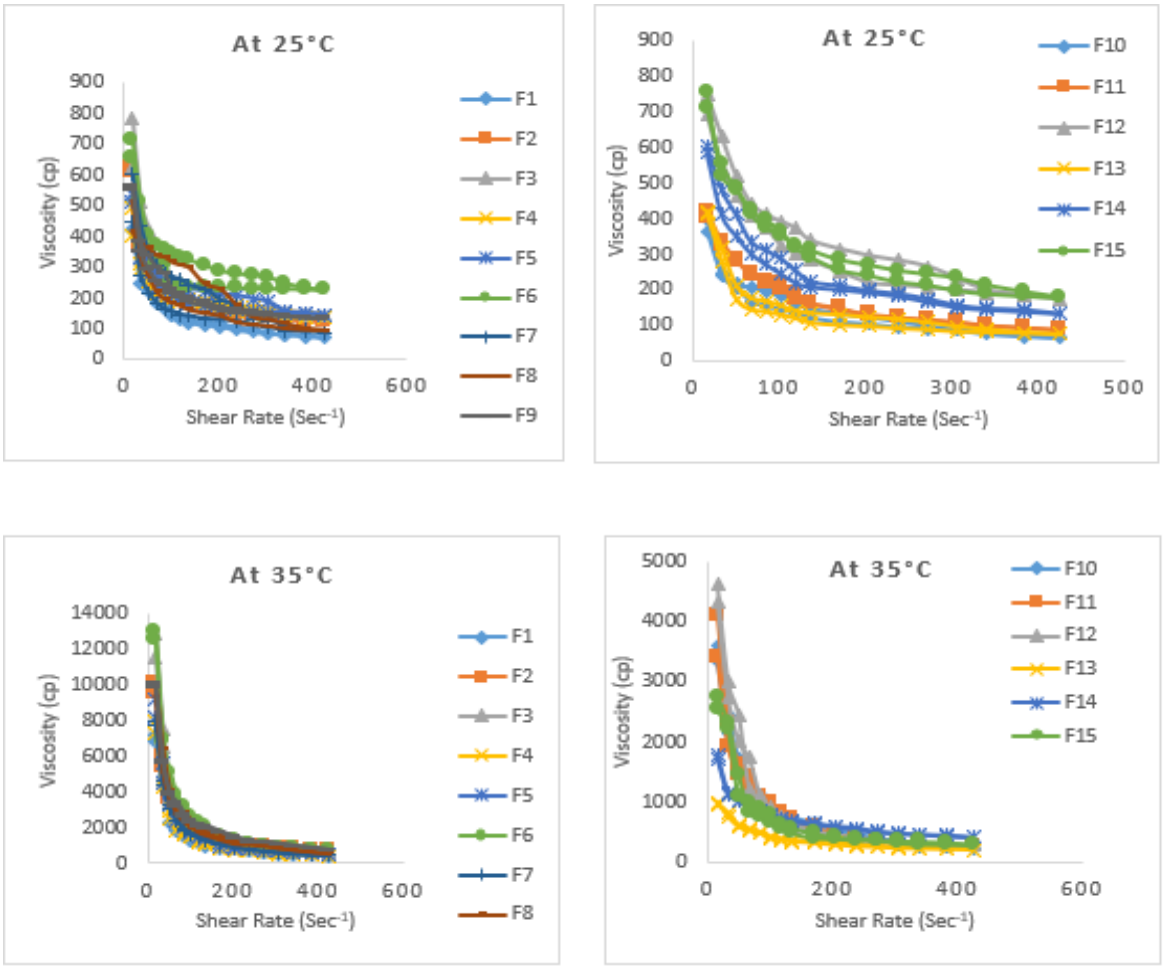

Figure 3. Correlation between the viscosity and shear rate to formulations of thermoreversible mucoadhesive otic gel at 25 and 35 ºC.

3.2.9. Stability studies

The stability study conducted for the selected formulations (F1, F2, F4, F7 and F10) at 4 and 25°C with RH 60±5% for 3 months. Figure (5) represents percent retained of OFX thermoreversible mucoadhesive otic gel formulations. It revealed that at 4°C the retained was ≥ 99.5% and at 25°C ≥ 96.8%. The degradation reaction was a zero-order reaction. The t_{90} values for the selected formulations F1, F2, F4, F7 and F10 were 965, 670, 398, 571 and 416 respectively as shown in table (5). F1 showed the highest stability results that was indicated by its t_{90} values after three months of storage at both 4**^{0}C** and 25 **^{0}C**. Table (6) represent a minimal change in pH after three months of storage at 4°C, the gelation temperature remained unchanged, but after three months at 25°C, there was a small decrease in the gelation temperature of the formulation under examination. Almost minimal change occurred in pH after three months of storage at 4°C, the gelation temperature remained unchanged, but after three months at 25°C, there was a small decrease in the gelation temperature of the formulation under examination, this might be resulted from the gel formulation's dehydration. This implies that formulations of thermoreversible mucoadhesive otic gels should be stored under refrigeration between 2 and 8 °C[20].

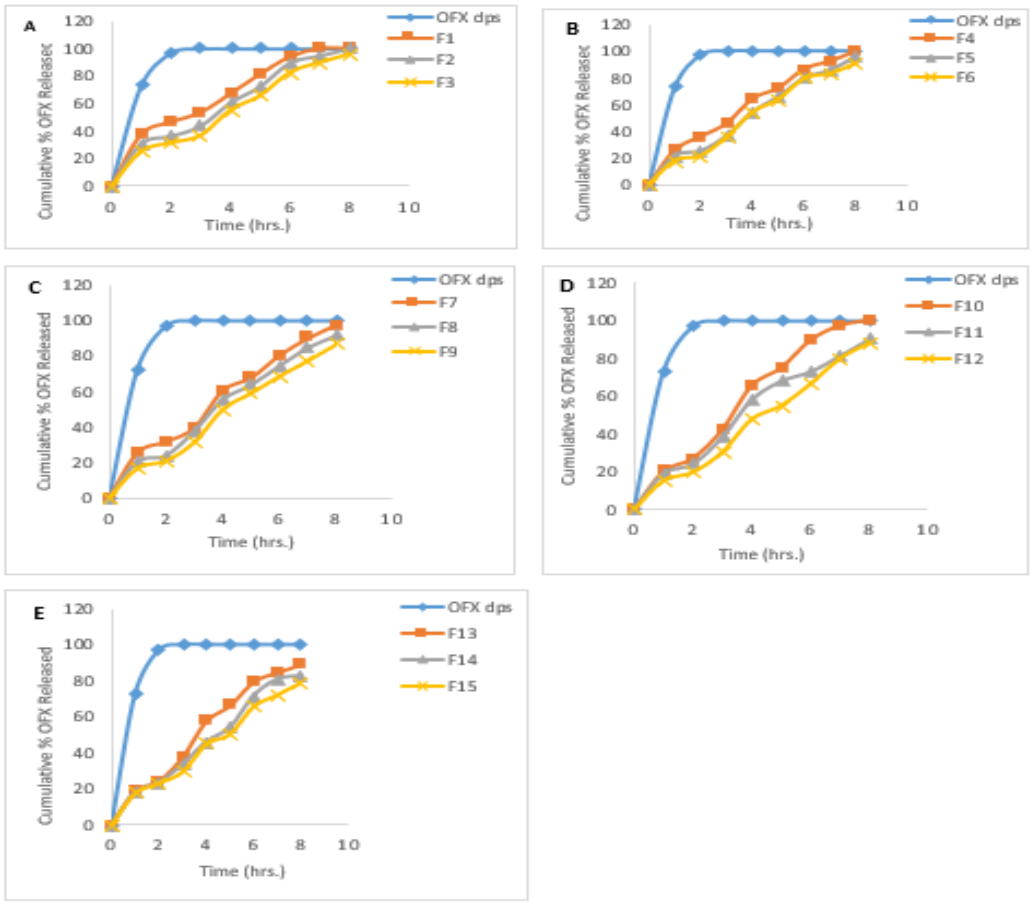

Figure 4. In vitro release of OFX thermoreversible otic gel formulations, (A) HPMC K100, (B) HEC, (C) Carrageenan, (D) Chitosan and (E) Carbopol 934.

Table 3. Kinetic analysis of OFX released from formulations of thermoreversible otic gels.

Formulae	Zero-order (r)	First-order (r)	Higuchi (r)	Korsmeyer–Peppas (n)
F1	**0.9840**	0.88996	0.9822	0.52494
F2	**0.9880**	0.84405	0.9766	0.6321
F3	**0.9897**	0.9516	0.9753	0.7034
F4	**0.9930**	0.8216	0.9913	0.6884
F5	**0.9925**	0.9353	0.9811	0.7944
F6	**0.9876**	0.9782	0.9833	0.8708
F7	**0.9927**	0.9257	0.9828	0.7017
F8	**0.9926**	0.9707	0.9856	0.7974
F9	**0.9932**	0.9766	0.9840	0.8657
F10	0.9833	0.8798	**0.9859**	0.8559
F11	0.9858	0.9775	**0.9872**	0.8124
F12	**0.9954**	0.9628	0.9808	0.9056
F13	0.9843	0.9818	**0.9857**	0.8331
F14	**0.9907**	0.9745	0.9792	0.8018
F15	**0.9934**	0.9810	0.9797	0.7806

* The bold value represents the highest correlation coefficient (r).

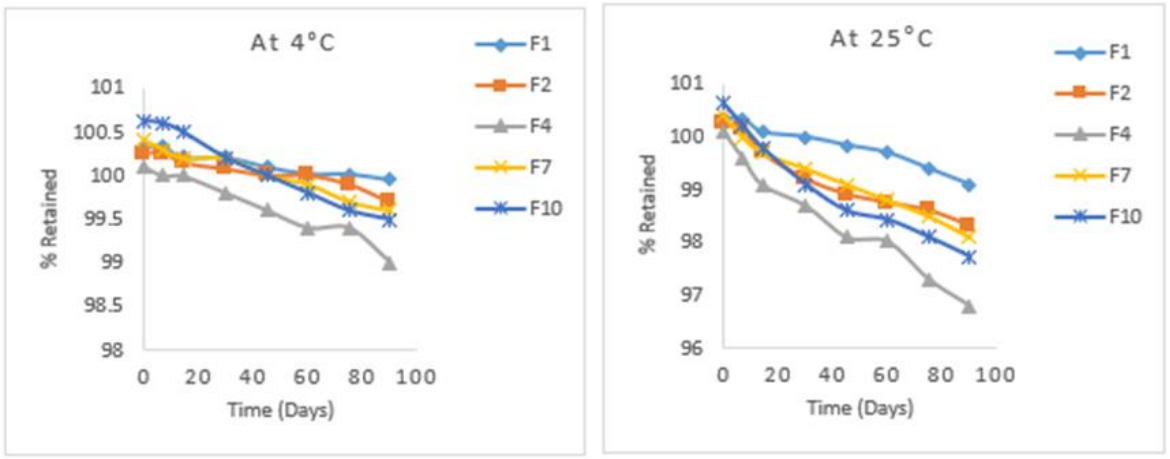

Figure 5. Percent retained of selected formulations of OFX thermoreversible otic gel stored at both 4 and 25°C/RH 60±5%.

Table 4. DE% value of OFX thermoreversible otic gel formulations for 8 h release.

Formulae code	DE% ± SD
F1	66.23 ± 0.21
F2	59.68 ± 0.14
F3	54.256 ± 0.31
F4	59.03 ± 0.22
F5	52.08 ± 0.18
F6	50 ± 0.33
F7	55.52 ± 0.26
F8	50.60 ± 0.11
F9	45.70 ± 0.42
F10	58.26 ± 0.15
F11	50.98 ± 0.32
F12	44.70 ± 0.13
F13	51.68 ± 0.24
F14	46.10 ± 0.35
F15	42.63 ± 0.12

Table 5. Values of t_{90} of selected formulations of OFX thermoreversible otic gel in stability testing

Formulations	T_{90} value (days)
F1	965
F2	670
F4	398
F7	571
F10	416

Table 6. Gelation temperature and pH of selected formulations of OFX thermoreversible otic gel after 3 months of storage at various temperature 4 and 25°C/RH 60±5%.

Formulae	Gelation temperatures ± SD			pH ± SD		
	Initial	After storage for 3 months		Initial	After storage for 3 months	
	time=0	4±2 °C	25 ± 2 °C	time=0	4±2 °C	25 ± 2 °C
F1	30.25±0.12	30.16±0.21	28.9±0.36	6.43±0.22	6.41±0.16	6.44±0.13
F2	29.37±0.24	29.2±0.13	28.2±0.24	6.51±0.13	6.5±0.2	6.52±0.24
F4	30.71±0.16	30.71±0.24	29.8±0.17	5.98±0.24	5.95±0.27	6±0.31
F7	31.42±0.33	31.3±0.25	30.2±0.22	6.78±0.11	6.76±0.18	6.8±0.26
F10	30.6±0.25	30.5±0.28	29±0.18	5.85±0.32	5.83±0.21	5.88±0.33

4. CONCLUSION

In order to minimize draw backs of conventional ear drops, poloxamer 407 based in-situ gels were successfully developed by cold process using five different types and concentration of mucoadhesive polymers. The formulations exhibited accepted pH, gelation temperature, mucoadhesive strength and drug content. According to the results of the rheological studies, all formulations displayed pseudoplastic rheology, which is demonstrated by a drop in viscosity with an increase in shear rate. The rheological study proved sol-gel transition at physiological ear temperature. In- situ gel sustained the drug release up to 8 h which was comparatively longer period than marketed ear drops. Formulation having the highest DE% (F1, F2, F4, F7 and F10) were selected for stability study. The result of short-term stability study indicated that F1 containing poloxamer 407 (18% w/v) and hydroxypropyl methyl cellulose K100 (0.5% w/v) is more stable according to the highest t_{90} value and the best storage condition for in-situ gel of OFX was at 4°C. The obtained results proved that the fabricated OFX in situ otic gel was a good alternative for conventional ear drops as it sustains the release of the drug for long period of time and may also reduce the number of applications of the drug. The authors think that this would be an important step forward the in vivo investigation.

Funding: This research received no specific grant from any funding agency.

Acknowledgments: I want to express my gratitude to all members of the faculty of pharmacy at Al-Azhar University, in particular to my co-authors, for providing excellent facilities and unwavering support for doing the research.

Conflicts of Interest: There is no Conflict of interest.

Author Contribution: **M**ethodology -writing, Reem A. Selim; review and editing, Amal A. E. Ammar and Seham M. Shawky. All authors have read and agreed to the published version of the manuscript.

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