

## Formulation and *in vitro* Evaluation of a Mucoadhesive Metronidazole Dental Gel for Oral Application

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**Periodontitis is inflammation of the supporting tissues of the teeth caused by microorganisms found in plaque. Metronidazole is commonly used for the treatment of periodontal diseases like gingivitis and periodontitis. Treatment of periodontal disease orally using metronidazole tablets for a prolonged period can cause gastrointestinal disturbances in patients leading to poor patient compliance. This project aimed at formulating a mucoadhesive metronidazole dental gel preparation safe for use on the gums as a form of prophylaxis against infections caused by anaerobic bacteria after dental procedures. Formulation of the gel was carried out in the laboratory using different concentrations of Carbopol® 971P NF. The gels obtained showed acceptable uniformity of content, pH, spreadability, *in vitro* diffusion characteristics as well as mucoadhesive and organoleptic properties.**

**Keywords:** Metronidazole, mucoadhesive gel, oral application

### INTRODUCTION

Periodontal diseases affect up to 90% of the global human population [1]. Gingivitis, the mildest form of periodontal disease, does not affect the underlying supportive tissue of the teeth [2]. On the other hand, periodontitis affects connective tissue and bone and can result in tooth loss in adults [2]. Although inflammatory and immune responses play an important role in periodontal disease [3] pathogenic microorganisms in plaque are necessary, but insufficient for disease causation [4]. Treatment modalities for periodontal disease include antimicrobial therapy, modulation of host immune response and tissue engineering [5].

Metronidazole is an antimicrobial agent available as both oral and parenteral formulations [6]. Topical metronidazole formulations, among them oral metronidazole gel, are relatively novel [7-8]. There is need to develop more metronidazole oral gel formulations for local use after dental procedures for the treatment and prophylaxis of anaerobic bacterial infections. Such formulations

will mitigate against the problem of gastrointestinal side effects experienced with the use of oral metronidazole tablets and ensure sustained release of the antimicrobial agent at the intended local site.

In the past few years, an increasing number of *in situ* gel forming systems have been investigated and many patents for their use in various biomedical applications including drug delivery have been reported [9]. *In situ* forming polymeric delivery systems, in particular those containing biodegradable and mucoadhesive polymers offer attractive clinical advantages such as ease of administration and reduced frequency of administration, improved patient compliance and comfort [10-11].

Various natural and synthetic polymers are used for formulation development of *in situ* forming drug delivery systems [11]. This project aimed at formulating a mucoadhesive metronidazole dental gel preparation safe for use on the gums as a form of prophylaxis against infections caused by anaerobic bacteria after dental procedures.

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## EXPERIMENTAL

### Materials

Metronidazole USP was donated by Cosmos Pharmaceutical Ltd, Nairobi, Kenya, Cellulose membrane was from Sartorius AG (Goettingen, Germany). Propylene glycol BP and glycerol GPR 98% v/v were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). A hot water bath (Baird and Tatlock Ltd, London, UK), a top loading balance (Sartorius AG, Goettingen, Germany), a thermometer (Brannan & Sons Ltd, Cumbria, England), an analytical balance (Shimadzu AUW ZZO D, Shimadzu Corporation, Tokyo, Japan), an ERWEKA DT 700 dissolution tester (Erweka GmbH, Heusenstamm, Germany), a T90+ double beam UV/VIS spectrometer (PG Instruments Ltd, Leicester, UK), a shaking water bath (GFL 1083, Gesellschaft fur Labortechnik mbH, Burgwedel, Germany) and a pH meter (WTW Microprocessor 537, WTW GmbH, Weilheim, Germany) were utilized. The other chemicals and reagents used were of analytical grade.

### Formulation of gels

The metronidazole mucoadhesive dental gel was prepared using dispersion method. Five different formulations weighing 50 g each were prepared: a blank formulation without the API (metronidazole), formulation A, formulation B, formulation C and formulation D. Each gel contained 1% of metronidazole with concentrations of Carbopol 971P NF polymer ranging from 0.5 to 2 % w/v. Propylene glycol was used as a co-solvent while triethanolamine was used as a gelling agent. The formulations were packed in round glass jars.

### Measurement of pH

The pH of the topical formulations was measured by inserting the electrode of the pH meter into the topical formulations (about 1cm deep) and allowing it to equilibrate for 1min. Three reading were taken randomly from different zones of the topical formulation.

### Spreadability study

A spreadability set up was used to predict the ease of spreading the formulated gel on skin [12]. The time taken to separate two slides with gel between them was measured and recorded.

### Content uniformity test

Metronidazole was analysed using a validated high performance liquid chromatography (HPLC) method on a Merck Hitachi L7 series (Merck Hitachi, UK) using a UV detector at 254 nm and a HPLC column (RP 4.6 mm × 25 mm with 5 µm L7 packing) with a mobile phase of 0.01 M monobasic potassium phosphate (pH 4) and absolute methanol (35:65% v/v) at a flow rate of 1 ml/min. A formulation containing an equivalent of 1.8 mg of the drug was weighed accurately and placed into a dry 50 ml volumetric flask and dissolved in 25 ml mobile phase. The contents were shaken using a mechanical stirrer for 20 min and diluted to volume using the mobile phase. The contents were further stirred for 1h and filtered through 0.45 µm nylon filter. The filtrate was analyzed by injecting 20 µl into the HPLC column and metronidazole content determined from a standard curve prepared using different concentrations (1-60 µg/ml) of reference metronidazole.

### *In vitro* mucoadhesion measurement

A significant characteristic of gel in the treatment of periodontitis is mucoadhesive strength for adhesion to the mucosa in the dental pocket. A modified tensiometry method using the Fisher tensiometer was employed to evaluate the mucoadhesive properties of gel formulations [13]. The limitation of the measurable surface tension or adhesive forces by this instrument was set at 0-100 dyne/cm<sup>2</sup>. A thin mica disk was placed on the tensiometer ring. A 1% (w/v) solution of sodium alginate was placed into the 37°C water-jacketed glass vial of the instrument. After calibrating the tensiometer with a standard weight, 200 mg from each gel formulation was placed on the mica disk and transferred to the

tensiometer. The gel formulation was left in contact with the 1% (w/v) solution of sodium alginate for 5 min and then detached from the solution at a speed of 0.2 inch/min. The adhesion force between mica disk and the solution of sodium alginate was used as the blank in all tests. The detachment force was measured in dyne/cm<sup>2</sup>. The test was performed six times for each formulation. After each experiment the glass plate was removed and the Palladium-Platinum ring of the tensiometer was washed with methanol, then acetone.

### ***In vitro* diffusion studies**

These studies were carried out according to a previously described method [14]. The cellulose membrane was cut into suitable sizes and soaked in distilled water overnight to hydrate and soften it. Hydrated cellulose membrane was used to simulate the epithelium layer of the skin. *In vitro* diffusion studies were carried out using the Erweka DT6 dissolution apparatus. About 1.8 g of drug-loaded gel formulation was placed on a marked area of 2cm<sup>2</sup> on a microscopic glass slide. This was completely covered with a hydrated cellulose membrane and firmly secured at the sides. The baskets were filled with 900 ml of dissolution medium (potassium phosphate buffer pH 7.2) and the temperature maintained at 37°C. The slides were slowly placed in the baskets and the speed of stirrer set at 100 rpm. The samples were prepared in triplicate for all the topical formulations as well as the innovator gel. Aliquots of 10ml were withdrawn at predetermined intervals and replaced with similar quantities of fresh dissolution fluid to maintain sink conditions. The drug content in each drawn sample was assayed using HPLC as mentioned above. All formulations were evaluated in triplicate (n=3).

## **RESULTS AND DISCUSSION**

### **pH of gel formulations**

It is important to know the pH for the prepared oral gel formulations since an acidic or alkaline formulation can cause irritation to the skin. The surface pH of the gel formulations was found to be close to neutral and ranged between 6.2

(formulation B) and 7.2 (formulation A) as shown in Table 1. Thus the gels are expected to be non-irritant to the oral mucosa.

### **Spreadability of gel formulations**

The variation in time required to separate the gel among the various formulations was found to correlate with the amount of Carbopol 971P NF used (Table 1). The slides with formulations containing 2 %w/v Carbopol 971P NF took longer to separate leading to the conclusion that increasing the amount of Carbopol 971P NF reduced ease of spreadability of the gel.

### **Metronidazole content uniformity of the formulations**

The percentage content of the gel formulations varied between 95.32% (formulation B) and 105.63% (formulation D) as shown in table 1. This finding complies with the United States Pharmacopeial specifications for the assay of metronidazole gel (90-110%) [15].

### ***In vitro* mucoadhesion of gel formulations**

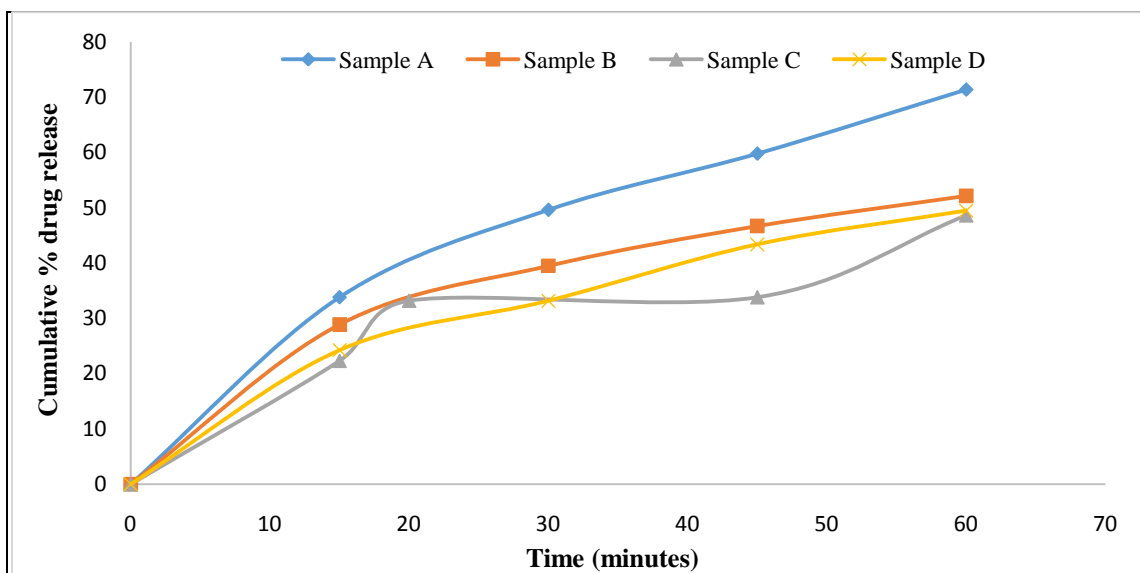
The mucoadhesion of formulation D (containing 2% carbopol 971P NF) was greater than that of formulation A (containing 0.5% carbopol 971P NF). The results showed that increasing the concentration of carbopol 971P NF could increase the mucoadhesion of the formulation. Carbopols have high molecular weights, swell in water up to 1000 times their original volume, creating a large adhesive surface. Carbopols also form secondary bioadhesion bonds with mucin lining the buccal cavity. Consequently, carbopols produce enhanced mucoadhesion. Incorporation of mucoadhesive polymers in formulations increases the contact time with mucosa and prolongs residence time improving clinical efficacy [16-17].

### ***In vitro* diffusion studies**

For each of the formulations, the amount of drug released at predetermined time intervals was determined and the results are as shown in the drug release profile in figure 1 below.

**Table1: pH, spreadability (time to separate slides) and content uniformity of gel formulations**

Formulation	pH	Time (s)	Mean % drug content $\pm$ SD
A	7.2	5	103.16 $\pm$ 0.2
B	6.2	12	95.32 $\pm$ 0.1
C	6.6	28	101.44 $\pm$ 0.6
D	6.4	103	105.63 $\pm$ 0.2

**Figure 1: Dissolution profile of formulated gels**

From the dissolution profile formulation A released metronidazole at a faster rate than the other gel formulations at the end of one hour. Formulations B, C and D exhibited relatively slower release (<50% drug release at end on one hour). The slow release of metronidazole may be attributed to the lower percentage of Carbopol in formulation A Compared to the other three formulations.

## CONCLUSION

Mucoadhesive metronidazole oral gels were successfully formulated using Carbopol® 971P NF polymers. The gels possessed acceptable surface pH, content uniformity, drug release profiles and mucoadhesive properties. The findings from this work can form the basis for further formulation studies for marketed products.

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## REFERENCES

- [1] B.L. Pihlstrom, B.S. Michalowicz and N.W. Johnson. *Lancet*. 366(9499), 2005, 1809-1820.
- [2] R.C. Williams. *New Engl. J. Med.* 322(6), 1990, 373-382.
- [3] D.L. Cochran. *J. Periodontol.* 79, 2008, 1569-1576.
- [4] T.E. Van Dyke and S. Dave. *J. Int. Acad. Periodontol.* 7(1), 2005, 3.
- [5] D.F. Kinane, P.G. Stathopoulou and P.N. Papapanou. *Nat. Rev. Dis. Primers.* 3, 2017, 17038.

- [6] H. Ravari, A. Jangjoo, J. Motamedifar and K. Moazzami. *Clin. Exper. Gastroenterol.* 4, 2011, 273.
- [7] K.J. McClellan and S. Noble. *Am. J. Clin. Dermatol.* 1(3), 2000, 191-199.
- [8] T.J. Nicholson and D. Armstrong. *Dis. Colon Rectum.* 47(5), 2004, 711-718.
- [9] D. Singh Malik, N. Mital and G. Kaur. *Expert Opin. Ther. Pat.* 26(2), 2016, 213-228.
- [10] M. Madan, A. Bajaj, S. Lewis, N. Udupa and J.A. Baig. *Ind. J. Pharm. Sci.* 71(3), 2009, 242.
- [11] A. Srivastava, T. Yadav, S. Sharma, A. Nayak, A. Kumari and N. Mishra. *J. Biosci. Med.* 4(01), 2016, 69-84.
- [12] K. Garala, P. Joshi, M. Shah, A. Ramkishan and J. Patel. *Int. J. Pharm. Investig.* 3(1), 2013, 29-41.
- [13] J. Varshosaz and Z. Dehghan. *Eur. J. Pharm. Biopharm.* 54(2), 2002, 135-141.
- [14] S. Maru, L. Gathu, A. Mathenge, A. Okaru, F. Kamau and H. Chepkwony. *East Cent. Afr. J. Pharm. Sci.* 15, 2012, 57-62.
- [15] U.S. Pharmacopoeia - National Formulary [USP 38 NF 33]. United States Pharmacopoeial Convention, Inc, Rockville, MD. 2015.
- [16] M. Paulsson. Controlled release gel formulations for mucosal drug delivery, Doctoral dissertation, Acta Universitatis Upsaliensis, 2001.
- [17] M. Jelvehgari, M.R. Rashidi and H.Samadi. *Iran. J. Pharm. Sci.* 2(4), 2006, 185-194.
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