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FTIR, Electrochemical Impedance and Iontophoretic Delivery Analysis of Guar Gum and Sesame Oil Based Bigels

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The objective of this study was to investigate the electrochemical properties of the prepared bigels and iontophoretic delivery of metronidazole for topical application. Bigels were prepared by mixing guar gum aquagel and Span 60-sesame oil based oleogel. FTIR spectra showed the formation of intermolecular hydrogen bonding. Impedance spectroscopy confirmed the conductive nature of bigels. The iontophoretic release study of metronidazole loaded bigels showed concentration dependent release of the drugs. The bigel containing higher concentrations of oleogel showed lower amount of drug release and followed zero order release kinetics. In conclusion, the results demonstrate that iontophoresis of metronidazole enables local enhanced topical delivery for treating various diseased conditions.

Keywords: iontophoresis, bigels, guar gum, impedance spectroscopy.

1. INTRODUCTION

Iontophoresis is an electrically mediated delivery system which has drawn attention of many scientists to deliver the active ingredients through topical (dermatological, cosmetics) or ocular route [1]. It involves the application of a mild electric current to increase the passage of the drug through the tissues [2]. This has major advantages for the drugs prone to first pass metabolism [3]. The rate of release can be controlled by having a control over formulation components, concentration of active ingredient, intensity of applied current, duration of current applications, surface area of the sample in contact with the active electrode. Hence, the system can be used for targeted delivery applications, reducing risk of irritation/adverse side effects [4]. Iontophoresis allows the transport of the active

medicament through the stratum corneum. The major limitation of iontophoretic delivery is its higher cost of administration [5].

Metronidazole [2-Methyl-5-nitroimidazole-1-ethanol, $C_6H_9N_3O_3$] is a nitroimidazole derivative used to treat various diseased conditions like amebiasis, vaginitis, trichomonas infections; giardiasis etc [6]. The oral treatment with metronidazole is associated with the side effects like metallic taste, nausea and a temporary lowered blood count. Metronidazole vaginal gel is one of the most effective treatments of the condition [7]. Bigels are novel gels which are composed of an aqueous gel and an oleogel mixed together in a predefined ratio. Bigels differ from emulsions as they do not need an emulsifier. In a bigel, both the phases (aqueous gel and oleogel) co-exist [8].

Guar Gum is a naturally occurring high molecular weight non-ionic hydro colloidal polysaccharide. It is a galactomannan consisting of (1-4) residual bonds of β-D-mannopyranose backbone with the side chain of associated (1-6) linked-α-D-galactopyranose. It has excellent cold water solubility because of the high galactose: mannose ratio. It is known as one of the best economical thickener, emulsifier and stabilizer. It shows high viscosity at low-shear rates, but possess strong shear-thinning behaviour [9].

Span-60 [(2R)-2-[(2R,3R,4S)-3,4-dihydroxyoxolan-2-yl]-2-hydroxyethyl octadecanoate, $C_{24}H_{46}O_6$] is a nonionic sorbitan monoester used as an emulsifier and stabilizer in medicine, food and cosmetic formulations [10-11]. Sesame oil is used in pharmaceuticals and cosmetic products due to its inherent anti-inflammatory, antiviral, anti-fungal and antibacterial properties. The fatty acid components, sesamol and sesamolin render strong antioxidant property [12].

Very limited literature is available regarding the use of gels in the field of iontophoretic drug delivery. In our previous study we reported a marked increase in percent release in iontophoretic delivery of metronidazole from olive oil based hydrogel [7]. Mannem *et al.* (2013) reported of fast and sustained concentration of amoxicillin and cefuroxime in skin using iontophoresis [13]. Manda *et al.* (2014) reported iontophoretic delivery of medicaments for the treatment of scars [14]. In this study bigels were developed by mixing the guar gum aqua gel and Span 60-sesame oil oleogel. The objective of the study was to investigate the iontophoretic delivery of antimicrobials. The bigels were loaded with a model antimicrobial drug, metronidazole and the iontophoretic delivery of the drug was carried out to investigate its possible application in the treatment of various topical diseases.

2. MATERIALS AND METHOD

2.1. Materials

Guargum and Span 60 were procured from Merck and LobaChemie Pvt. Ltd, India, respectively. Sesame oil was purchased from Tilsona®, Recon Oil Industries Pvt Ltd, India. Metronidazole was a gift sample from Aarti drugs, Mumbai, India.

2.2. Methods

2.2.1. Preparation of gels

The aquagel was prepared by dissolving required quantity of guar gum in hot water (1% w/w) maintained at 70°C under stirring (1000 rpm). The stirring was continued until a smooth, transparent mixture was obtained. A preservative, propyl paraben (0.02% w/w) was added to the water before addition of guar gum to prevent bacterial contamination to the formulations. The hot mixture upon cooling to room temperature formed highly viscous aquagel.

The oleogel was prepared by adding required quantity of Span 60 (15% w/w) in warm sesame oil (60-70°C) kept under continuous stirring (500 rpm). The hot mixture turned into oleogel when left undisturbed and cooled down to room temperature. The drug containing oleogels were prepared by dispersing metronidazole, followed by addition of Span 60.

The required quantity of molten oleogel was transferred drop-wise in aquagel previously maintained at 60-70°C under stirring at 1000 rpm. The stirring was continued until a homogenous mixture was obtained (Table 1). The mixture formed bigel when cooled to room temperature. Metronidazole loaded bigels (1% w/w) were prepared by adding metronidazole loaded oleogel in the aquagel.

2.2.2. Molecular properties

The molecular properties of the bigels were studied by infrared spectroscopy, X-Ray diffraction analysis and diffuse reflectance spectroscopy. The functional group identification and presence of interaction, if any, was studied by FTIR spectroscopy (AlphaE ATR-FTIR, Bruker, USA) operated in the Attenuated Total Reflectance (ATR) mode. The samples were scanned in the range 4000 – 400 cm^{-1} at a spectral resolution of 1 cm^{-1} [15].

The stable bigels were subjected to spectroscopic measurement in the range 200 to 800 nm by UV-Vis spectrophotometer (Lambda 35 UV/Vis spectrophotometer, Perkin Elmer, USA) equipped with a solid state detector using a spectral resolution of 1 nm. The percent reflectance was plotted against wavelength.

2.2.3. Impedance spectroscopy

The conductivity profile of the developed bigels was measured using a computer-controlled impedance analyzer (Phase sensitive multimeter, PSM1735, Numetriq, Japan). The experimental data was recorded as a function of frequency (0.1Hz–1MHz) at room temperature [16].

2.2.4. Ionotophoretic drug delivery

The metronidazole loaded bigels were investigated for their iontophoretic drug release studies. The release study was conducted using an in-house developed ionotophoretic drug delivery setup. The

passive form of release was also performed along with the active form to know if there is a synergistic action of electrically mediated set up. The drug delivery set up contained two compartments, a donor and a receptor compartment. The donor compartment was tied with a previously activated dialysis membrane (MW cut-off - 60 kDa, Himedia, Mumbai) at one end. The receptor compartment was filled with 25 ml distilled water (37 ^{o}C, 100 rpm). The drug loaded samples were accurately weighed (2.15 g) in the donor compartment which was slightly dipped in the receptor volume during the release study so that the drug gets released from the bigel in the receptor fluid through the dialysis membrane. Two stainless steel electrodes (diameter 1.4 cm) were used to connect the donor and the receptor compartments. The measurements were done using an a.c. current (32.13 μA, I_{rms}), which supplied a current density of 20.88 μA/cm^2. A sinusoidal voltage of 0.707 V (V_{rms}) was generated by a standard signal generator using a constant current source. The release study was conducted for 2 h and samples were collected at regular intervals (0.25, 0.5, 0.75, 1, 1.5 and 2h). The amount of sample collected was subsequently replaced with 3 ml of fresh water to maintain the overall receptor volume to 25 ml. The collected samples were analysed spectroscopically using a UV-spectrophotometer (UV 3200 double beam, Labindia) at 321 nm [17-19].

3. RESULTS AND DISCUSSION

3.1. Preparation of the bigels

The aquagel was whitish, viscous and translucent whereas the oleogel was slightly yellow, highly viscous and opaque. Both the gels were smooth in texture. The bigels were milky white, viscous and opaque. They possessed water based consistency upon touching which was associated with their very high water content (Figure 1).

Figure 1. Stable bigel formation upon cooling to room temperature

Table 1. Bigel compositions (% w/w)

Formulations	Aquagel	Oleogel	Metronidazole
BG1	72.73	27.27	-
BG1M	72.73	26.27	1
BG2	66.67	33.33	-
BG2M	66.67	32.33	1

The bigels containing higher oleogel content showed higher consistency, firmness and viscosity. At the same time, the bigel containing lower concentrations of oleogel were easy to spread and were less sticky.

3.2. Molecular properties

FTIR analysis was used to evaluate the possible intermolecular interactions between metronidazole and the raw material. The spectra of blank and drug loaded bigels are shown in Figure 2(a). The principal peaks present in the raw materials (guar gum, Span 60 and sesame oil) were preserved in the blank as well as the metronidazole loaded bigels. Both the bigels showed a strong absorption band at ~3,300 cm^{-1} which can be assigned to the O–H stretching vibrations due to the presence of intra/intermolecular hydrogen bonding [19]. Very weak absorption bands were present at ~2,930 cm^{-1}, ~2,850 cm^{-1}, and at ~1,748 cm^{-1} in the blank bigels, which can be attributed to the presence of O-H stretching vibration, C-H stretching vibration in CH_2 and CH_3 present in the alkanes and C=O stretching vibration of the carbonyl group of ester respectively [20]. The above peaks were absent in the drug loaded bigels suggesting addition of drug affected the structural organization of the bigel system. A prominent peak at ~1465 cm^{-1} is associated with the (O-C-O) symmetric stretching vibrations [21].

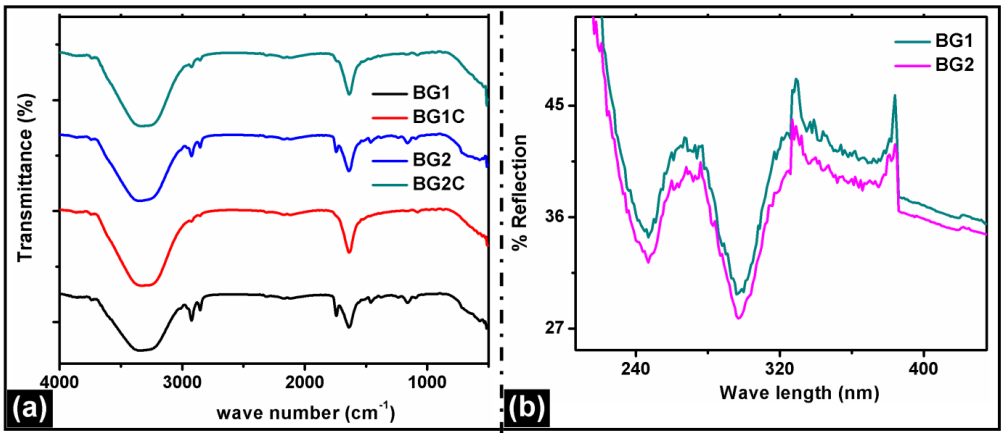

Figure 2. Molecular properties; (a) FTIR spectra of blank and drug loaded bigels and (b) Diffuse reflectance spectroscopy of bigels

The extent of intermolecular hydrogen bonding was quantified by calculating the area under the curve (AUC) from the broad absorption band obtained in the range 3700-2950 cm^{-1} (Table S1) [22]. The results suggested that the difference in AUC between the blank bigels was insignificant whereas the addition of drugs has increased the AUC of the gels.

Table S1. AUC for the peak at ~3300 cm^{-1} (between 3700-2950 cm^{-1})

Sample	AUC
BG1	1031.58
BG1M	1212.40
BG2	1106.95
BG2M	1187.28

Reflectance spectroscopy is based on the principle of appearance of dips at certain points due to scattering of light while passing through the sample surface. These dips are drop in reflection which is characteristic of certain ions and molecules. The reason behind the appearance of drops in reflection is due to the absorbed light by the electrons. These drops of reflection are recorded as a function of wavelength and plotted as percent reflectance in the UV-visible range (Figure 2(b)). The apparent depth (D) of absorption was calculated by using the formula:

$D = 1 - R_b/R_c$

where, R_b is the maximum reflectance recorded at the bottom of the dip, and R_c is the reflectance of the continuum at the same wavelength as R_b.

BG2 showed a slightly higher depth of absorption in comparison to BG1which is inversely related to the dispersed droplet sizes (Table 2). Higher depth of absorption in BG2 bigel resulted due to more scattering from its droplet surfaces. As BG2 possessed smaller droplet size in comparison to BG1, hence more surface area was available resulting in more scattering.

Table 2. Band depth obtained from diffuse reflectance spectroscopy

Formulations	R_b	R_c	Band Depth (D)
BG1	29.93	42.19	0.29
BG2	27.91	40.65	0.31

3.3. Electrical properties

The electrical properties of the developed bigels were analysed by impedance spectroscopy study. An equivalent circuit was proposed to model the Nyquist plot. The design of the equivalent circuit was decided based on combined impedance (Z″) and modulus (M″) plots with respect to frequency (Figure 3(a) and (b)). M″ plot has shown appearance of two peaks whereas Z″ plot has shown a single peak in the high frequency region. The low frequency peak was due to the grain

boundary and the high frequency peak was associated with the grain effects [23]. The appearance of the peak is associated with the relaxation in the conductivity of the gels. The broadened peak in lower frequency was due to the spread of relaxation times resulting in smaller conductivity. Similarly the sharp peak at high frequency was associated with the shorter relaxation time resulting in higher conductivity.

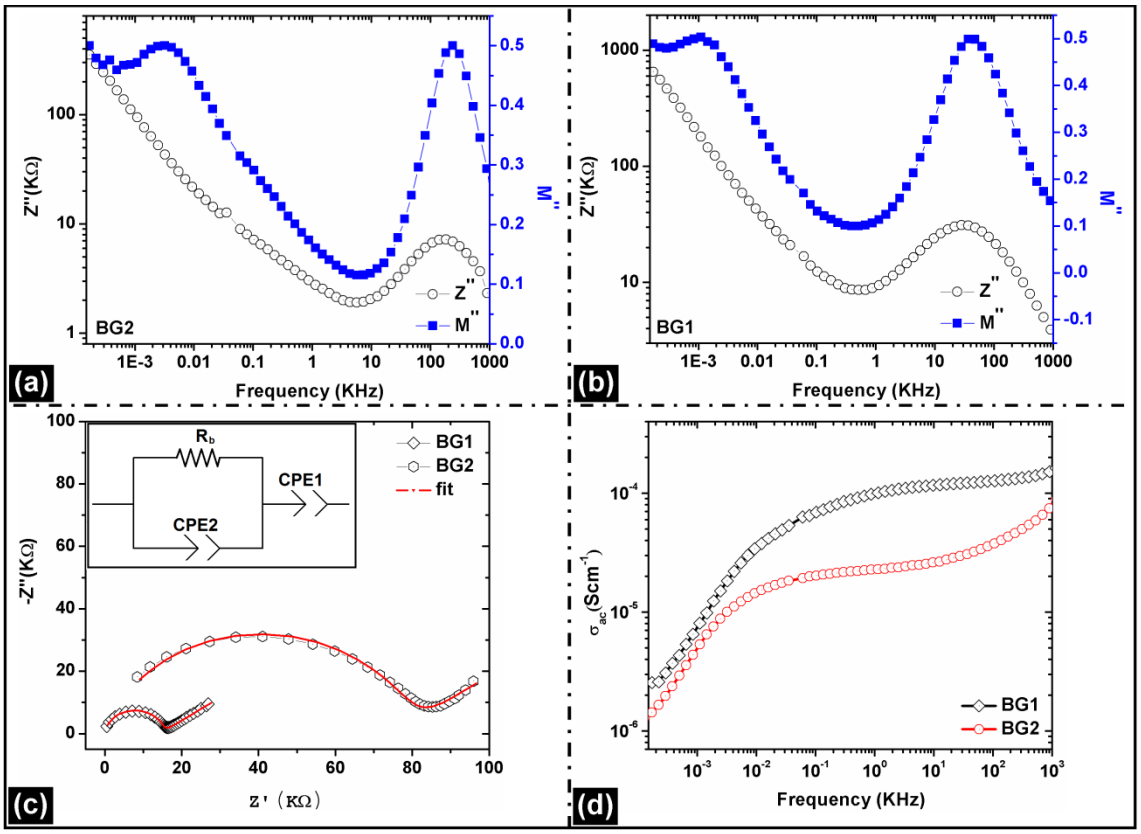

Figure 3. Electrochemical characterization of the bigels (a-b) impedance (Z″) and modulus (M″) plots (c) Nyquist plot and (d) a.c. conductivity.

Due to the localized movement of the charge carriers, the peak obtained from the impedance (Z″) and modulus (M″) spectra did not coincide [24]. This suggested deviation from the ideal behavior of the system (non-Debye behaviour). Hence, a constant phase element (CPE) is to be added in the equivalent circuit diagram to compensate this deviation [25]. A CPE element is generally used in a circuit diagram in place of capacitor to compensate the inhomogeneity of the system.

Two constant phase elements were introduced in the equivalent circuit diagram named as CPE1 and CPE2 (Figure 3(c)). CPE1 depicts the double-layer capacitance behaviour between electrode and electrolyte interfaces, whereas CPE2 describes the bulk properties of the system. When a semicircle is depressed, the CPE is placed parallel to a resistor. The semicircle obtained in the high-frequency region was due to the parallel combination of bulk resistance and CPE2, whereas the non-vertical spike obtained after the semicircle was due to CPE1.

The Nyquist plot exhibited two well-defined regions, namely, a semicircle in high frequency region, which was due to the bulk effect of electrolytes, and a non-vertical spike in the low frequency range which is attributed to the effect of blocking electrodes (Figure 3(c)) [26]. The low frequencies intercept on the real axis giving the bulk electrical resistance (R_b) value [27]. The bulk resistance increased with increased concentration of oleogel. The improved ionic conductivity of BG1 was due to the enhancement of the ionic mobility and number of carrier ions. The conductivity decreased with an increase in the oleogel concentration [28].

The change in a.c. conductivity of the bigels was plotted against frequency (Figure 3(d)). Three distinct zones were observed; a low frequency dispersion zone, an intermediate frequency plateau zone and a high frequency dispersion zone [29]. The frequency independent plateau gives the d.c. conductivity of the sample. BG1 showed higher conductivity in comparison to BG2. The non-conducting nature of oleogel resulted in decreased conductivity of BG2. The low frequency dispersion is due to the polarization effect on the sample-electrode interface. The conductivity of the bigels increased with increase in frequency. This confirmed the capacitive behavior of the bigels [29]. Jonschers power law is used to explain the conductivity of the system which is given by the equation [30]:

$$\Sigma_{ac} = \sigma_{dc} + A\omega^s$$

where, σ_{ac} is a.c. conductivity; σ_{dc} is d.c. conductivity; A is a pre-exponential constant; ω is the angular frequency and s is power law exponent, where $0 < s < 1$

The d.c. conductivity of the bigels was calculated by the formula:

$$\sigma_{dc}=(1/R_b)* (l/A)$$

where, R_b is bulk resistance, l is the thickness and A is the area of the sample (Table 3). BG1 showed higher d.c. conductivity as compared to BG2.

Table 3. Electrical properties of the bigels

Formulations	R_b (Ω) (10^4)	C_{CPE1} (F) (10^{-11})	n_{CPE1}	C_{CPE2} (F) (10^{-6})	n_{CPE2}	бdc (Scm^{-1}) (10^{-5})
BG1	1.44	7.59	0.99	6.01	0.40	8.85
BG2	7.75	50.60	0.85	3.35	0.43	1.64

3.4. Iontophoretic drug delivery

The impedance analysis confirmed the conductive nature the developed bigels. The conductivity behavior may improve the release of drug from the formulation. Topical formulations need to get absorbed at a faster rate for immediate effects. The faster release of metronidazole may improve the treatment efficiency in many diseased conditions. Hence, the metronidazole loaded bigels were evaluated as their possible application in iontophoretic drug delivery. The release of drug is enhanced during iontophoresis when an electric field is applied [31-32]. BG1M showed higher release

of metronidazole compared to BG2M in both active and passive conditions. The higher amount of water in BG1M might have enhanced the diffusion of drug through the dialysis membrane. The release rate in the active condition was higher compared to the passive condition in both the bigels. The drug molecule might have got charged due to the presence of an electrical field resulting in a higher amount of drug release from the bigel.

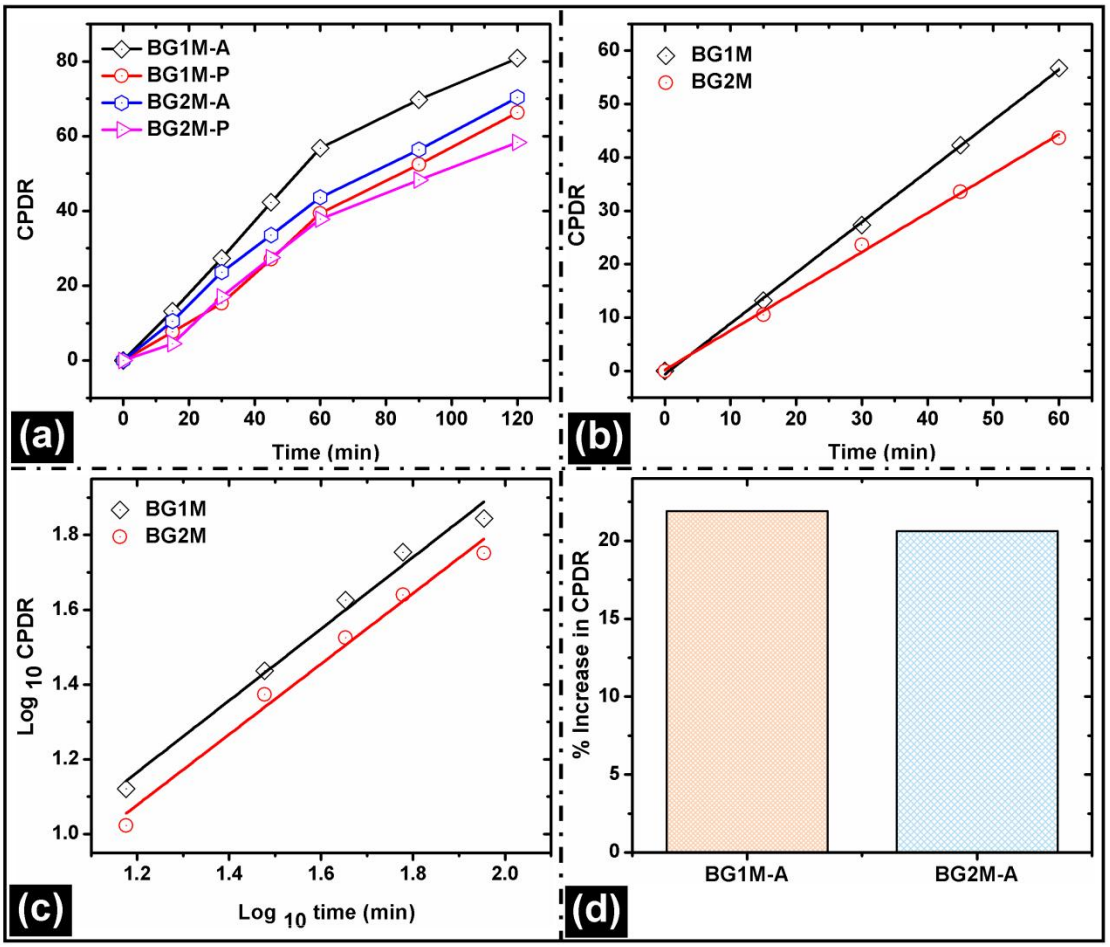

Figure 4. Drug release studies (a) cumulative percent drug release, (b) zero order fitting, (c) KP model fitting and (d) increase in iontophoretic drug release.

BG1M showed ~22%, whereas BG2M showed ~21% percent increase in the release of metronidazole over a period of 2 h (Figure 4(a), Table 4). The higher increase in drug release in BG1M may be accounted to its higher water content in comparison to BG2M (Figure 4(d)). The higher aqueous component might have increased the transfer of electrically charged drug molecule at a faster rate in BG1M. Metronidazole shows higher partitioning in water compared to oil. This property might have also helped in higher release of the drug in the presence of higher aqueous content. Hence, it may be concluded that the bigels can be tried as carriers for iontophoretic drug delivery [33].

Table 4. Drug release kinetics of the developed gels

Formulations	BG1M	BG2M
CPDR		
Active	80.86±3.25	70.38±3.12
Passive	66.33±2.89	46.31±1.28
Zero order		
Adj.R-Square	0.999	0.997
KP Model		
Adj. R-Square	0.982	0.981
n-value	0.96	0.94

The release kinetics of metronidazole from the bigels was examined by various kinetic models. The release of metronidazole followed zero-order kinetics which confirmed that the release of the drug was concentration independent and diffusion mediated (Figure 4(b)). The diffusion coefficient (n) value was estimated by fitting the release data with Korsmeyer-Peppas model (Figure 4(c)). It was in between 0.94 and 0.96 for both bigels suggesting non-Fickian case-II diffusion transport mechanism [34]. The non-Fickian diffusion behavior further confirmed that more than one mechanism (swelling and diffusion) was involved in the release of drug from the bigel matrix [35]. The difference in polymer relaxation behavior of the oleogels was the major reason behind the change of drug diffusion pattern.

4. CONCLUSION

The current study discussed about potential bigel matrices for iontophoretic drug delivery. The aquagel, oleogel and the bigels were prepared using easy and economical methods. The bigels were milky white and possessed smooth texture. The bigels containing higher aqueous content showed higher conductivity. The iontophoretic delivery application was evaluated by loading an antimicrobial drug, metronidazole in the gels. Higher aqueous contents in the bigel resulted in higher release of drug due to its higher partitioning in water compared to oil. The release of the drug followed zero order release kinetics. The electrically mediated release study showed a considerable increase in the release of drug under a constant current source. The preliminary studies suggested that the developed bigels possess the potential to be utilized as matrices for iontophoretic drug delivery.

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