Original Research Article

Development and characterization of k-carrageenan platforms as periodontal intra-pocket films

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Abstract

Purpose: *To prepare emulsion-based Intrapocket polymeric films for the treatment of periodontitis.*
Method: *Films were fabricated by dehydration of an emulsion containing k-carrageenan (KC) in aqueous phase and Compritol® 888 ATO (Compritol®) or Dimodan® UJ (DU®) or different ratios of both. The resulting films were characterized by mechanical texture analyser to determine Young's modulus and tensile strength. Glass transition temperature (T_g) of the films was evaluated by dynamic mechanical and thermal analyser while surface morphology was evaluated using scanning electron microscope. In-vitro drug release was conducted in pre-warmed phosphate buffer. Bacterial adherence was assessed after 24 h.*
Results: *Young's modulus was highest for KC films to which no lipid was added (5.33 ± 0.38 GPa) and decreased following lipid incorporation. T_g was highest in KC films (106.25 ± 4.53 ° C) but decreased upon addition of lipids. The surface of KC was smooth but roughness increased with increasing Compritol® load. Drug release from KC films was complete (99.80 ± 8.43 %) after 2 h; however, upon adding lipid, the release was extended 8 h and was affected by lipid type and ratio. Microbiologic assay demonstrated noticeable reduction in viable count compared to control and was affected by lipid type and ratio. The film formulated from a combination of DU® and Compritol® in a ratio of 80:20 was strong, flexible and reduced microbial adherence. Moreover, it showed a smooth surface and extended release for over 8 h.*
Conclusion: *Intra-pocket films were prepared by drying emulsion-based films. Resulted films were strong, flexible, prolonged drug release over 8 h and could lower bacterial growth. The prepared film may offer efficient treatment in periodontitis patients.*

Keywords: *K-carrageenan, Periodontitis, Biofilm, Dynamic mechanical thermal analysis, Pharmaceutical formulations*

INTRODUCTION

Periodontitis is a chronic disease [1] characterized by the formation of periodontal pockets deeper than 4 mm [2]. Periodontal disease is associated with degeneration of gum and may extend to alveolar bones or gingiva [3]. Prescribing antimicrobial agents is used in the treatment of periodontitis. However, it is deemed

necessary to employ high oral doses to achieve effective drug levels in the gingival crevicular fluid [1]. The limitation observed with high doses microbicide necessitated the need to develop localized delivery systems [4]. The development of intra-pocket delivery systems offer great potential because periodontal pockets represent a reservoir for the microbial organisms [5]. K-carrageenan (KC) was employed in pharmaceutical industry as an orodispersible film carrier and a common gelling agent [6]. KC forms fast dissolving films and thus may not be suitable for long periods of administration. As a result, an emulsion-based KC film would delay the fast dissolving nature of KC and subsequently retard drug release. Lipid excipients can serve two pronged approaches, firstly modify the drug release and secondly improve the brittleness observed in KC films. Kulkarni proposed that the addition of various types of lipids may affect the properties of the films and control the rate of drug release from its matrices [7]. As a result, it was decided to incorporate two lipids; Compritol® 888 ATO (Compritol®) and Dimodan® UJ (DU®) into KC films.

The films were loaded with metronidazole (MNZ) as an antimicrobial agent that has been used for the treatment of periodontitis in the forms of gels and films [5]. Clinical manifestation demonstrated slower rate of relapse of clinical parameters associated with administration of MNZ loaded films [8]. Subsequently, the films composed from KC, MNZ and lipids were subjected to further characterization. Films were prepared with or without the addition of varying amount of Compritol® and DU® followed by mechanical testing, drug release testing, and microbial assays to evaluate their use as potential intra-pocket films.

EXPERIMENTAL

Materials

(Compritol 888® ATO (glycerol dibehenate EP) lipid matrix was kindly donated from (Gattefossé, France). (Dimodan U/J lipid distilled monoglyceride was kindly donated by Du Pont Nutrition Biosciences, Denmark). Pluronic® F127 (Cat. no. P2443) and k-carrageenan (KC) (Cat. No. 22048) were purchased from Sigma Aldrich, Germany). (Metronidazole was purchased from Acros, USA (Cat. No. 210340050).

Preparation of dried film hydrogel

Films were produced using the modified procedure of Kulkarni *et al* [9]. Aqueous layer was prepared by stirring 10 mL KC (2 % w / w)

with 5mg MNZ at 60°C. The lipid layer was prepared by stabilizing 500 mg molten lipid in 10 mL of Pluronic® F127 (0. 5 w/v %). To prepare drug-loaded lipid particles, 500 mg molten lipid was added to a 20 mL glass vial containing a stabilizer solution (prepared separately by dissolving 0.5 % Pluronic® F127 in 100 mL water) to reach a mass of 1 g. The mixture was ultrasonicated (Omni Sonic Ruptor 400, Omni International, Inc, USA) for 10 min with a continuous pulse at 35% power. MNZ was loaded by stirring 5 mg for 15 min to ensure the drug was dissolved into the "milky" emulsion.

The composition of each of the prepared is illustrated in Table 1. KC solution (4 %) was prepared by stirring 0.4 g KC powder in 9.6 mL water at 60 °C for 20 min. A molten solution was combined with an equal volume of drug loaded in lipid particle dispersion and gently stirred for 20 min while heating until homogenization was visually achieved. The resultant gel contained 2 % KC. KC gel (without lipid particles) was prepared by stirring 5 mg MNZ with 2% KC solution at 60°C. Afterward, the gels were poured in Petri dishes (7 cm diameter) and left to dry overnight. The now dried films (intra-pocket films) were weighed three successive days to ensure complete dryness to ensure no water residual was present. The dried films were removed from the Petri dish and kept in plastic bags for further investigation.

Table 1: Composition and ratio of lipid-KC composite hydrogels. DU®, Compritol®. * Denotes KC with no lipid incorporated or "neat"

Formulation	DU® (%)	Compritol® (%)
F1	*	*
F2	100	0
F3	80	20
F4	50	50
F5	20	80
F6	0	100

Determination of glass transition temperature

Measurements of the glass transition temperature (T_g) of the intra-pocket (dried) films was performed using the Q800 DMTA (TA, USA), the mode selected was tensile and the selected oscillatory frequency was 1Hz to T_g. A heating rate of 3° C min^{-1} was employed over a temperature range of -20 °C to 140 °C. Dried films (n=3) were cut into rectangular shapes (30 mm length of the films, 5 mm width of the films and 0.11 mm and measurement of the thickness was recorded via a digitalized calliper). The T_g was detected from the peaks of the tan δ lines [10].

Evaluation of mechanical features

The film's tensile properties were analysed using a Stable Micro Systems TA-XT2 texture analyser (Goldaming, Surrey, UK). Dried films (3 cm length ✕ 0.5 cm width x 0.11 cm thickness, n = 5) were clamped between the static (lower) and moveable (upper) grips, to make sure the stressed films are kept under constant length of 20 mm. Inducing fracture on the films was done by raising the upper clamp at a fixed rate of 0.5 mms^{-1}. The mechanical features were retracted from the resulting plot of the stress-strain. The measurements of the various mechanical features were calculated as described by McAvoy *et al* [11], including the Young's modulus, the elongation (%) recoded at the break point and the Ultimate Tensile Strength (UTS) [11].

In vitro drug release

In-vitro drug release was determined by immersing dried films (average dimensions 30 mm length x 5 mm width x 0.11 cm thickness, n = 3) in 10 mL of pre-warmed phosphate buffer (pH 7.4) at 37°C in glass vials. At certain determined time intervals; media (10 mL) were taken and a fresh 10 mL warmed buffer was added.

The resulted amounts were measured using a previously plotted curve of r^2 value of > 0.99. Plots of measured concentration of released drug against time were plotted as previously recommended [12] using the mechanism of releasing was depicted by implementing the Korsmeyer-Peppas equation [13] as in Eq 1.

$$Mt/M_\infty = Kt^n \ldots\ldots\ldots\ldots (1)$$

where Mt/M_∞ is the fractional solute release, k is a kinetic constant, t is the release time, and n is the release exponent relates the pattern involved in drug release, where Fickian diffusion is suggested when reaching a value of around 0.5, while non Fickian or anomalous diffusion are denoted by values within the range of 0.5 – 1.0 [13]. The release study was continued to 24 h to ensure more than 80 % of the drug release in all formulations.

Microbiological assay

The ability of the prepared formulations to minimize microbial growth on their surfaces was studied using two standard microorganisms: *Candida albicans* (ATCC 10231) and *Streptococcus oralis* (ATCC 6249). The dried films were exposed to high concentration of

microbial growth (circa 1 X1 0^9 CFU / mL, confirmed by viable count) and were left for 24 h, then the adhered bacteria were dislodged and viable count was computed.

Microbial adhesion experiment was done according to Jones *et al* with modifications. Dried films (n = 6, mean disc thickness of 0.11 mm and mean diameter of 2.8 mm) were swabbed with microorganism suspensions and immediately placed on an Agar Petri dish and left for 24 h. Afterwards, the loose microorganisms were washed out using Quarter Strength Ringer Solution (QSRS) after 3 x washing of 20 mL for 30 s. Dislodgement of adherent microbes was done by sonicating the films separately in sealed test tubes filled with 10 mL QSRS. The films were removed and subsequent calculation for the viable microbes was conducted after serially diluting of the liquid phase, and the measures were quantified as CFU/mm^2. Each film was compared to a control containing similar formulation components but devoid from MNZ [12].

Assessment of film morphology

Surface topography of the dried films was examined by SEM using a FEI Quanta FEG 450 (ThermoFisher Scientific, USA). Samples were mounted on slides and gold-coated, and images were taken using a 10 keV acceleration voltage of the secondary electron (SE) mode.

Statistical analysis

Simple results were expressed as mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) was implemented for advanced statistical analysis, and the Tukey Kramer test was performed for all post hoc tests. A statistical value of *p < 0.05* was denoted significant for all types of statistics.

RESULTS

Glass transition temperature (T$_g$)

Table 2 summarizes the recorded T$_g$ of the dried films. The T$_g$ of F1 lipid-free films was 106.25 ± 4.53 °C. Incorporation of lipids in KC films decreased T$_g$ in all cases (Table 2). However, the reduction in T$_g$ was more profound upon increasing the relative content of DU®. For instance F2; KC films containing 100 % DU® demonstrated a T$_g$ of 65.81 ± 3.86 °C (*p = 0.021*) compared to F1. The addition of Compritol® affected T$_g$ as well; F4 which is KC film loaded with equal ratio of DU®: Compritol® exhibited a T$_g$ of 79.38 ± 2.27 °C. Whereas KC films which

included 100% Compritol ®showed T of 83.91 ± 2.58 °C (F6, KC film loaded with lipid, 100 % Compritol®).

Mechanical properties

The mechanical properties (Young's modulus, UTS and % elongation) of the medical films are presented in Table 3. KC neat films (F1) displayed a high Young's modulus (5.33 ± 0.38 GPa). The inclusion of lipids and the type of lipid to KC films resulted in a significant reduction in Young's modulus. For instance, F6 films, which contained the highest amount of Compritol® had Young's modulus value of 1.21 ± 0.20 GPa (significantly lower than F1 films, $p = 0.006$) and F2 which had the highest load of DU® has Young's modulus of 0.54±0.02 ($P = 0.022$ compared to F6)**.**

UTS for F1 (KC neat film) was high (41.59 ± 8.83 MPa). The addition of lipids significantly reduced UTS; where F2 (100 % DU® containing KC films) showed UTS of 6.53 ± 0.43 MPa. Moreover, increasing the ratio of Compritol® increased UTS amongst the lipid-containing films (F6, 100 % Compritol® had 25.96 ± 2.37 MPa, $P = 0.000$ against all lipid containing films).

Furthermore, the % elongation at break for F1 (KC neat films) was 1.19 ± 0.25 %. The addition of DU® significantly increased the flexibility of the films; F2 had the highest % of elongation value

3.68 ± 0.90 % in ($p = 0.024$ compared to F1 neat KC film). The addition of Compritol® in all ratios did not enhance the elasticity significantly where the % of elongation at break observed was similar to that for KC (neat film) (p = 1.000 compared to F1).

In vitro drug release

Figure 1 illustrates the *in- vitro* release profile of MNZ from the prepared dried films. Clearly, the polymer type and lipid ratio dictated the release profile. The KC films F1 (with no lipids incorporated) exhibited complete drug release (99.8 ± 8.43 %) in the first hour of the experiment. The incorporation of lipids extended the drug release; the initial stage of the release experiment exhibited a burst in the lipid containg films, where more than 25 % of the drug was released after one hour in all formulations. Furthermore, the lipid type had significantly affected the rate of the drug release, where the incrorporation of Compritol® to the KC films showed a slower release rate compared to F1 KC film and films containing DU®.

For example, 6.40 ± 0.63 % MNZ was released from F6 (100 % of Compritol®) at 10 minutes, whereas F2 (100 % of DU®) illustrated a release of 9.87 ± 0.81 % at the same time point ($p < 0.05$). After 8 h, the observed release in F6 was 57.16 ± 3.7 % and for F2 the MNZ release was

Table 2: Effect of varying lipid content ratio (ratios of included DU® and/or Compritol®) on T_g of dried KC films (n = 3)

Formulation	DU® (%)	Compritol® (%)	Glass transition temperature T_g (°C) (mean ± SD)
F1	_*	__*	106.25 ±4.53
F2	100	0	65.81 ±3.86
F3	80	20	62.29 ±5.12
F4	50	50	79.38 ±2.27
F5	20	80	86.15 ±0.18
F6	0	100	83.91 ±2.58

Note: *All dried films containing 5 mg MNZ. T_g was determined using DMTA at 1Hz, heating ramp of 3 ° C / min. *Denotes KC with no inclusion of lipids*

Table 3: Effect of lipid content ratio (ratios of DU® and/or Compritol®) on the mechanical properties of KC films. Note that all formulations contained 5 mg MNZ (n = 5). *Denotes KC with no inclusion of lipids

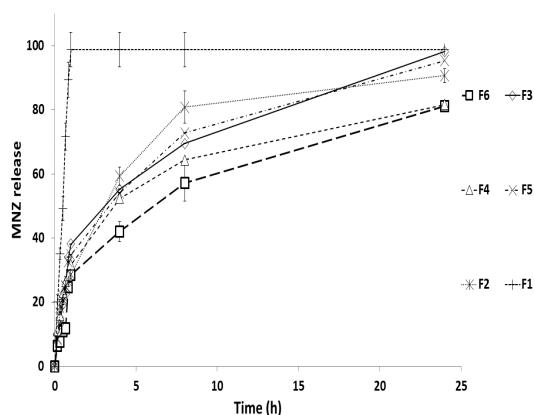| Formulation	DU®	Compritol®	Mechanical properties (mean ± SD)		
			Young's modulus (GPa)	UTS (MPa)	Elongation (%)
F1	-*	-*	5.33 ±0.38	41.59 ±8.83	1.19 ±0.25
F2	100%	0%	0.54 ±0.02	6.53 ±0.43	3.68 ±0.90
F3	80%	20%	0.76 ±0.10	16.37 ±2.26	1.51 ±0.63
F4	50%	50%	0.84 ±0.18	13.71 ±4.27	1.20 ±0.14
F5	20%	80%	0.72 ±0.89	16.37 ±2.23	1.68 ±0.59
F6	0%	100%	1.21 ±0.20	25.96 ±2.37	0.99 ±0.26

Figure 1: *In vitro* release profiles of MNZ (5 mg) from KC films of variable lipid content ratios F1 film with no lipid inclusion. F2: KC film loaded with 100% DU®. F3: KC film loaded with 80 % DU® and 20 % Compritol®. F4 KC films loaded with 50 % DU® and 50 % Compritol®. F5 KC films loaded with 20 % DU® and 80 % Compritol®. F6 KC films loaded with 100 % Compritol®

80.89 ± 4.97 %. The release constant (K) was the fastest in F1 (KC alone without incorporation of lipids, k = 8.7, Table 4) and was slowest in F6 (KC films containing 100 % Compritol®) having a K value of 1.4. Table 4 demonstrates that the release exponent (n) was found to be within the range of 0.47 to 0.81 suggesting that the release mechanism was non-Fickian dominated by erosion and diffusion. The release study was continued to 24 hours to ensure more than 80 % of the drug was released in all formulations.

Microbiologic properties

Figure 2 and Figure 3 demonstrate the growth of *C. albicans* (ATCC 10231) and *S. oralis* (ATCC 6249) respectively on the dried films surface (The data is presented in supplementary file and show microbial growth compared to control of the same formulation component but devoid from MNZ).

Considering *C. albicans*, neat KC films (F1) disintegrated to gels and were not eligible for viable count procedure therefore; it is not presented in Figure 2. Clearly all dried films had lower growth compared to their control. Considering the prepared films, the highest

candida growth was observed with increasing the load of Compritol® in F6 (loaded with 100 % Compritol®) and F5 (loaded with 80 % Compritol®) having a growth of 14070 and 9323 CFU / mm^2, respectively. On the other hand, increasing DU® ratio demonstrated lower growth.

As observed in the F1 films mixed with *C. albicans,* KC films F1 and F2 had disintegrated to gel and were not eligible for viable count. The addition of Compritol® improved the integrity of the films. However, raising the amount of added Compritol® resulted in an increase in the number of viable *S. oralis* (Figure 3). For example, the loading of 100 % Compritol® (F6) demonstrated growth of 7055 CFU/mm^2. On the other hand, the lowest bacterial growth was observed with the highest employed DU® loading (F3, 80 % DU®, *P < 0.05* compared to all formulations).
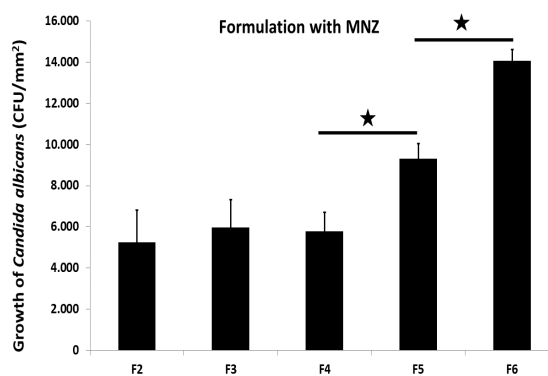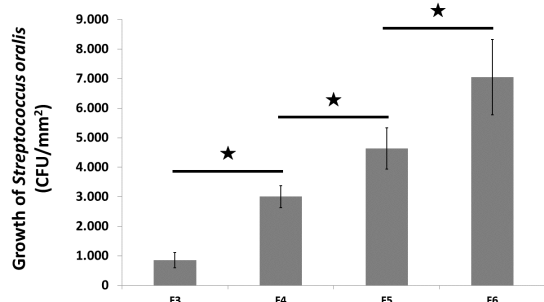

Figure 2: Mean (± S.D.) of viable count for adhered *C. albicans* (ATCC 10231) on KC films of variable; lipid content ratios after 24 h. F2: KC film loaded with 100% DU®. F3: KC film loaded with 80 % DU® and 20 % Compritol®. F4 KC films loaded with 50 % DU® and 50 % Compritol®. F5 KC films loaded with 20 % DU® and 80 % Compritol®. F6 KC films loaded with 100 % Compritol®

Film morphology

The micrograph illustrates the surface of KC neat film F1 (Figure 4 -A) where it appears smooth. Figures 4-B and 4-C illustrate the SEM micrographs of F2 (100% DU®) and F3 (80% DU®); both surfaces appear smooth. However, the employment of higher loadings of Compritol® led to a rough surface. For example, Figure 4- D

Table 4: *In vitro* drug release of MNZ, as indicated by the Korsmeyer–Peppas release exponent (n) and rate constant (k). F1 film with no lipid inclusion. F2: KC film loaded with 100% DU®. F3: KC film loaded with 80 % DU® and 20 % Compritol ®. F4 KC films loaded with 50 % DU® and 50 % Compritol ®. F5 KC films loaded with 20 % DU® and 80 % Compritol ®. F6 KC films loaded with 100 % Compritol ® Note: Films are loaded with 5 mg MTN and variable lipid ratio

Parameter	F1	F2	F3	F4	F5	F6
N	0.81	0.77	0.77	0.65	0.61	0.47
K	8.7	3.6	3.6	3.5	2.8	1.4

DISCUSSION

Following successful preparation of the intra-pocket films, different characterization techniques were carried out to evaluate their efficiency. *C. albicans* viable count of tested films was significantly lower than their corresponding control (P < 0.05 in all cases). KC neat films (F1) turned into gel and therefore it was difficult to dislodge the organisms. Furthermore, increasing the load of Compritol® increased viable count as observed in F6. The ability of MNZ to lower the viable count may have an impact on reducing the complications of chronic periodontitis as explained by Larsen [8]. *S. oralis adherence* was significantly lower than control (*p = 0.000*, supplementary data). KC neat film disintegrated to gel after 24. Raising the amount of added Compritol® resulted in marked increase in the adherence of bacteria.

Wake *et al* was able to grow dental biofilms and found the viable count after 24 h incubation ranging from 1×10^9 to 10^{10} CFU/mL [14]. KC lipid-loaded films exhibited an exceptional reduction in viable count, which was 10^6 times lower compared to the reported values at the same time point.

Rough surfaces would encourage the attachment of microbes to adhere better [15]. The SEM micrographs illustrated a smooth surface for KC neat and DU®-containing KC films (Figure 4). Conversely, Compritol® exhibited a rough surface. The variation in melting point between DU® and Compritol® may attribute to the roughness of the surface, as Compritol® has a higher melting point which means it solidifies slower than DU® and this may suggest that compritol lipid particles have a higher probability to arrange themselves into spheres.

SEM micrographs suggested employing Compritol® alone may not be proper due to rough surfaces. In addition, the high number of microbes that grew on the surface of F6 suggested that the addition of Compritol® in low ratio compared to DU® in KC films is preferred.

The incorporation of lipids significantly affected T_g of KC films. T_g of F1 was 106 ° C consistent with the value reported in the literature (108.9 ° C) [16]. The incorporation of lipids significantly reduced T_g of KC (*P<0.05*) from 106.25 ± 4.53 °C to a T_g range (62 to 86 ° C). The reduction in T_g was influenced by the type of lipid and melting temperature [17]. This reduction is attributed to the lower melting point of the lipid that will allow the lipid molecules in their melted form to penetrate between the polymer chains and

Figure 3: Mean (± SD) of viable count for adhered *S. oralis* (ATCC 6249) on KC films of variable lipid content ratios after 24 h. F3: KC film loaded with 80 % DU® and 20 % Compritol®. F4 KC films loaded with 50 % DU® and 50 % Compritol®. F5 KC films loaded with 20 % DU® and 80 % Compritol ®. F6 KC films loaded with 100 % Compritol®
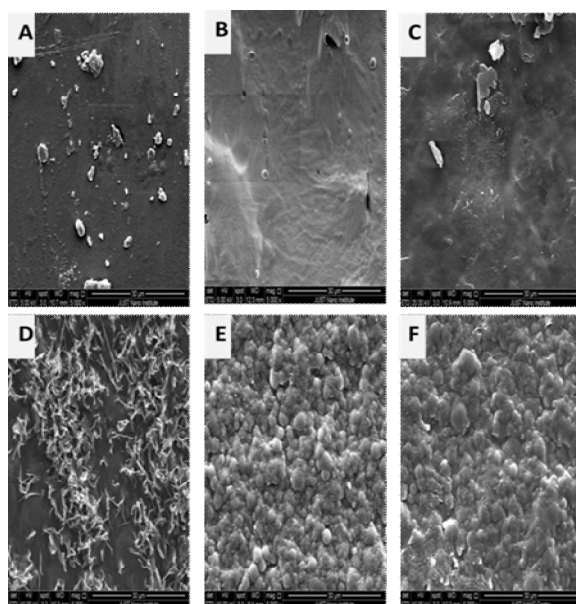
Figure 4: Surface topography of KC films of variable lipid content ratio. A: F1 film with no lipid inclusion. B: F2 KC film loaded with 100% DU®. C: F3 KC film loaded with 80 % DU® and 20 % Compritol®. D: F4 KC films loaded with 50 % DU® and 50 % Compritol®. E: F5 KC films loaded with 20 % DU® and 80 % Compritol®. F: F6 KC films loaded with 100 % Compritol®. Micrographs were taken at 5000 X magnification and 5.00 kv

which represents F4 (50% Compritol®) shows a unique morphological charac-teristics of the film as the surface appeared rough with sharp objects. Furthermore, films loaded with a higher load of Compritol® F5 (80 % Compritol®) and F6 (100 % Compritol®) in Figure 4- E and 4- F, respectively illustrate a rough surface with sphere-like particles that may be developed throughout the cooling process.

create more void leading to a lowering in T_g as supported by Omelczuk *et al* [18].

The mechanical properties of polymers are strongly dependent on their T_g [18]. Films with lower T_g are more flexible and demonstrate higher percentage of elongation. [19]. High T_g is observed with high Young's modulus of elasticity [19]. KC neat films demonstrated the highest Young's modulus value. The further decrease in Young's modulus was consistent to the addition of DU[®] and Compritol[®] which acted like plasticisers. The inclusion of the aforementioned lipids led to an increase in the void volume, thus rendering the polymer chain more flexible (increased % elongation) and weaker (decreased tensile strength). These observations are compatible with findings reported by Oladzadabbasabadi and collegues [20].

The employment of Compritol[®] to retard drug release was extensively investigated in the literature [21]. Lipid-containing formulations released more than 25% of drug during the first hour. The release at the 8 h time point has reached 80%. The intra-pocket films contain approximately 250 µg of MNZ. Considering that 25 % of drug content was released (corresponding to 62.5 µg) in the first hour and the remaining drug continued to elute over the next 8 h indicates that MIC was reached during the entire period of administration. The drug release experiments illustrated significant differences between lipid-containing KC films. F6 was able to retard drug release more than F2 and demonstrated the lowest release constant K as observed in Table 4. In addition, the high T_g of the KC films loaded with Compritol[®] would retard the release of the drug as the gelation and erosion of the KC chains will be delayed.

CONCLUSION

This work reports the successful preparation of Intra-pocket films for the treatment of periodontitis. The films have been fabricated by drying an emulsion-based KC gel. The films showed variation in their characteristics: ability to reduce microbial growth, surface morphology, mechanical properties and drug release. The use of DU[®]: Compritol[®] 80: 20 in the emulsion based dried films met the demand of this project where the resulting films are strong, flexible, prolonged the drug release over 8 h and reduced the growth of micro-organisms. This indicates potential for further investigation as a commercial product.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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REFERENCES

1. Khan G, Yadav SK, Patel RR, Nath G, Bansal M, Mishra B. Development and evaluation of biodegradable chitosan films of metronidazole and levofloxacin for the management of periodontitis. AAPS PharmSciTech 2016; 17 (6): 1312-1325.

2. Kaur S, White S, Bartold P. Periodontal disease and rheumatoid arthritis: a systematic review. J Dent Res 2013; 92 (5): 399-408.

3. Kebschull M, Demmer R, Papapanou P. "Gum bug, leave my heart alone!"—epidemiologic and mechanistic evidence linking periodontal infections and atherosclerosis. J Dent Res 2010; 89 (9): 879-902

4. Chen X, Wu G, Feng Z, Dong Y, Zhou W, Li B, Bai S, Zhao Y. Advanced biomaterials and their potential applications in the treatment of periodontal disease. Crit Rev Biotechnol 2016; 36 (4): 760-75.

5. *Jain N, Jain GK, Javed S, Iqbal Z, Talegaonkar S, Ahmad FJ, Khar RK. Recent approaches for the treatment of periodontitis. Drug Discov Today 2008; 13 (21-22): 932-943.*

6. *Hoffmann EM, Breitenbach A, Breitkreutz J. Advances in orodispersible films for drug delivery. Expert Opin Drug Deliv 2011; 8 (3): 299-316.*

7. *Kulkarni CV, Tomšič M, Glatter O. Immobilization of nanostructured lipid particles in polysaccharide films. Langmuir 2011; 27 (15): 9541-9550. Kulkarni CV, Tomšič M, Glatter O. Immobilization of nanostructured lipid particles in polysaccharide films. Langmuir 2011; 27 (15): 9541-9550.*

8. *Larsen T. In vitro release of doxycycline from bioabsorbable materials and acrylic strips. J Periodontol 1990; 61 (1): 30-34.*

9. *Kulkarni CV, Moinuddin Z, Patil-Sen Y, Littlefield R, Hood M. Lipid-hydrogel films for sustained drug release. Int J Pharm 2015; 479 (2): 416-421.*

10. *Backfolk K, Holmes R, Ihalainen P, Sirviö P, Triantafillopoulos N, Peltonen J. Determination of the glass transition temperature of latex films: Comparison of various methods. Polym Test 2007; 26 (8): 1031-1040.*

11. *McAvoy K, Jones D, Thakur RRS. Synthesis and Characterisation of Photocrosslinked poly (ethylene glycol) diacrylate Implants for Sustained Ocular Drug Delivery. Pharm Res 2018; 35 (2): 36.*

12. *Jones DS, Lorimer CP, McCoy CP, Gorman SP. Characterization of the physicochemical, antimicrobial, and drug release properties of thermoresponsive hydrogel copolymers designed for medical device applications. J Biomed Mater Res B Appl Biomater: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials 2008; 85 (2): 417-426.*

13. *Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm 1983; 15 (1): 25-35.*

14. *Wake N, Asahi Y, Noiri Y, Hayashi M, Motooka D, Nakamura S, Gotoh K, Miura J, Machi H, Iida T. Temporal dynamics of bacterial microbiota in the human oral cavity determined using an in situ model of dental biofilms. NPJ Biofilms Microbiomes 2016; 2: 16018.*

15. *Roehling S, Astasov-Frauenhoffer M, Hauser-Gerspach I, Braissant O, Woelfler H, Waltimo T, Kniha H, Gahlert M. In vitro biofilm formation on titanium and zirconia implant surfaces. J Periodontol 2017; 88 (3): 298-307.*

16. *Mahmood WAK, Khan MMR, Yee TC. Effects of reaction temperature on the synthesis and thermal properties of carrageenan ester. J Phys Sci 2014; 25 (1): 123-138.*

17. *Roblegg E, Ulbing S, Zeissmann S, Zimmer A. Development of lipophilic calcium stearate pellets using ibuprofen as model drug. Eur J Pharm Biopharm 2010; 75 (1): 56-62.*

18. *Omelczuk MO, McGinity JW. The influence of polymer glass transition temperature and molecular weight on drug release from tablets containing poly (DL-lactic acid). Pharm Res 1992; 9.1: 26-32.*

19. *Repka MA, Gerding TG, Repka SL, McGinity JW. Influence of plasticizers and drugs on the physical-mechanical properties of hydroxypropylcellulose films prepared by hot melt extrusion. Drug Dev Ind Pharm 1999; 25 (5): 625–633.*

20. *Oladzadabbasabadi N, Ebadi S, Mohammadi Nafchi A, Karim AA, Kiahosseini SR. Functional properties of dually modified sago starch/κ-carrageenan films: An alternative to gelatin in pharmaceutical capsules. Carbohydr Polym 2017; 160: 43-51.*

21. *Barakat NS, Elbagory IM, Almurshedi AS. Controlled-release carbamazepine matrix granules and tablets comprising lipophilic and hydrophilic components. Drug Delivery 2009; 16 (1): 57-65.*