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Posaconazole loaded ocular inserts for antifungal activity

Kadir Aykaç[1,2], Evrim Yenilmez[2], Müzeyyen Demirel[2], Ebru Başaran[2*]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Erzincan, Turkey
[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey

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*Corresponding author:
ebcengiz@anadolu.edu.tr

ABSTRACT

Corneal and conjunctival infections are common ocular diseases; however, sometimes lead to blindness when neglected. Despite most of the ocular drug delivery systems are in eye drop form, they suffer from poor retention on the ocular surface and low ocular bioavailability leading to unsatisfactory results even with repeated treatment. Therefore, there is a need for more effective drug delivery systems for the ocular application. The present study was carried out to demonstrate that ocular inserts effectively delivers a significant concentration of drug with topical administration for the treatment of fungal infections with the help of extended residence time on the ocular surface. Chitosan-based inserts were prepared by the freeze-drying method. The prepared inserts were evaluated for various parameters. Layered structures were revealed with scanning electron microscopy analyses. Thermal and structural behaviors were analyzed by differential scanning calorimetry and Fourier-transform infrared spectroscopy with nuclear magnetic resonance analyses, respectively. Drug contents were evaluated by a validated HPLC method. *In vitro* release studies were also performed in simulated tear fluid at 34±1°C for 48 hours. Analyses results revealed that chitosan-based ocular inserts were suitable systems for posaconazole delivery for the treatment of severe ocular fungal infections.

1. INTRODUCTION

Ocular diseases require immediate treatment due to vision threatening critical clinical reasons [1]. Topical instillation is the most widely preferred non-invasive route of drug administration to treat diseases affecting mostly the anterior segment [2]. However, the treatment of ocular infections with traditional drug delivery systems (eye drop, etc.), especially with the topical application, is challenging due to the unique structural properties of the eye. The eye is segmented into two parts, anterior and posterior segments. The anterior segment of the eye comprises cornea, aqueous humour, iris, ciliary body, and lens, whereas the posterior segment includes retina and vitreous humour [3]. Cornea, which is regarded as the main penetration site, acts as a barrier for both hydrophilic and lipophilic drugs [4,5]. Treatment approaches differ considering the target sites of the eye; therefore, the main strategies for the enhancement of ocular bioavailability are the extension of residence time on the corneal surface and the enhancement of corneal permeability by penetration enhancers [6]. In our study, ocular inserts were formulated for the maintenance of extended duration on the ocular surface. Chitosan was used as a polymeric lattice for the structural integrity of the inserts [7]. Chitosan is a biodegradable and non-toxic biomaterial, which has excellent mucoadhesive strength and has been routinely explored for controlled drug delivery at various mucosal sites of the body [5-8]. Chitosan is a polycationic polymer due to the positively charged amino groups [9-11]. Considering the negative charge of mucin layers at mucosal membranes, cationic drug delivery systems electrostatically interact with mucosal surfaces, which results in increased bioavailability with topical application [12-16].

Posaconazole is a second-generation triazole group member like voriconazole, ravuconazole, isavuconazole, and albaconazole, has greater potency, and possesses increased activity against resistance and emerging pathogens [17]. Posaconazole has also been investigated in phase III studies and approved by the regulatory agencies for the treatment and prophylaxis of invasive fungal infections; therefore, posaconazole was selected as an active agent considering its broad-spectrum activity [18].

In this study, posaconazole loaded chitosan inserts were prepared by the lyophilization method. Besides the longer duration period of the ocular inserts, electrostatic attractions between the negatively charged ocular surface and chitosan-based cationic inserts will enhance the ocular bioavailability of the active agent. Sustained release of drugs from polymeric network gives the possibility to reduce dose and dosing frequency while maintaining the effective topical treatment of sight-threatening severe ocular fungal infections.

2. MATERIALS AND METHODS

2.1. Materials

Posaconazole was gifted by Abdi İbrahim İlaç (İstanbul, Turkey). Chitosan (high molecular weighted; 310000-375000 Da) and Acetic acid (glacial, ≥99%) were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol, methanol, and acetonitrile were the products of Merck (Darmstadt, Germany). All other chemicals were in analytical grade.

2.2. Preparation of Ocular Inserts

Ocular inserts were prepared by lyophilization method [19]. Briefly, chitosan was dissolved (40 mg/mL) in acetic acid solution (2%, v/v), and posaconazole was dissolved in ethanol:acetonitrile (5:1) mixture. Formulations were prepared by mixing these solutions with different concentrations (**Table 1**) and stirred for 24h for the evaporation of the organic solvents.

The solutions were stored at -80±5°C, and lyophilization (3L, -86°C, Operon Freeze Dryer, Gimpo-City, Korea) method was applied. A uniform film was achieved and cut into pieces and were stored in well-closed containers until being analyzed (**Figure 1**).

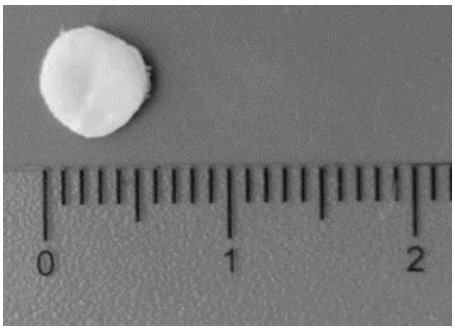

Figure 1. *Ocular insert prepared by lyophilization method*

2.3. Characterization Studies of Ocular Inserts

2.3.1. Morphological analyses

The morphological properties of posaconazole loaded inserts were investigated by scanning electron microscopy (SEM) analysis (Zeiss Ultra Plus Fesem, Germany).

2.3.2. Differential scanning calorimetry analyses

Structural and crystallinity changes of posaconazole and chitosan were evaluated using differential scanning calorimetry (DSC) (DSC-60, Shimadzu Scientific Instruments, Columbia, USA). Analyses were performed under nitrogen (flow rate of 50 mL/min) at 30-300°C. Thermograms of pure posaconazole and polymer were used as references.

2.3.3. Fourier transform infrared spectrophotometry analyses

For the structural analyzes, Fourier transform infrared (FT-IR; IRAffinity-1S Shimadzu, Tokyo, Japan) analyses were performed. High-sensitivity DLATGS detector was used with Germanium-coated KBr Beam splitter at 7800 to 350 cm^{-1} wavenumber range. FT-IR spectra of pure posaconazole and chitosan were used as references.

2.3.4. Nuclear magnetic resonance analyses

For the evaluation of the interactions between the active agent and the polymer, ^{1}H-NMR analyses were performed on Fourier 300 NMR (Brucker, Germany). Spectra of pure posaconazole and chitosan were used as references.

2.3.5. Determination of posaconazole

A modified high-performance liquid chromatography (HPLC) method was used for the determination of posaconazole [20]. Shimadzu 20 A (Tokyo, Japan) with Shimadzu Shim-Pack CLC-ODS column (Tokyo, Japan; column diameter: 4.6 mm, column length: 25.0 cm, particle diameter: 5 µm, and particle size: 100 Å) was used as the instrument. Acetonitrile:distilled water (60:40, v/v) was used as the mobile phase with a flow rate of 1.0 mL/min. 20 µL constant amount of samples were injected via an autosampler (SIL-20A, Shimadzu, Tokyo, Japan) and a photodiode array detector (SPD-M20A, Shimadzu, Tokyo, Japan) was used at 262 nm. The column temperature was set to 25°C. (CTO-10AS-VP, Shimadzu, Tokyo, Japan) Validation studies were performed for data reliability [21].

2.3.6. *In vitro* drug release studies

In vitro drug release studies were carried out by Apparatus 1 method with Pharma Test-PTWS820D (Hainburg, Germany) [22]. In this study, pH 7.4 simulated tear fluid (STF; 500 mL) was used as the release medium at 34±1°C [23]. At predetermined time intervals (0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 9, 24, and 48 hours), 1 mL samples were taken from the release medium, and the equivalent volumes of fresh medium were added back for the maintenance of sink conditions. The amount of active substance in the samples was determined with HPLC. Each analysis was repeated three times.

3. RESULTS AND DISCUSSION

Compositions of the formulations were given in **Table 1**. Formulations were kept in well-closed containers until being analyzed.

Table 1. *Compositions of the formulations*

Ingredients	Formulation Code		
	CPS0	CPS1	CPS2
Chitosan HMW (mg)	40.0	40.0	40.0
Posaconazole (mg)	-	16.7	25.0
Ethanol:acetonitrile (5:1 (mL)	-	2.0	3.0
Acetic acid (2%) (mL)	9.6	9.6	9.6

3.1. Characterization Studies of Ocular Inserts

Morphological and structural analyses were performed for the characterization of inserts.

3.1.1. Morphological analyses

SEM is regarded as a reference method for the determination dimensional properties of the samples [24], therefore in this study, morphological analyses of the inserts were determined by SEM analyses, and micrographs were presented in **Figure 2**.

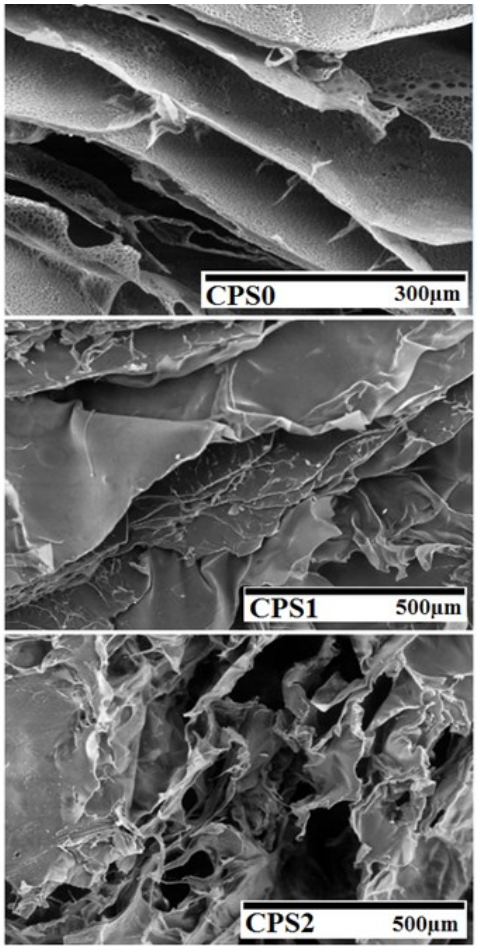

Figure 2. *SEM micrographs of the formulations*

Analyses results revealed that the inserts were formed in layers, which gives the possibility to enhance the tear sorption capacity of the formulations [25].

3.1.2. Differential scanning calorimetry analyses

DSC gives details about the thermal properties of the polymers, which is one of the most essential data for the processing of materials and also predicting the shelf life of the final product [26].

DSC thermogram of posaconazole exhibited a sharp endothermic peak at 174.59°C. Also, the melting peak was revealed in the thermogram of the physical mixture of posaconazole and chitosan, showing no chemical interaction was revealed between the active agent and polymer (**Figure 3**). No peaks were revealed in the thermograms of chitosan, showing that the amorphous structure of the polymer while the posaconazole peak was disappeared in the thermograms of formulations showing that active agent was molecularly dispersed within the polymeric layers [27]. And also, water forms intermolecular hydrogen bonding with various chitosan and posaconazole's amine and hydroxyl groups, which helps in molecular rearrangement resulting in ease of chain mobility as well as crystallinity (**Figure 3**) [25].

3.1.3. Fourier transform infrared spectrophotometry analyses

FT-IR spectroscopy was used to investigate the interactions between posaconazole and chitosan (**Figure 4**).

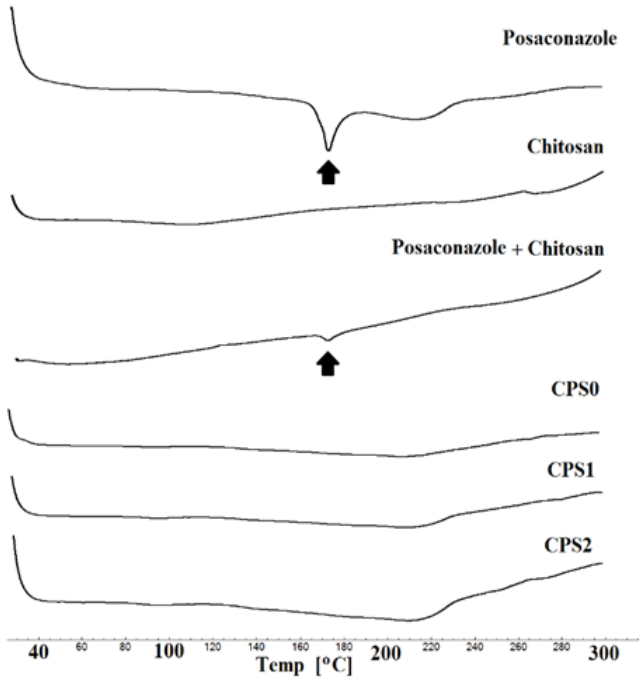

Figure 3. *DSC thermograms of the pure materials, physical mixture, and formulations*

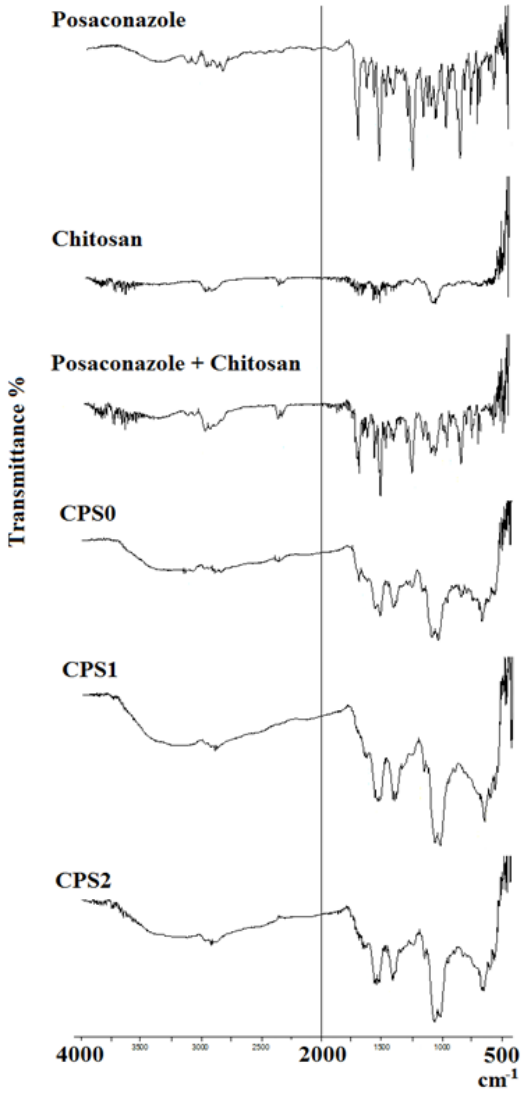

Figure 4. *FT-IR spectra of the pure materials, physical mixture, and formulations*

FT-IR spectrum of posaconazole shows high-intensity absorption peaks at 3061, 2966, 1685, 1508, and 1224 cm^{-1} corresponding to the stretching vibrations of OH, CH_2, C=O, C=N, and C–N, respectively. The spectra of the physical mixtures corresponded to the spectra of individual components. Characteristic absorption peaks of posaconazole were revealed in the spectrum, indicating that posaconazole remained in the physical mixture without any interactions with chitosan [28]. However, characteristic signals of posaconazole could not be detected in the spectra of the formulations showing that active agent was molecularly dispersed within the polymeric layers in correlation with DSC analyses (**Figure 3**) [27,28].

3.1.4. Nuclear magnetic resonance analyses

For the evaluation of the interactions between the active agent and the polymer ^{1}H-NMR analyses were performed, and analyses results were presented in **Figure 5**. Analyses results revealed that posaconazole signals were detected in the spectra of formulations with increased intensity (marked with arrows) in the range of 1-4 ppm showing that presence of posaconazole within the polymeric network without any chemical interaction [23,29].

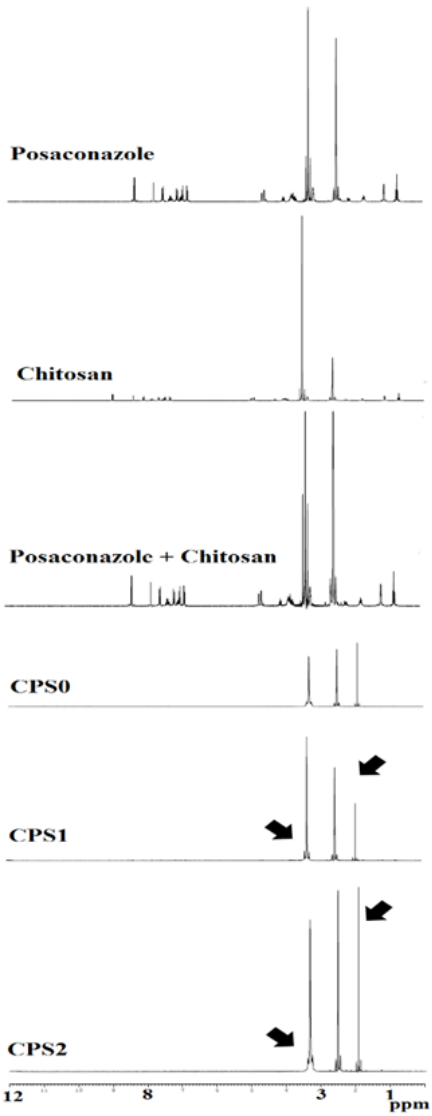

Figure 5. *^{1}H-NMR spectra of the pure materials, physical mixture, and formulations*

3.1.5. Determination of posaconazole

Validation studies of the HPLC method were carried out in accordance with the guidelines of ICH within the range of 5-200 µg/mL [21]. R^2 was 0.9999, accuracy values were in the range of 98.96-102.58%, while RSD value was less than 2% as a result of precision studies. The selectivity of the method was analyzed in comparison with placebo formulation (CPS0) and with all the components of the study (**Figure 6**).

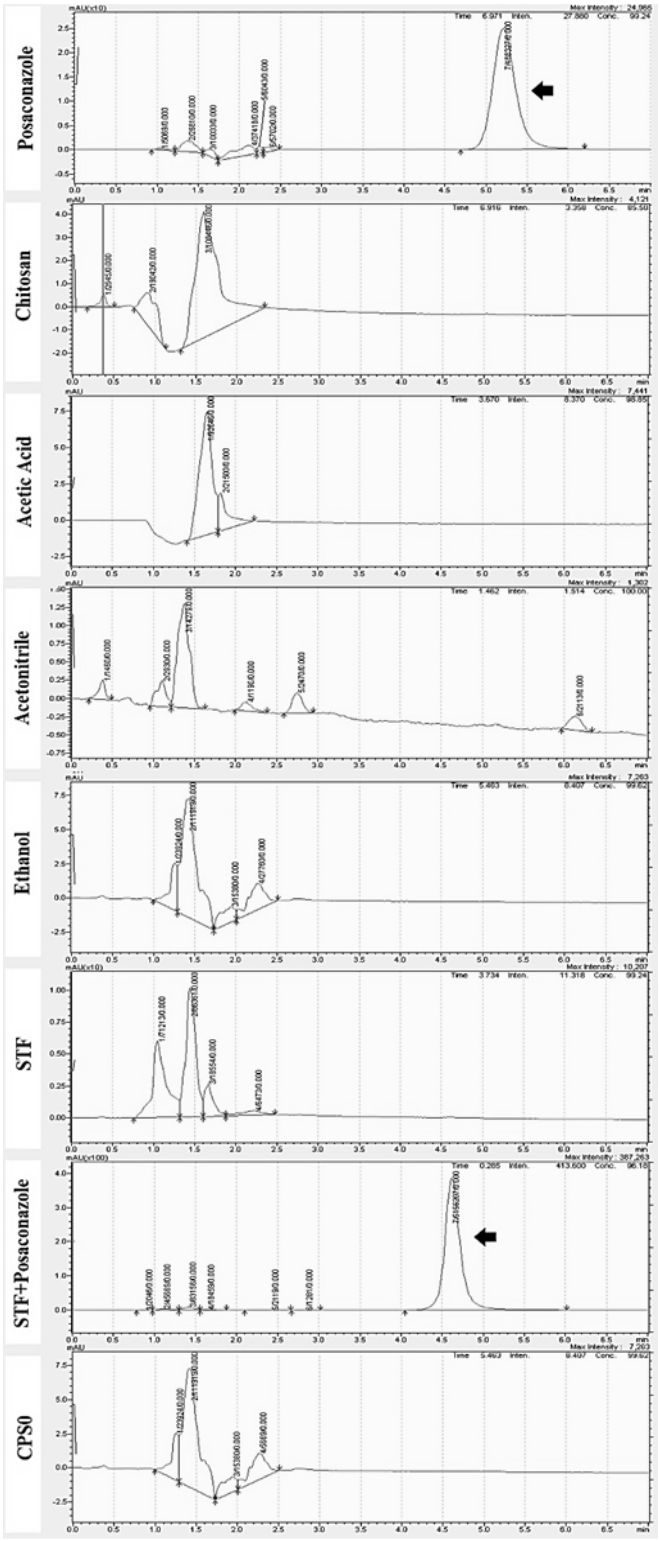

Figure 6. *HPLC analyses signals of posaconazole in selectivity studies*

Analyses results revealed that no other substance did give signals at the point where the active substance was determined. For the evaluation of the posaconazole amount, a constant amount of formulation was dissolved in acetic acid (2%; v/v):ethanol:acetonitrile mixture (1:1:1, v/v) and were analyzed by HPLC. Analyses results revealed posaconazole amounts were 4.01±0.02% and 5.90±0.01% (mean±SE; n=3) for the CPS1 and CPS2 formulations respectively (**Table 1**)

3.1.6. *In vitro* release studies

In vitro release studies were performed in STF at pH 7.4 for 48 hours, and analyzes results were presented in **Figure 7** [23,29,30]. According to the analysis results, the release rate of the active agent from CPS1 has reached the highest point of 40.40% while it was 49.09% for CPS2 after 48 hours. Despite the release rates could not reach 100%, the analyses were conducted for 48 hours considering the residence time on the ocular surface.

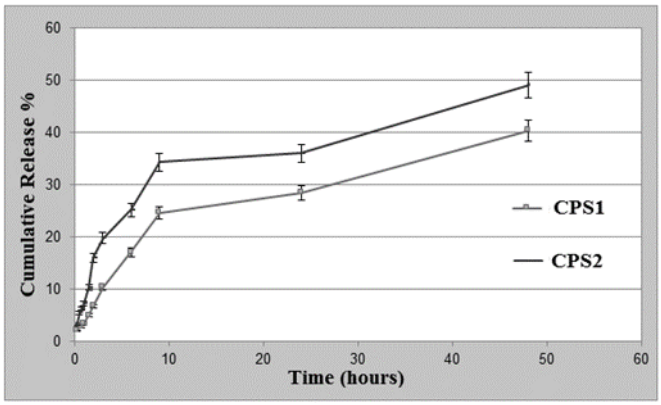

Figure 7. *In vitro release profiles of formulations (mean±SE; n=3)*

Posaconazole was practically insoluble in water (0.012 mg/mL); therefore, for the maintenance of sink conditions, *in vitro* release analyses were performed using Apparatus 1 (in 500 mL STF at 34±1°C) and release studies of the pure drug could not be performed with same method [23,29,31]. As the commercial products of posaconazole were in tablet, oral suspension, and injection forms, in *in vitro* release analyses comparison with commercial products could not be performed considering the results of the analyses will not contribute the ocular application of posaconazole via chitosan-based inserts [31].

Since most of the ocular formulations have poor performance due to their uncontrollable and undesirable burst releases with rapid removal from the ocular surface; biphasic release systems can maintain an initial burst release followed by a relatively steady release which enhances the ocular performance and ocular bioavailability of the applied formulation resulting ineffective treatment of severe ocular disorders [30,32].

4. CONCLUSION

The present study describes the development of posaconazole loaded chitosan inserts by the freeze-drying method. *In vitro* characterization analysis results revealed the characteristic properties of inserts in detail. SEM analyses revealed the layered structure of the system, which gives the possibility to increase the tear sorption of the system. Considering the cationic character of the polymeric structure enhanced ocular bioavailability with the help of electrostatic interactions between the oppositely charged ocular and polymeric surfaces as well as enhanced corneal duration period of the inserts.

AUTHOR CONTRIBUTIONS

Concept: EY, MD, EB; Design: EY, MD, EB; Supervision: MD, EB; Materials: EB; Data Collection and/or Processing: KA, EB; Analysis and/or Interpretation: KA, EB; Literature Search: KA, EB; Writing: MD, EB; Critical Reviews: EY, MD, EB.

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CONFLICT OF INTEREST DECLARATION

The authors declared no conflict of interest.

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