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Research Paper

Reformulation of extemporaneous tetracycline mouthwash to improve its stability for pemphigus patients

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ABSTRACT

Patients with pemphigus vulgaris are prescribed an extemporaneously prepared antimicrobial mouthwash containing hydrocortisone, nystatin, diphenhydramine and tetracycline (HNDT) to prevent opportunistic infections. Literature shows that tetracycline has poorer aqueous stability compared to doxycycline. This study investigated how temperature and pH affect tetracycline stability in HNDT mouthwash and explored the feasibility of reformulating with doxycycline to attain a longer beyond-use date. The original and pH-modified tetracycline-formulated and doxycycline-formulated mouthwashes were stored at 5ºC and 30ºC for 7–21 days. The amounts of tetracycline or doxycycline remaining were quantified using a high-performance liquid chromatography (HPLC) method developed. The pH-modified mouthwashes generally showed lower stability than the original mouthwashes. Overall, doxycycline showed a better stability profile than tetracycline. The doxycycline-formulated mouthwash (without pH adjustment) was found to be most stable when stored at 5ºC and enabled pemphigus patients to use the mouthwash for an extended period of 3–4 weeks.

Key words: pemphigus vulgaris, tetracycline, doxycycline, antimicrobial mouthwash, stability

1. Introduction

Pemphigus vulgaris is a chronic autoimmune skin disease that results in painful blisters and erosions on the skin and mucous membranes, commonly inside the mouth. Patients suffering from oral lesions will require a mouthwash to maintain good oral hygiene and prevent opportunistic infections (Amagai et al., 1991; Budimir et al., 2008). The mouthwash also acts as a supportive therapy to reduce pain in the oral cavity caused by ulceration (Meurer et al., 1977; Papakonstantinou et al., 2018). Currently, in a local health-care institution, an extemporaneous preparation, referred to as HNDT mouthwash, containing hydrocortisone, nystatin, diphenhydramine and tetracycline hydrochloride (TC) is dispensed to pemphigus outpatients. TC refers to the hydrochloride salt of tetracycline while tetracycline free base refers to the non-ionized form (Miyazaki et al., 1975).

The poor aqueous stability of TC (Yi et al., 2016) coupled with no local study on the stability and efficacy of the HNDT mouthwash raised concern on the arbitrarily assigned beyond-use date of two weeks. Patients are also inconvenienced as they have to return fortnightly to collect fresh supplies of the mouthwash. It was reported that degradation rate of TC increased with temperature when raised from 25°C to 37°C while TC in phosphate buffer solutions degraded the most rapidly at pH 2.0, followed by pH 6.0, then pH 4.0 (Lund, 1994; Wu and Fassihi, 2005). In general, less acidic mouthwashes of pH 6-7 are ideal as they minimize demineralization of the enamel and pain to the mouth with oral lesions (Kalyani and Leelavathi, 2019). However, it is also important to select a pH where the stability of the drug can be maintained.

Doxycycline, a second-generation tetracycline antibiotic, has increasingly been prescribed (Grantham et al., 2017; Kimura and Kawai, 2020; Maibach, 1991). It was reported to be more stable than TC at an elevated temperature (Hassani et al., 2008). In addition, doxycycline has better antimicrobial activity with less side effects such as reduced gastrointestinal disturbances, diminished tooth discoloration and potentially lower hepatotoxicity (Heaton et al., 2007; Sweetman, 2009).

Moreover, doxycycline has anti-inflammatory activities and bacterial susceptibility at lower dose (Di Caprio et al., 2015). It was therefore postulated that doxycycline would be a good substitute for the less stable tetracycline in the mouthwash.

Doxycycline hyclate (DH) is a semisynthetic compound and it is a more soluble salt form of doxycycline (Sweetman, 2009). Factors such as pH and temperature could affect the stability of DH as it exists in different forms under different pH conditions and epimerization is slower at the pH range of 5.0–6.0 (Colaizzi and Klink, 1969; Libinson and Ushakova, 1976). Another study showed that pure DH underwent thermal degradation (Injac et al., 2007). However, little is known about the effects of storage temperature on the stability profile of DH in a mouthwash comprising multiple components.

This study aimed to investigate how pH and storage temperature would affect the stability of TC and DH in mouthwashes that also comprised other components such as hydrocortisone, nystatin and diphenhydramine. The feasibility of replacing tetracycline with doxycycline in order to formulate a more stable antimicrobial mouthwash with longer beyond-use date was evaluated. TC was reported to be most stable at pH 4.0 (Wu and Fassihi, 2005). Hence, TC-formulated mouthwashes, with unadjusted pH (original) and pH adjusted to pH 4.0–4.5 (modified), were studied. DH epimerization was reported to occur slower at pH range of 5.0–6.0 (Colaizzi and Klink, 1969; Libinson and Ushakova, 1976). Consequently, DH-formulated mouthwashes with unadjusted pH (original) and pH adjusted to pH 5.0–6.0 (modified), were also evaluated. The original and modified mouthwashes were stored at refrigeration (5°C) and ambient (30°C) temperature and stability profiles of TC or DH evaluated over the storage period.

2. Materials and Methods

2.1. Materials

TC (Sigma Aldrich, St. Louis, USA), which complied with USP testing specifications, was used as a reference standard. TC powder (ICM Pharma, Singapore), hydrocortisone tablets (Orion Pharma, Espoo, Finland), DH, nystatin and diphenhydramine hydrochloride (USP grade, Medisca, Plattsburgh, USA) were purchased to prepare the mouthwashes. Di-sodium hydrogenphosphate, sodium hydroxide, ortho-phosphoric acid 85% (Merck, Darmstadt, Germany), sodium di-hydrogenphosphate and potassium dihydrogenphosphate (Nacalai Tesque, Kyoto, Japan) were used to modify the pH of the mouthwashes.

Acetonitrile (HPLC grade, Fisher Scientific, Geel, Belgium), 1.0 M hydrochloric acid, ortho-phosphoric acid 85% (Merck, Darmstadt, Germany), tetrahydrofuran (HPLC grade, Fulltime, Anqing, China and purified water were used in the HPLC analysis.

2.2. Mouthwashes formulation

The mouthwashes consisted of 0.50 mg/mL hydrocortisone, 1.81 mg/mL nystatin and 2.20 mg/mL diphenhydramine hydrochloride, together with 6.25 mg/mL TC or 0.20 mg/mL DH. The original mouthwashes were prepared by dissolving TC or DH completely in purified water before addition of other components.

The modified TC-formulated mouthwashes were prepared by dissolving the ingredients in pH 6.0 phosphate buffer and the resultant mixture was adjusted to pH 4.0–4.5 using sodium hydroxide and ortho-phosphoric acid. The modified DH-formulated mouthwashes were prepared by dissolving the ingredients in a phosphate buffer. The latter was produced by diluting 50 mL of 0.2 M potassium dihydrogenphosphate solution to 200 mL with purified water, followed by pH adjustment to 5.5 using 0.2 M sodium hydroxide solution. A pH range of 5.0–5.5 was attained in the DH-formulated mouthwash after adding all the components.

2.3. Determination of pH of mouthwashes

The pH meter (PB-11, Sartorius, Goettingen, Germany) was pre-calibrated and used to determine the measurements.

2.4. UV spectrophotometric analysis of components in the mouthwash formulations

A UV spectrophotometer (SHD0150, Shimadzu, Kyoto, Japan) was used to scan the absorbance of each component in the mouthwash over wavelength range of 200-400 nm. The maximum absorbance wavelength (λ_{max}) of TC and DH with the least absorbance by other components was subsequently utilized in the HPLC method to assay TC and DH respectively.

2.5. Development of analytical methods for TC and DH

A HPLC system (LC-20AT, Shimadzu, Kyoto, Japan) with a diode array detector (SPD-M10A, Shimadzu, Kyoto, Japan) was used. The HPLC method employed by Hussien (Hussien, 2014) to assay TC was adapted as good separation of TC and its degradants was obtained. The C18 reversed phase column (YMC-Pack ODS-AQ, 250 mm × 4.6 mm i.d., 5 μm, YMC America, Allentown, USA) was maintained at 50°C. The mobile phase consisted of acetonitrile and purified water acidified to pH 2.8 using ortho-phosphoric acid to suppress ionization of TC and mixed separation mechanisms (Anderson et al., 2005). The amount of acetonitrile was varied from 15% to 40% in 7.5 min, back to 15% in 0.1 min, and held at 15% for 2.4 min. The flow rate was 1.0 mL/min and the injection volume was 50 μL. The detection wavelength used was determined based on the UV spectrophotometric results.

The HPLC method employed by Injac et al (Injac et al., 2007) to assay DH was adapted. A symmetry C8 reversed phase column (250 mm × 4.6 mm i.d., 5 μm, Waters Corporation, Milford, Massachusetts) was used. The mobile phase

comprised acetonitrile-purified water-tetrahydrofuran (28:67:5 v/v/v) acidified to pH 2.5 with 1.0 M hydrochloric acid for reducing mixed separation mechanisms of DH (Anderson et al., 2005). The mobile phase was delivered at 0.5 mL/min into a column maintained at 27°C and injection volume was 10 µL. The detection wavelength used was determined based on the UV spectrophotometric results.

Prior to HPLC analysis, the samples were equilibrated to room temperature by leaving to stand for 30 min. They were pre-filtered through a 1 µm syringe filter (Acrodisc, Pall Corporation, New York, USA) and diluted to the working concentration range of the HPLC analysis method. The samples were then passed through a 0.45 µm RC filter (Sartorius, Gottingen, Germany) and analyzed by HPLC.

2.6. Determination of solubility of TC and DH in mouthwashes

Excess amounts of TC and DH were added separately into purified water as well as original and modified mouthwashes at room temperature (i.e. air-conditioned laboratory temperature) of 22°C and left to stir continuously to determine the solubility of TC and DH in the respective medium. Samples were withdrawn and concentrations of TC and DH dissolved were determined by the HPLC methods described in the previous section.

2.7. Stability studies of TC and DH in mouthwashes

The original and modified TC-formulated and DH-formulated mouthwashes were prepared and stored in amber glass bottles to protect from light. The mouthwashes were stored separately in a refrigerator maintained at temperature of 5°C and in a stability chamber (WTC binder, Fisher Scientific, Singapore) maintained at temperature of 30°C, which represents the typical local ambient temperature. Aliquot samples were taken immediately after preparation and at pre-determined time points over a period of 7–21 days.

2.8. Assay of TC and DH in stability studies

Aliquot samples of the mouthwashes withdrawn were pre-filtered (0.45 µm RC filter, Sartorius, Gottingen, Germany), diluted appropriately and stored at –20°C in a freezer (H700L, Kelvinator, Mascot, Australia) to minimize further degradation prior to HPLC analysis. The frozen samples were thawed to room temperature and passed through a 0.45 µm RC filter (Sartorius, Gottingen, Germany) prior to injection for HPLC analysis. The concentrations of TC and DH present were determined by the HPLC methods described previously.

2.9. Statistical analysis

Two sample t-test was performed using Microsoft Excel™ 2011 (Microsoft Corp, Redmond, USA) to determine any significant differences in TC and DH content in the samples of the stability study. The difference was considered significant if p value is < 0.05.

3. Results and Discussion

3.1. pH values of the mouthwashes

The pH of TC in purified water was 2.46 ± 0.05, while those of the original TC-formulated and modified TC-formulated mouthwashes were 2.75 ± 0.02 and 4.27 ± 0.25, respectively. On the other hand, the pH of DH in purified water was 3.06 ± 0.01, while those of the original DH-formulated and modified DH-formulated mouthwashes were 3.38 ± 0.04 and 5.37 ± 0.03, respectively. Acidic mouthwashes of low pH were likely to cause pain when used with blisters or lesions in the mouth. Besides, low pH can adversely affect the stability of TC and DH and therefore it is important to alter the pH of the mouthwashes appropriately. Reported studies have suggested that the stability of the other components (i.e. hydrocortisone, nystatin and diphenhydramine) would not be significantly affected by the modified pH conditions used (Manchanda et al., 2018).

3.2. UV absorbance of components in the mouthwashes

Individual components were dissolved in purified water according to the concentrations used in the mouthwash and diluted appropriately for UV analysis. TC had two absorbance peaks, at 277 nm and 357 nm. At 357 nm, other components had minimal absorbance, thus 357 nm was chosen for UV quantification of TC in the HPLC analysis. DH also showed two absorbance peaks, at 274 nm and 347 nm. At 347 nm, hydrocortisone and nystatin showed no absorbance while a slight absorbance was present for diphenhydramine. Therefore, 347 nm was chosen for UV quantification of DH in the HPLC analysis.

3.3. HPLC method development for assay of TC and DH

During storage, TC and DH may degrade in the mouthwash. For accurate assay of TC or DH, the HPLC method should be able separate the antibiotics from their degradants. Forced degradation was carried out by heating aqueous solutions of the antibiotics in an oven at temperatures ranging from 30ºC to 100ºC (U10, Memmert, Schwabach, Germany) for a period of 3 days to 2 weeks, before HPLC analysis. Figure 1a shows the typical chromatogram of TC obtained at the detection wavelength of 357 nm. The peaks at retention time of 7.8 min and 5.7 min correspond to TC and a degradation product, respectively. The resolution value between the peaks of TC and its degradation product was 6.61. The relative standard deviation of five replicate injections was 0.094%.

The method to assay DH was adapted from Injac et al (Injac et al., 2007). A longer column was used to improve column efficiency and separation of compounds. It was also reported in another HPLC method that 3–10% of tetrahydrofuran had been used to elute DH (Cherlet et al., 2003), thus the inclusion of 5% tetrahydrofuran in the mobile phase composition aided in the separation of DH from its

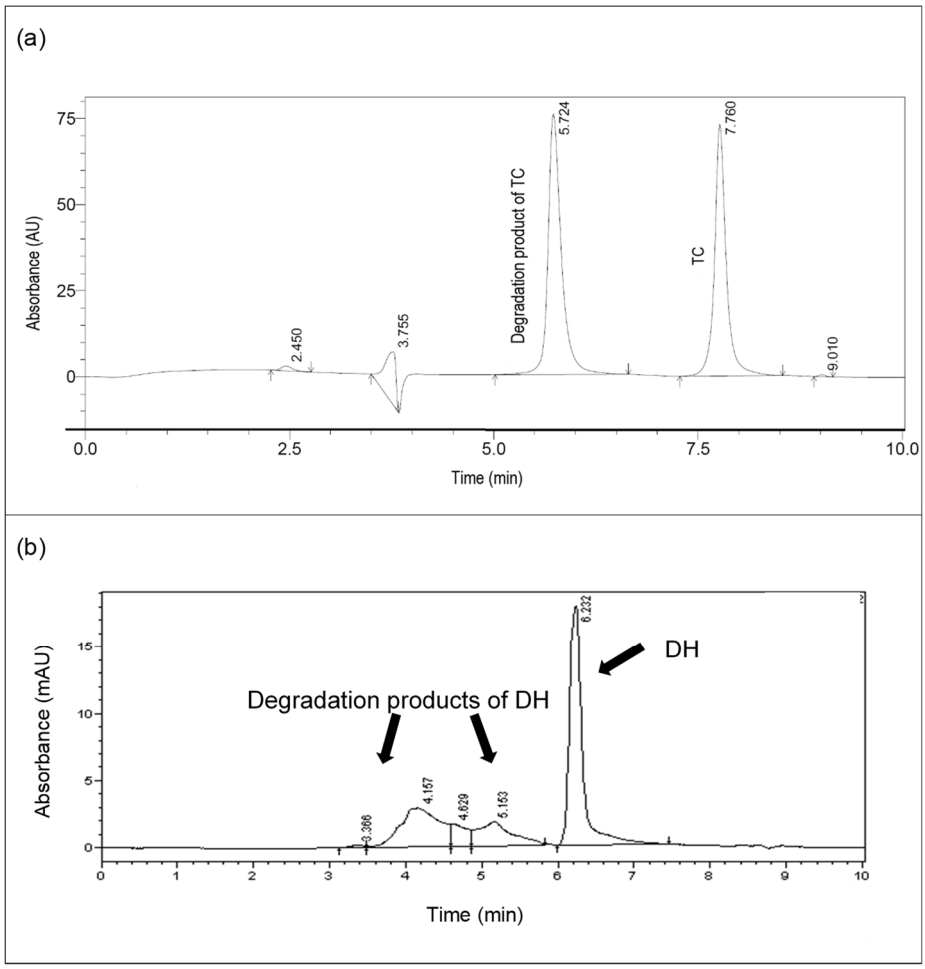

Figure 1. A typical HPLC chromatogram showing (a) tetracycline hydrochloride (TC) and (b) doxyclycline hyclate (DH) with its degradants. The original and modified TC mouthwashes had pH values of 2.75 and 4.27 respectively while the original and modified DH mouthwashes had pH values of 3.38 and 5.37 respectively. All mouthwashes were stored at 5°C and 30°C for a period of 7–21 days.

degradation products, resulting in better resolution and tailing factor. Figure 1b shows the typical chromatogram of DH obtained at the detection wavelength of 347 nm, with peak retention time of 6.2 min for DH.

3.4. Solubility studies of TC and DH in the mouthwashes

The solubility studies of TC and DH in purified water, original and modified mouthwashes were conducted to establish theoretically the highest concentrations of soluble antibiotics that could be achieved in the respective media.

The TC concentration was found to decrease significantly over time ($p < 0.05$). In literature, it was reported that tetracycline free base precipitated from its aqueous solution on standing (Reynolds, 1996). This might partially account for the decrease in TC concentration over time. Another possible reason is that transformation or degradation of TC began upon its dissolution in the aqueous media. Hence, the maximum concentration of TC had to be determined from samples withdrawn immediately after TC dissolved in the medium (t = 0 min).

The highest concentration of TC that could be dissolved in purified water (152.02 mg/mL) was comparable to that in the original mouthwash (157.71 mg/mL). In contrast, the highest concentration of TC that could dissolve in the modified mouthwash was only 0.937 mg/mL, which is less than 1% of the solubility of TC in purified water or in the original mouthwash. TC has multiple pKa values (3.3, 7.7 and 9.7) (Lund, 1994) and exists mainly as zwitterions with lower aqueous solubility at pH 4.0–4.5 (Hawker et al., 2015). It should be recalled that the aqueous solution of TC and the original mouthwash had comparable pH values, of 2.46 and 2.75, respectively, while the modified mouthwash had a significantly higher pH of 4.27. Moreover, sodium hydroxide, which was used to prepare the phosphate buffer in the modified mouthwash, has been reported to cause hydrolysis and precipitation of tetracycline free base.

Solubility of DH in purified water, original and modified mouthwashes were all found to be more than 425 mg/mL. From the solubility studies, the concentration of TC used (6.25 mg/mL) could all dissolve in the original mouthwash but only a very small portion in the modified mouthwash. However, for DH at 0.20 mg/mL, all could dissolve in both

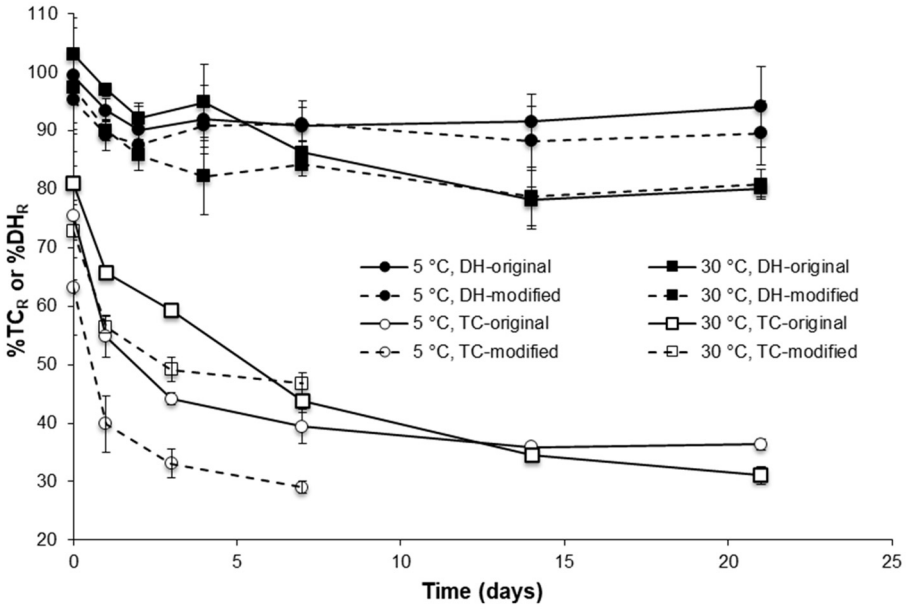

Figure 2. Stability of TC and DH in mouthwashes at different storage temperatures. The data represents the mean values with standard deviation.

the original and modified mouthwashes. The solubility studies were conducted in an air-conditioned laboratory with temperature of 22°C, which is lower than the typical ambient temperature of 30°C. The solubility of DH had been reported to increase by fourfold as temperature increased from 4°C to 37°C (Giovagnoli et al., 2010). Hence, the concentration of soluble TC that could be achieved in the mouthwash is very much restricted compared to DH.

3.5. Stability studies of TC and DH in the original mouthwashes

According to the formulation, the mouthwashes contained 6.25 mg/mL of TC or 0.20 mg/mL of DH, which were deemed to be effective concentrations of the compounds for antimicrobial action (Dean et al., 2003; Soni et al., 2017). As degradation mainly involves the dissolved compound, the concentration of TC or DH dissolved could be considered as the baseline concentration in the stability study. For accuracy, the baseline concentrations (i.e. TC_0 and DH_0) were determined by withdrawing samples of the mouthwashes immediately after preparation for assay. The concentrations of TC and DH remaining at different time intervals (i.e. TC_t and DH_t) were also determined. The percent of TC remaining (% TC_R), was calculated from $(TC_t/TC_0) \times 100\%$ and plotted against time. The same applied to DH (Figure 2).

The TC_0 and DH_0 determined were very close to the concentrations of TC and DH used in the mouthwashes. The % TC_R and % DH_R generally decreased over 21 days as TC and DH underwent degradation. The original DH-formulated mouthwash stored at 5°C showed the greatest stability (Figure 2). At day 21, mouthwashes stored at 5°C had significantly higher % TC_R and % DH_R than corresponding mouthwashes stored at 30°C ($p < 0.05$). Over time, the degradation rate was also lower for mouthwashes stored at 5°C as compared to 30°C. The trend for TC may be partially explained by the precipitation of tetracycline free base on standing (Reynolds, 1996). The water solubility of tetracycline free base and TC were also reported to decrease markedly when temperature fell from about 30°C to 15°C (Caço et al., 2008; Varanda et al., 2006). Collectively, these phenomena would reduce the concentration of TC in solution, manifesting as a greater extent of reduction in % TC_R at lower temperature over the first 7 days. Degradation rate of TC was reported to increase with temperature due to the increase in molecular collisions and reactivity (Loftin et al., 2008). It was also reported that more degradants formed at elevated temperature (Kelly, 1964; Kühne et al., 2001; Lindauer et al., 1976). While degradation products were not quantified in this study, it was observed that the peaks in the HPLC chromatogram corresponding to degradants were larger at higher temperature. This accounted for the greater extent of reduction in % TC_R at higher temperature over time and the lower % TC_R at higher temperature on day 21. The findings for DH was consistent with another study reporting that DH underwent thermal degradation (Injac et al., 2007). At high temperature, the main degradation products identified were methacycline and 6-epidoxycycline (Injac et al., 2007). Methacycline, 4-epidoxycycline and 6-epidoxycycline were reported to have lower antimicrobial efficacy and may even cause hepatotoxicity (de Barros et al., 2017; Injac et al., 2007; Virolainen et al., 2008). Therefore, it is beneficial to limit the degradation of DH by storing at 5°C.

Compared to TC, DH had a better stability profile. At the end of 21 days, the % DH_R in both the original and modified mouthwashes were all above 70% while TC in the modified mouthwash degraded to less than 50% at day 7 (Figure 2). The results of this study showed that DH underwent

degradation at a much slower rate than TC. These findings corroborate with another study which found that DH had a better stability profile than tetracycline (Hassani et al., 2008). The better stability profile of DH could be attributed to the chemical structure of DH where a hydroxyl group at C5 of its molecule reduced the propensity of epimerization that could occur at C4 to form 4-epidoxycycline. Besides, DH does not have a hydroxyl group at C6. Unlike tetracycline, DH will not degrade to anhydro derivatives (Dihuidi et al., 1985). The replacement of tetracycline with DH resulted in a more stable mouthwash formulation with other benefits of DH such as anti-inflammatory activities (Di Caprio et al., 2015).

3.6. Stability studies of TC and DH in the modified mouthwashes

In general, the modified mouthwashes showed significantly lower stability than the original mouthwashes (Figure 2, $p < 0.05$). Similar to the original TC-formulated mouthwashes, the modified counterpart stored at lower temperature of 5°C showed lower % TC_R due to the decreased concentration of TC in solution at the lower temperature. The stability study for the modified TC-formulated mouthwashes was terminated after 7 days as the % TC_R was less than 30% and 50% for 5°C and 30°C, respectively (Figure 2). Hence, modifying pH of the mouthwash significantly reduced the stability of both TC and DH.

Like TC, DH has multiple pKa values (3.02, 7.97 and 9.15) (Kogawa and Salgado, 2012). DH mainly exists in zwitterion state from pH 3.02 to 7.97 with an isoelectric point near pH 5.5, at which DH should be most stable (Giovagnoli et al., 2010). However, the results of the stability studies (Figure 2) show that DH was relatively more stable in the original mouthwash with pH 3.38 than the modified mouthwash with pH 5.37. The phosphate buffer with added sodium hydroxide, used in the modified mouthwash, seemed to cause an initial loss of DH. Nevertheless, when adjusting the pH of the mouthwash, it should be noted that pH should not exceed 5.5 as hydrocortisone degrades markedly beyond pH 5.5 (Manchanda et al., 2018).

Despite some early loss, the % DH_R was still above 80% at day 21 for both the original and modified mouthwashes. The concentrations of DH in the modified mouthwashes at day 21, when stored at 5°C and 30°C were at least 0.179 ± 0.011 mg/mL and 0.162 ± 0.005 mg/mL respectively. These concentrations were more than 10 times the minimum inhibitory concentration (MIC) of 0.016 mg/mL of DH against *Pseudomonas aeruginosa*, a common and less susceptible opportunistic bacteria in the oral cavity (Dean et al., 2003; Soni et al., 2017). According to earlier studies, DH attained optimal dose-dependent antimicrobial activity when its concentration remained at 8–16 times its MIC (Cunha et al., 2000). Thus, the original or modified DH-formulated mouthwashes were most likely to maintain their antimicrobial activity for at least 3–4 weeks. Further studies should be conducted to affirm this. If needed, the concentration of DH in the formulation may be increased to ensure that the remaining concentration is effective. Although the beyond-use date of the mouthwash could be extended to at least a month even when stored at ambient temperature of 30°C, nystatin has been reported to have maximum stability when kept refrigerated at 2–8°C (Vermerie et al., 1997). Thus, patients should ideally be advised to keep the mouthwash refrigerated at 2–8°C to minimize the degradation rate of DH and maintain the anti-fungal activity of nystatin at the same time. It should be noted that the modified mouthwash is less acidic (pH 5.37) and will probably cause less demineralization of the enamel and pain to the mouth with oral lesions (Marsh et al., 2009). Consequently, the modified DH-formulated mouthwash is more advantageous than the original DH-formulated mouthwash.

As DH-formulated mouthwash appeared to be more stable than TC-formulated mouthwash as shown in Figure 2, the degradation kinetics of the DH-formulated mouthwashes were further evaluated. As shown in Figure 2, degradation kinetics of DH in the mouthwashes appeared to follow a first order kinetics model ($R^2 = 0.808$ and 0.540, respectively, for original and modified mouthwashes stored at 30°C). The approximate first order kinetics rate constant (k) was obtained from the slope of the graph, natural logarithm of DH_R against time. The k values of the original and modified mouthwashes stored at 5°C and 30°C respectively are shown in Table 1. At MIC and above, DH is expected to have bacteriostatic activity. DH in the mouthwash stored at different temperature degraded at different rates. The predicted duration for DH concentration to degrade and decrease to its MIC, 5× and 10× of its MIC, respectively was calculated using the equation of the regression line ($\ln DH_R$ =

Table 1. Degradation kinetics of the DH-formulated mouthwashes.

Type of mouthwash	Storage temperature (°C)	k (h^{-1})	Predicted duration (days) for DH* to reach		
			10 × MIC	5 × MIC	MIC
Original	5	–0.00003	215	1178	3413
	30	–0.00049	17	76	213
pH-Modified	5	–0.00005	107	685	2026
	30	–0.00029	16	116	347

* DH: doxycycline hyclate

k*time + constant) of the first order degradation kinetics model. Based on 5× MIC of DH, the beyond-use date could be extended to 76 days for the original mouthwash and 116 days for the pH-modified mouthwash stored at 30°C. Further studies to determine the antimicrobial activity of the mouthwash at extended period are recommended to validate these findings.

4. Conclusion

The stability of tetracycline and doxycycline in the formulated mouthwashes was investigated to determine if the beyond-use date of the mouthwashes could be extended. DH was found to have a better stability profile compared to tetracycline. Based on the results obtained, the DH-formulated mouthwash was more stable when stored at refrigeration temperature of 5°C and the beyond-use date could be extended to a month and beyond. The pH of the mouthwash could also be increased to 5.0–5.5 with a slight decrease in the stability of DH but an important advantage of reducing acidity and pain to the mouth lesions. With this re-formulated mouthwash, pemphigus patients will only need to collect fresh supplies of the mouthwash every month or longer and this will greatly benefit them. The potential anti-inflammatory effect of DH could be further investigated to widen the use of this mouthwash formulation to other similar conditions. Further studies are recommended to investigate the antimicrobial activity of the mouthwash when stored at 5°C and 30°C, and the effectiveness of additives to further enhance the stability of doxycycline.

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Conflicts of Interest

The authors report no conflict of interest.

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