# Physicochemical Characterization and Preliminary in Vivo Efficacy of Bioadhesive, Semisolid Formulations Containing Flurbiprofen for the Treatment of Gingivitis

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**Abstract** □ In this study, the physicochemical properties and preliminary in vivo clinical performance of formulations containing hydroxyethylcellulose (HEC; 3, 5, 10% w/w), poly(vinylpyrrolidone) (PVP; 3, 5% w/w), polycarbophil (PC; 1, 3, 5% w/w), and flurbiprofen (5% w/w) were examined. Flurbiprofen release into PBS pH 7.4 was performed at 37 °C. The mechanical properties (hardness, compressibility, adhesiveness, initial stress) and syringeability of formulations were determined using a texture analyzer in texture profile analysis (TPA) and compression modes, respectively. In general, the time required for release of 10 and 30% of the original mass of flurbiprofen ($t_{10\%}$, $t_{30\%}$) increased as the concentration of each polymeric component increased. However, in the presence of either 5 or 10% HEC and 5% PC, increased PVP concentration decreased both $t_{10\%}$, $t_{30\%}$ due to excessive swelling (and disintegration) of these formulations. Increased concentrations of HEC, PVP, and PC significantly increased formulation hardness, compressibility, work of syringe expression, and initial stress due to the effects of these polymers on formulation viscoelasticity. Similarly, increased concentrations of PC (primarily), HEC, and PVP increased formulation adhesiveness due to the known bioadhesive properties of these polymers. Clinical efficacies of formulations containing 3% HEC, 3% PVP, 3% PC, and either 0% (control) or 5% (test) flurbiprofen, selected to offer optimal drug release and mechanical properties, were evaluated and clinically compared in an experimental gingivitis model. The test (flurbiprofen-containing) formulation significantly reduced gingival inflammation, as evaluated using the gingival index, and the gingival crevicular fluid volume, whereas, these clinical parameters were generally increased in volunteers who had received the control formulation. There were no observed differences in the plaque indices of the two subject groups, confirming that the observed differences in gingival inflammation could not be accredited to differences in plaque accummulation. This study has shown both the applicability of the in vitro methods used, particularly TPA, for the rational selection of formulations for clinical evaluation and, additionally, the clinical benefits of the topical application of a bioadhesive semisolid flurbiprofen-containing formulation for the treatment of experimental gingivitis.

## Introduction

Periodontal diseases are a group of inflammatory conditions affecting the supportive structures of the teeth, the gingiva, periodontal ligament, and alveolar bone. Inflammation of the gingiva is referred to as gingivitis whereas extension of inflammation into deeper tissues is termed periodontitis.[1] The inflammation is in response to plaque bacteria residing on both the tooth surface and beneath the gingiva in periodontal pockets. Treatment of the disease, aimed at arresting the progression of the destructive process and preventing recurrence, is mainly through the mechanical cleaning of the tooth surface. However, as specific bacteria are thought to play a major role in the disease process, antimicrobial agents have also been used as adjuncts to treatment, particularly in early-onset and refractory cases.[2−4] The potential side-effects of administering systemic antibiotics, and the inability of antiseptic mouthwashes to penetrate the periodontal pocket, have fueled interest in the sustained delivery of such agents within the pocket. The attributes of, and indeed problems associated with, such drug delivery systems have been described by several authors.[5−7] One particular problem common to many drug delivery systems designed for use in the oral cavity is poor retention at the site of application.[1,8] This problem may be resolved by the incorporation of bioadhesive polymers, i.e., polymers that exhibit characteristic adhesive interactions with biological membranes, within the drug delivery system.[8,9] In so doing, several authors have reported improved retention and, hence, clinical performance of topical formulations designed for the oral cavity.[10,11]

New insights into the mechanisms underlying periodontal disease have placed greater emphasis on the role of the host response, rather than bacterial aetiology, as the primary determinant of disease progression. Inflammatory mediators, including the arachadonic acid derivative prostaglandin $E_2$ ($PGE_2$), have been associated with the condition. Levels of $PGE_2$ in the periodontal tissues, which are significantly increased at diseased sites, are reduced following successful treatment and may be used as predictors of further tissue destruction.[12,13] As a result of these findings, a number of studies have investigated the potential use of nonsteroidal antiinflammatory drugs (NSAIDs) as an adjunct to mechanical cleaning in the treatment of periodontal diseases. Results from both animal[14,15] and human[16−19] studies have shown the clinical benefit of flurbiprofen in the treatment of periodontal diseases. Given the potential problems associated with systemic NSAID usage, topical application of these agents may be of clinical value in the treatment of periodontal disease.

Therefore, this study describes the physicochemical properties, i.e., drug release and mechanical properties, of candidate flurbiprofen-containing, controlled-release bioadhesive semisolids, important determinants of clinical performance. In addition, a preliminary in vivo evaluation of a formulation exhibiting optimal physiochemical proper-

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ties for the treatment of gingivitis, one of the periodontal diseases, is described.

## Experimental Section

**Materials**—Flurbiprofen was a gift from Boots plc., Nottingham, England. Hydroxyethylcellulose (Natrosol HHX 250-Pharm), poly(vinylpyrrolidone) (Kollidon K90), and polycarbophil (Noveon AA-1) were donated by Aqualon LTD (Warrington, England), BASF (Ludwigshafen, Germany), and B. F. Goodrich Company, Cleveland, OH, respectively. All other chemicals were purchased from BDH Laboratory Supplies, Poole, England and were AnalaR, or equivalent, quality.

**Methods**—*Preparation of Flurbiprofen-Containing Formulations*—Initially, hydroxyethylcellulose (HEC; 3, 5, 10%w/w) was dissolved with stirring in the required amount of phosphate-buffered saline (PBS; pH 6.8, 0.03 M) using a mechanical stirrer. Poly(vinylpyrrolidone) (PVP; 3, 5% w/w), polycarbophil (1, 3, 5% w/w) and, finally, flurbiprofen (5.0% w/w, particle size <63 $\mu$m) were mixed thoroughly into this gel to form pharmaceutical semisolids. All formulations were stored in amber ointment jars at 4 °C until required.

*In Vitro Release of Flurbiprofen*—The release of flurbiprofen from the bioadhesive formulations into PBS (pH 7.2) at 37 °C was determined in triplicate using a Caleva 7ST dissolution apparatus with paddle stirrers (100 rev $min^{-1}$), as previously reported.[7,20] Formulations were retained within three-sided plastic molds and anchored to the bottom of the dissolution vessels, thus ensuring that drug release occurred principally from the top of the molds. Samples of the dissolution fluid were removed at predetermined intervals and their absorbances determined using ultraviolet spectroscopy at 290 nm. Flurbiprofen release was determined using a calibration curve, which was linear over the concentration range 1.0−100.0 $\mu$g $mL^{-1}$ ($r > 0.99$, with zero intercept). The presence of formulation excipients was observed not to interfere with the analysis.

Drug release data generated from dissolution experiments were fitted to the general release equation (eq 1) using logarithmic transformations and least squares regression analysis, as previously described.[20−22]

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

where $M_t/M_\infty$ = the proportion of flurbiprofen released at time $t$, $k$ = a constant incorporating structural and geometrical characteristics of the delivery system, and $n$ = the release exponent, a measure of the primary mechanism of drug release.

*Characterization of Bioadhesive, Flurbiprofen-Containing Semisolids Using Texture Profile Analysis*—The method employed to characterize the mechanical properties of each formulation was texture profile analysis.[7,9,20] In brief, formulations were packed into identical 60 mL ointment jars to a fixed height, avoiding the introduction of air into the samples, and texture profile analysis performed using a Stable Micro Systems TA-XT2 texture analyzer (Haslemere, Surrey, UK). The analysis involved the double compression of the analytical probe (10 mm diameter) into each sample. The depth and rate of each compression were 15 mm and 2.0 mm $s^{-1}$, respectively, and a 15 s delay period between the end of the first and beginning of the second compression was allowed. All analyses were performed on four replicate samples. From the resultant force−time plot, several mechanical parameters may be determined, including[8] (1) product hardness (force required to attain a given deformation); (2) product adhesiveness (the work required to overcome the attractive forces between the surface of the sample and the surface of the probe with which the sample comes into contact) and compressibility (the force per unit time required to deform the product during the first compression of the probe); (3) product compressibility (the work required to deform the product during the first compression cycle of the probe); (4) initial stress (the resistance to probe compression over a defined time period, i.e., from 0.5 to 1.5 s of the initial compression.

*Determination of the Work Required to Expel the Bioadhesive, Semisolid Formulations from a Syringe*—The method employed to determine the work required to expel the formulations from a syringe (syringeability) has previously been reported by us.[7,20] Each formulation was packed into plastic syringes (of identical dimensions) to a preselected height (3 cm). The formulation was then expressed from the syringe using the Stable Micro Systems Texture Analyzer in compression mode and the work done determined by measuring the area under the resultant force−distance plot. Increased area under the force−distance plot is indicative of decreased ease of syringeability. All measurements were performed, at least, in quadruplicate.

*Clinical Evaluation of Bioadhesive Semisolid Formulations in Experimental Gingivitis*—The effects of two formulations containing 3% w/w HEC, 3% w/w PVP, 3% w/w PC and either 5% or 0% w/w (control) flurbiprofen on gingival inflammation were determined using an experimental gingivitis model, as described by Heasman et al.[17] Ethical approval for the study was obtained from the Faculty of Medicine Ethics Committee, The Queen's University of Belfast. Exclusion criteria for the study included use of, or allergy to, nonsteroidal, antiinflammatory drugs, recent antibiotic therapy, use of the contraceptive pill, pregnancy, gastric upset, smoking, inadequately attached gingiva, and crowding of the lower anterior teeth. Ten subjects were recruited, of whom nine successfully completed the study. Each subject was examined and, where required, dental prophylaxis was performed to ensure health of the gingival tissues. Each subject was provided with an individual bite guard designed to cover their lower six anterior teeth and associated attached gingiva. Subjects were instructed on normal toothbrushing techniques and asked to brush their teeth twice daily. Bite guards were worn during toothbrushing to allow plaque accumulation in the lower anterior region. During the third week of the study, subjects were given the test formulations and asked to apply 0.5 mL evenly over the attached gingiva around the lower six anterior teeth following toothbrushing at night for seven consecutive nights. Subjects continued to wear the bite guard during toothbrushing. Five subjects received the flurbiprofen-containing formulation, whereas the remainder received the control formulation, i.e., devoid of flurbiprofen. Both clinician and subject were unaware as to which formulation was administered. Clinical assessments were performed at weekly intervals over a three-week period. Plaque deposits were assessed at four surfaces (mesial, distal, labial, lingual) of each lower lateral incisor using the plaque index.[23] The level of inflammation in the gingival tissues was scored at the labial surfaces of the four lower incisor teeth using the gingival index (GI) on a ordinal scale of 0 to 3, as described by Loe and Silness.[24] The degree of inflammation was then recorded as the mean GI score for these four sites. Gingival crevicular fluid (GCF) was collected from the distal surfaces of the lower lateral incisors. Briefly, the area was isolated with cotton wool rolls and dried with a continuous air stream, and the GCF was collected onto a Periopaper strip inserted into the gingival sulcus for 30 s. The volume of GCF collected was determined using a Periotron 6000 (Louisville, KY).

*Statistical Analysis of Results*—The effects of HEC (3, 5, and 10% w/w), PVP (3 and 5% w/w), and PC (1 and 5% w/w) on the times required for the release of defined percentages (i.e., 10 and 30%) of the original mass of flurbiprofen from each formulation, formulation hardness, adhesiveness, compressibility, initial stress, and syringeability were evaluated statistically using a three-way Analysis of Variance (ANOVA, $p < 0.05$ denoting significance). In a subsequent analysis, the effects of increasing concentrations of PC from 1 to 3 to 5% w/w and HEC from 3 to 5 to 10% w/w on the release and mechanical properties of formulations containing 3% PVP were statistically evaluated using a two-way ANOVA.[8] Post-hoc statistical analyses of the means of individual groups were performed using Fischer's LSD test. In clinical studies, both the gingival indices, plaque indices, and gingival crevicular fluid of the two patient groups following the initial two-week study period, and the effects of flurbiprofen treatments and control treatments on gingival inflammation, gingival crevicular fluid volume, and plaque indices, were statistically compared and evaluated using a Mann−Whitney U-test. Finally, the differences in gingival inflammation, gingival crevicular fluid volume, and plaque indices of the patients over week two to three of the study period were statistically assessed using a Wilcoxon signed rank test. In all analyses, $p < 0.05$ denoted significance.

## Results

Application of the generalized release equation to the flurbiprofen release data allowed calculation of the release

**Table 1—Effects of Hydroxyethylcellulose (HEC), Poly(vinylpyrrolidone) (PVP), and Polycarbophil (PC) on the Time Required for the Release of Flurbprofen (10 and 30% of original drug loading) from Bioadhesive, Semisolid Formulations**

| concn of PC (% w/w) | concn of PVP (% w/w) | concn of HEC (% w/w) | time (h) required for release of flurbiprofen (mean ± sd) | |
|---|---|---|---|---|
| | | | 10% | 30% |
| 1 | 3 | 3 | 1.27 ± 0.05 | 3.19 ± 0.12 |
| 1 | 3 | 5 | 3.26 ± 0.11 | 13.51 ± 0.81 |
| 1 | 3 | 10 | 4.90 ± 0.21 | 24.57 ± 1.99 |
| 1 | 5 | 3 | 1.72 ± 0.09 | 4.73 ± 0.19 |
| 1 | 5 | 5 | 5.52 ± 0.25 | 17.10 ± 1.11 |
| 1 | 5 | 10 | 10.00 ± 0.56 | 32.33 ± 1.92 |
| 3 | 3 | 3 | 2.17 ± 0.17 | 8.73 ± 0.56 |
| 3 | 3 | 5 | 7.08 ± 0.61 | 21.21 ± 3.32 |
| 3 | 3 | 10 | 17.11 ± 0.99 | 40.96 ± 2.27 |
| 5 | 3 | 3 | 3.30 ± 0.22 | 10.99 ± 1.01 |
| 5 | 3 | 5 | 14.27 ± 0.91 | 40.45 ± 1.69 |
| 5 | 3 | 10 | 28.41 ± 3.01 | 101.11 ± 7.22 |
| 5 | 5 | 3 | 5.54 ± 0.21 | 16.52 ± 1.11 |
| 5 | 5 | 5 | 6.17 ± 0.51 | 20.17 ± 1.24 |
| 5 | 5 | 10 | 16.53 ± 1.34 | 40.51 ± 2.51 |

exponent ($n$) which, for the formulations under investigation, ranged from 0.6 to 1.0. Therefore, in light of the disparity of release rates exhibited by these formulations, statistical analyses were performed on the times required for the release of 10 and 30% ($t_{10\%}$, $t_{30\%}$, Table 1) of the original loading of flurbiprofen from each formulation, as previously reported by us.[20] A wide range of values of $t_{10\%}$ and $t_{30\%}$ were exhibited by the formulations under examination. Maximum $t_{10\%}$ and $t_{30\%}$ values were observed in the formulation containing 10% HEC, 3% PVP, and 5% PC and were 28.41 ± 3.01 and 101.11 ± 2.22 h, respectively. Conversely, minimum $t_{10\%}$ and $t_{30\%}$ values were 1.27 ± 0.05 and 3.19 ± 0.12 h, respectively, and were associated with semisolids containing 3% HEC, 3% PVP, and 1% PC. Increasing the concentration of either HEC (from 3 to 5 and from 5 to 10% w/w) or alternatively PC (from 1 to 3 and from 5 to 10% w/w) significantly increased both $t_{10\%}$ and $t_{30\%}$. Conversely, the effect of PVP on the release of flurbiprofen from the semisolid formulations under examination was dependent on the concentrations of both HEC and PC. Thus, in formulations containing either 3, 5, or 10% HEC and 1, 3, or 5% PC, increasing the concentration of PVP from 3 to 5% w/w significantly increased $t_{10\%}$, $t_{30\%}$. Conversely, increasing the concentration of PVP in the formulations containing 5 or 10% HEC and 5% PC significantly reduced $t_{10\%}$, $t_{30\%}$. These disparate effects accounted for the observed interactions in the statistical analysis of the effects of polymeric components on these release parameters.

The effects of HEC, PVP, and PC on the mechanical (textural) properties and syringeability of the formulations under examination are presented in Tables 2 and 3, respectively. Increasing concentrations of each polymeric component (HEC, PVP, and PC) significantly increased each parameter investigated. Hence, the minimum values of hardness, adhesiveness, compressibility, initial stress, and work of syringeability were exhibited by the formulation containing 3% HEC, 3% PVP, and 1% PC and were 0.52 ± 0.02 N, 2.24 ± 0.14 N mm, 2.36 ± 0.16 N mm, 0.50 ± 0.06 × $10^6$ dynes $cm^{-2}$, and 19.58 ± 0.46 N mm, respectively. Conversely, maximum values of hardness, adhesiveness, compressibility, initial stress, and work of syringeability were 9.12 ± 0.20 N, 13.02 ± 0.48 N mm, 49.82 ± 1.12, 10.01 ± 0.61 dynes $cm^{-2}$, and 113.78 ± 2.82 N mm, respectively, observed in the formulation containing the highest concentration of each polymer investigated, namely 10% HEC, 5% PVP and PC 5%. Interestingly, statistical interactions were observed in the Analysis of Variance concerning the effects of these polymeric components on each mechanical parameter. In these, the effects of PVP and PC on the mechanical properties of semisolids containing 10% HEC were significantly (and disproportionately) greater than those containing either 3 or 5% HEC.

Following the initial two week period, the gingival indices of the two patient groups were 0.75 ± 0.44 and 0.81 ± 0.54. This difference in the two patient groups was insignificant. The comparative effects of seven daily (nightly) applications of two formulations, each containing 3% HEC, 3% PVP, 3% PC and either 0 or 5% flurbiprofen on the level of inflammation, as determined using the gingival index, are presented in Figure 1. As determined by the gingival index, the level of gingival inflammation increased in two of the four volunteers treated with the control formulation, but reduced in four out of five subjects treated with the test formulation containing flurbiprofen. Mean gingival crevicular fluid levels were also reduced in four out of five subjects treated with the flurbiprofen-containing formulation, but also in three out of four control subjects (Table 4). However, the mean gingival crevicular fluid volume of subjects treated with flurbiprofen-treated formulations was significantly lower when compared to that of subjects in the control group. Finally, the plaque indices did not significantly change between week two and three of the study period in either patient group (Table 4). No patients reported any side-effects following application of either formulation.

## Discussion

The formulations examined in this study exhibited wide ranges of both drug release and mechanical properties that were dependent on the concentrations of each polymeric component present. In light of the relative structural complexity of these systems, it is appropriate to discuss the observed physicochemical properties in relation to the physical state of each polymeric component. In all formulations, HEC (3, 5, 10% w/w) was initially dissolved to form a primary gel into which PVP was added until the saturation solubility of this component in the primary gel was exceeded. Further additions of PVP resulted in the emergence of a two-phase semisolid system in which this polymer was present both in solution and as a suspended solid within the HEC gel. Hence, in gels containing 3% HEC, PVP (3 and 5%) was soluble, whereas in gels containing 5% and 10% HEC, PVP existed both in solution and suspension, the ratio of the mass of dissolved to suspended PVP decreasing as the concentration of HEC was increased. Following its addition, as a direct consequence of its cross-linked structure, PC did not dissolve in the formulation but exhibited swelling, the extent of which was dependent on the amount of available water present in the formulations. Thus, as the concentrations of HEC (primarily) and PVP increased, the degree of swelling of PC decreased. The range of physicochemical properties exhibited by the formulations under examination may be due to the states of PVP and PC within the primary HEC gel. Similarly, in a previous publication, the states of these polymeric components, namely dissolved/dispersed (in the cases of HEC and PVP) or swollen/unswollen (PC) were reported to directly influence the viscoelastic properties of related formulations containing chlorhexidine designed for the treatment of localized infection.[25] Thus, the effects of each polymeric component on both the release of flurbiprofen and the mechanical properties of each formulation will be considered in light of the state of each polymeric component in these formulations.

**Table 2—The Effects of Concentration of Hydroxyethylcellulose (HEC), Poly(vinylpyrrolidone) (PVP), and Polycarbophil (PC) on the Hardness, Adhesiveness, Compressibility, and Initial Stress of Formulations Containing Flurbiprofen (5% w/w), As Determined Using Texture Profile Analysis**

| concn of PC (% w/w) | concn of PVP (% w/w) | concn of HEC (% w/w) | hardness (N) | adhesiveness (N mm) | compressibility (N mm) | initial stress (dyn $cm^{-2} \times 10^6$) |
|---|---|---|---|---|---|---|
| 1 | 3 | 3 | 0.52 ± 0.02 | 2.24 ± 0.14 | 2.36 ± 0.16 | 0.50 ± 0.06 |
| 1 | 3 | 5 | 1.18 ± 0.02 | 5.56 ± 0.08 | 5.74 ± 0.08 | 1.58 ± 0.12 |
| 1 | 3 | 10 | 6.00 ± 0.18 | 7.86 ± 0.28 | 14.74 ± 1.42 | 4.69 ± 0.46 |
| 1 | 5 | 3 | 0.64 ± 0.02 | 3.06 ± 0.20 | 2.96 ± 0.04 | 0.76 ± 0.06 |
| 1 | 5 | 5 | 1.38 ± 0.06 | 7.10 ± 0.38 | 8.82 ± 0.18 | 2.00 ± 0.03 |
| 1 | 5 | 10 | 6.62 ± 0.14 | 12.04 ± 0.76 | 29.94 ± 1.42 | 5.75 ± 0.12 |
| 3 | 3 | 3 | 0.91 ± 0.04 | 3.14 ± 0.21 | 4.00 ± 0.23 | 0.81 ± 0.02 |
| 3 | 3 | 5 | 1.89 ± 1.11 | 6.91 ± 0.42 | 7.23 ± 0.54 | 1.88 ± 0.11 |
| 3 | 3 | 10 | 7.14 ± 0.32 | 8.84 ± 1.04 | 17.84 ± 1.01 | 5.99 ± 0.33 |
| 5 | 3 | 3 | 1.40 ± 0.12 | 4.84 ± 0.30 | 6.70 ± 0.56 | 1.48 ± 0.10 |
| 5 | 3 | 5 | 2.71 ± 0.06 | 8.86 ± 0.28 | 22.54 ± 0.86 | 2.69 ± 0.31 |
| 5 | 3 | 10 | 8.88 ± 0.26 | 10.84 ± 1.04 | 42.98 ± 1.18 | 7.32 ± 0.50 |
| 5 | 5 | 3 | 1.64 ± 0.06 | 5.78 ± 0.22 | 7.90 ± 0.10 | 1.72 ± 0.05 |
| 5 | 5 | 5 | 3.41 ± 0.04 | 8.26 ± 0.62 | 16.84 ± 0.24 | 3.10 ± 0.30 |
| 5 | 5 | 10 | 9.12 ± 0.20 | 13.02 ± 0.48 | 49.82 ± 1.12 | 10.01 ± 0.61 |

**Table 3—The Effects of Hydroxyethylcellulose (HEC), Poly(vinylpyrrolidone) (PVP), and Polycarbophil (PC) on the Syringeability[a] of Bioadhesive, Semisolid Formulations Containing Flurbiprofen**

| mean ± sd work (N mm) required to syringe formulations containing | | | | |
|---|---|---|---|---|
| PC (% w/w) | PVP (% w/w) | HEC 3% w/w | HEC 5% w/w | HEC 10% w/w |
| 1 | 3 | 58.74 ± 3.46 | 123.61 ± 10.22 | 241.47 ± 14.70 |
| 1 | 5 | 74.14 ± 4.01 | 151.05 ± 3.57 | 279.49 ± 10.24 |
| 3 | 3 | 79.99 ± 3.54 | 168.11 ± 7.11 | 298.31 ± 12.12 |
| 5 | 3 | 139.47 ± 14.16 | 191.40 ± 8.19 | 341.34 ± 8.46 |
| 5 | 5 | 163.91 ± 9.06 | 221.41 ± 9.27 | 379.00 ± 3.99 |

[a] Measured as the work required to express a defined amount of the formulation from a syringe of specified dimensions, as described in Materials and Methods.

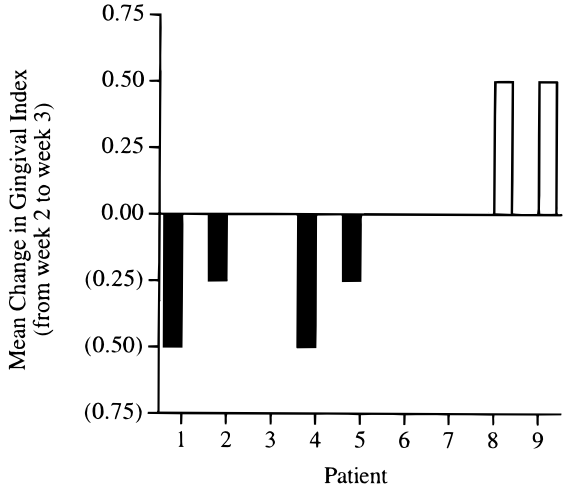


**Figure 1**—The effect of topical application of a test formulation (containing 5% w/w flurbiprofen, 3% w/w hydroxyethylcellulose, 3% w/w poly(vinylpyrrolidone), and 3% w/w polycarbophil) or a control formulation (containing 3% w/w hydroxyethylcellulose, 3% w/w poly(vinylpyrrolidone), and 3% w/w polycarbophil) for one week on the level of gingival inflammation (as evaluated using the gingival index) in clinical subjects. Subjects 1–5 received the test formulation whereas subjects 6–9 received the control formulation.

Increasing the concentration of HEC in aqueous gel systems has previously been reported to increase both gel elasticity (storage modulus) and, additionally, viscosity due to increased polymeric chain entanglement.[25,26] One consequence of these rheological alterations was observed in the effects of HEC on the release of flurbiprofen, in which the rate of drug diffusion through the polymer matrix and, hence, drug release was reduced. Similarly, in formulations containing 3% HEC (in which PVP was dissolved), increasing the concentration of PVP from 3 to 5% decreased flurbiprofen release due to the greater entanglement of dissolved polymeric chains. In general, the reduced release of flurbiprofen observed following increased formulation concentrations of PVP, in which this polymer was present in both the solid and solution phases in the primary HEC gel, may be due to the effects of both the increasing semisolid nature, and, hence, increased elasticity of these formulations.[25,26] A further retarding effect of PVP on drug release may be due to swelling following contact with dissolution fluid. These two mechanisms serve to enhance the elastic structure of the formulations, thereby decreasing the rate of drug diffusion through the semisolid matrix. Similarly, incorporation of PC into these formulations decreased flurbiprofen release, observations that may also be explained by the state of this polymer in each formulation. The extent of swelling of PC in the formulations under investigation was greatest in those formulations containing 3% HEC, 3% PVP, and 1% PC, as, first, the amount of free water, i.e., water not associated with the other polymeric components, was greatest in this system, and, second, the ratio of mass of free water to mass of PC was largest, thus allowing a greater percentage swelling of this polymer.

**Table 4—The Effects of Application of Either a Control (devoid of flurbiprofen) or Test Formulation on the Gingival Crevicular Fluid Volume and Plaque Index of Subjects**

| | | gingival crevicular fluid volume (periotron units)[c] | | plaque index[c] | |
|---|---|---|---|---|---|
| treatment | patient number | prestudy (2 weeks)[d] | poststudy (3 weeks)[e] | prestudy (2 weeks)[d] | poststudy (3 weeks)[e] |
| flurbiprofen[a] | 1 | 32.5 | 8.5 | 1.2 | 1.2 |
| flurbiprofen[a] | 2 | 12.5 | 4.5 | 1.0 | 0.6 |
| flurbiprofen[a] | 3 | 7.5 | 1.0 | 0.5 | 0.6 |
| flurbiprofen[a] | 4 | 14.0 | 12.0 | 0.8 | 0.8 |
| flurbiprofen[a] | 5 | 21.0 | 16.5 | 0.5 | 0.5 |
| control[b] | 6 | 38.0 | 16.0 | 1 | 1.1 |
| control[b] | 7 | 19.0 | 6.5 | 1 | 0.8 |
| control[b] | 8 | 14.5 | 35.0 | 0.5 | 0.5 |
| control[b] | 9 | 12.0 | 11.0 | 0.9 | 0.7 |

[a] Formulation contained 5% w/w flurbiprofen, 3% w/w hydroxyethylcellulose, 3% w/w poly(vinylpyrrolidone), and 3% w/w polycarbophil. [b] Formulation contained 3% w/w hydroxyethylcellulose, 3% w/w poly(vinylpyrrolidone), and 3% w/w polycarbophil. [c] All values are means. The coefficient of variation in all cases <6%. [d] Gingival crevicular fluid volume and plaque index at the end of the second week of the study. [e] Gingival crevicular fluid volume and plaque index at the end of the third week of the study.

In the swollen state, PC imparts both greater elasticity to the formulation, due to entanglement of the chains of poly(acrylic acid) with those of HEC and PVP, and also greater viscosity.[25] Thus, flurbiprofen release was further retarded. As the concentrations of HEC and PVP were increased in the formulations, the mass of free water available for swelling of PC decreased, and hence the percentage of unswollen PC particles increased. In this scenario, the greatest mass of unswollen particles of PC occurred in formulations containing 10% HEC, 5% PVP, and 5% PC. Following immersion in dissolution fluid, the unswollen particles of PC imbibed fluid and commenced swelling, the extent of which was dependent on the initial percentage of unswollen PC. This swelling process established the presence of a swelling front that opposed further ingress of dissolution fluid and hence retarded drug dissolution and diffusion. The presence of PC as dispersed, unswollen particles had a significantly greater retarding effect on drug release than when primarily present as swollen or partly swollen particles due to the influence of this extensive swelling front. In certain formulations, namely those in which the mass of unneutralized PC was greatest (i.e., HEC 5 or 10%, PVP 5%, PC 5%), the extent of swelling resulted in extension of the formulation beyond the retaining molds, leading to disintegration of the formulation. In this fashion, the surface area for drug dissolution and diffusion was significantly increased, and, as a result, the release of flurbiprofen from these formulations was greater than their counterparts containing 3% PVP. The role of formulation swelling on flurbiprofen release accounted for the statistical interactions between HEC and PC and between PVP and PC, as the concentration of HEC (primarily) and PVP directly influenced the state of PC in, and hence swelling of, the formulations. Therefore, as the mass of suspended solids within the formulations increased, there was a significant (and statistically unpredicted) effect on the release of flurbiprofen.

It is accepted that the mechanical properties of topical formulations will directly influence their clinical performance. Therefore, in the design of such systems, it is important to fully characterize these properties. Recently, we have successfully reported the use of texture profile analysis (TPA) for the mechanical characterization of a range of topical formulations, e.g., semisolids,[7,8] gels,[26] and creams,[27] and, consequently, this technique was employed for the characterization of the formulations examined in this study. In particular, TPA provides information for several relevant rheological parameters, including formulation hardness and compressibility, properties that may be related to both mouth feel and ease of removal of the formulation from the container and also ease of application of the formulation onto the proposed substrate,[8,26] initial stress, a measure of the stress required to initiate flow, and adhesiveness, a property that is related to bioadhesion.[8] Thus, both the versatility of sample consistencies that may be analyzed by TPA and the type of information provided by this technique have established its use in the development of topical formulations. In this current study, TPA has been successfully employed to characterize the range of flurbiprofen-containing formulations. Furthermore, the syringeabilities of formulations have been measured using a Texture Analyzer, as, in many cases, it is appropriate to apply formulations to remote supragingival sites/periodontal pockets using syringe systems. Once more, the wide ranges of hardness, compressibility, adhesiveness, initial stress, and work of syringeability exhibited by these formulations may be attributed to the state of the polymeric components.

Formulation hardness, compressibility, initial stress, and work of syringing are parameters that measure the resistance of the product to probe compression, and, indeed, their relationships with product viscosity have been reported previously.[8,29,30] Hence, the effects of HEC, PVP, and PC on these parameters may be directly related to their effects on product viscosity and, indeed, elasticity. Consequently, increasing the amount of dissolved HEC and PVP increased these mechanical properties due to increased elasticity and viscosity of the formulations,[25,26] resulting from the increasing entanglement of the polymeric chains. Increasing the mass of solid particles in the formulation, however, offers greater semisolid properties to the formulations and, hence, greater resistance to probe compression. Interestingly, statistical interactions were observed between HEC and PVP and between HEC and PC with respect to hardness, compressibility and work of syringeability that may again be explained by the state of each polymeric component in the formulations. In these interactions, the effects of PC and PVP on these parameters were significantly (and unexpectedly) greater in the presence of 10% HEC than either 3 and indeed 5% HEC. Thus, as the concentration of HEC increases, the mass of suspended PVP and unswollen PC increase, and thus the formulations adopted greater semisolid nature due to the increasing content of suspended solids. At lower concentrations of HEC, the effects of PVP and PC on formulation hardness, compressibility, and work to syringe may be attributed, in part, to the entanglement of the dissolved chains of HEC with PVP and also the swollen chains of PC. As the mass of solid particles increases, the relative contribution of chain entanglement to these mechanical properties decreases due to the overwhelming effects of the increasing mass of dispersed polymeric solid materials. The observed imbalance of the contribution of polymeric chain entanglement and suspended solid particles accounted for the statistical interactions.

In textural analysis, adhesiveness refers to the work required to overcome the attractive bonds between the sample and probe and is determined from the area under the force−distance curve in the tension section of the plot. However, at this point it is useful to record that the removal of the probe from the sample may involve the destruction of cohesive bonds within the sample in addition to the cleavage of bonds between the sample and probe. Interestingly, a correlation has been reported between the mucoadhesive properties of formulations and their adhesiveness,[8,26] and, consequently, quantification of adhesiveness provides a useful, rapid method by which the mucoadhesive properties of related formulations may be reliably ranked. In this current study, increasing concentrations of HEC, PVP, and PC significantly increased formulation adhesiveness. As these polymers have been described as bioadhesive,[9] these observations may be due to both their ability to form adhesive bonds (in a concentration-dependent fashion) with the probe in TPA, and to increase viscosity (tack) of the formulations, a property that has been reported to influence the adhesiveness of certain formulations.[8]

It is important to consider specifically the role of PC in the adhesiveness of the formulations under examination. PC possesses the ability to strongly interact with biological substrates, the degree of interaction being dependent on the number of unneutralized carboxylic acid groups on the poly(acrylic acid) chains.[8,9] In this study, and indeed in related earlier publications,[8,31] the contribution of unneutralized carboxylic acid groups to adhesiveness in TPA was confirmed. Hence, in formulations containing 3% HEC, 3% PVP, and 1% PC, the extent of neutralization, and hence swelling of PC, was greatest, and this formulation exhibited lowest adhesiveness. Conversely, in formulations where the amount of water available to neutralize PC is lowest, i.e.,

10% HEC, 5% PVP, and 5% PC, the mass of unneutralized (and hence unswollen) particles of PC was greatest, and hence adhesiveness is maximal. Given the correlation with mucoadhesion, this formulation would be expected to possess the greatest mucoadhesive properties of all formulations studied.[26] The statistical interactions observed between HEC and PC, PVP and HEC, and PVP and PC reflect once more the effects of the state of each polymeric component on formulation adhesiveness. Thus, the presence of solid polymeric particles may be associated with dramatic increases in adhesiveness due both to the effects on formulation viscosity (and hence tack) and additionally to the effects on the mass of free water present and, hence, the mass of unneutralized carboxylic acid groups in PC.

The ideal formulation for clinical examination for the treatment of periodontal diseases should possess a number of desirable attributes, including adequate prolonged and controlled release of therapeutic agent, ease of removal from the container, and ease of application to, and good retention at, the desired site. In addition, it should be comfortable to the patient during the residence period of the formulation. The various in vitro methods employed in this study have allowed quantification of all of these attributes. Hence, in vitro release methods defined the nature of the release of flurbiprofen from all formulations. The TPA parameters of hardness/compressibility and adhesiveness described the ease of removal from the container/ease of application to the desired site and product retention, respectively. Finally, acceptability of the product by the patient during the period of residence in the oral cavity may be conveniently described by hardness measurements in TPA, as previously reported,[28,31] whereas the syringeability of the formulations to, e.g., subgingival or remote supragingival regions, was conveniently described using a modified compression test.

Ideally, candidate formulations for clinical examination should exhibit low hardness, initial stress, compressibility, work of syringeability, yet high adhesiveness. However, as may be observed in Tables 2 and 3, increased formulation adhesiveness (and hence increased retention at the site of application) may be achieved by increasing the concentration of PC (primarily), HEC, and PVP. However, such increases also resulted in increased formulation hardness, compressibility, and work of syringing, undesirable formulation attributes. Therefore, in terms of mechanical properties, a compromise must be attained between formulation adhesiveness and formulation hardness, compressibility, and syringeability. Examples of formulations that exhibited acceptable mechanical properties included HEC 3%/PVP 3 or 5%/PC 1, 3 or 5% and HEC 5%/PVP 3%, PC 1 or 3%. Furthermore, in light of the proposed daily (nightly) administration of the formulation and based on information from related clinical studies,[14,17] the formulations containing 3% HEC, 3% PVP, and either 3 or 5% PC exhibited the required release properties, namely $t_{30\%}$ values of 8.73 $\pm$ 0.56 and 10.99 $\pm$ 1.01 h, respectively. Therefore, following consideration of both the mechanical and release properties, the formulation selected for clinical examination contained 3% HEC, 3% PVP, 3% PC, and 5% flurbiprofen. Initial clinical assessment of this formulation, using an experimental gingivitis model, indicated significantly beneficial effects in the treatment of gingivitis, as denoted by the improvement in the gingival index and the gingival crevicular fluid volume. Of further interest were the insiginificant differences in the plaque indices of the two patient groups during weeks 2 to 3 of this study period. Therefore, changes in gingival crevicular fluid volume could not be explained by differences in plaque accumulation. It is known that levels of the cyclooxygenase metabolites of arachadonic acid, $PGE_2$ and $TxB_2$, both of which are potent vasodilators, are elevated during the onset of gingival inflammation.[17] The clinical effects of flurbiprofen, an inhibitor of the cyclooxygenase pathway, observed in this study may thus be attributed to prolonged reduced levels of both mediators in the gingival tissue. Quantification of these levels is the subject of current clinical investigations.

In conclusion, a series of formulations containing HEC, PVP, PC, and flurbiprofen has been designed and characterized in vitro in terms of both flurbiprofen release and mechanical properties. The use of texture profile analysis to characterize the mechanical properties has been shown to be particularly useful as the mechanical parameters defined by this technique (e.g., hardness, compressibility, syringeability, adhesiveness) have direct relevance to both the potential clinical and sensory performance of these formulations.[8] A candidate formulation was chosen for clinical evaluation containing 3% HEC, 3% PVP, 3% PC, and 5% flurbiprofen that offered optimal flurbiprofen release over the proposed daily application of the product and, additionally, offered a compromise between formulation adhesiveness (and hence persistence at the site of application) and hardness (comfort during the period of application), and compressibility/syringeability (ease of removal from the container and ease of spreading/application to the inflamed site).

The comparative clinical efficacies of this chosen flurbiprofen-containing formulation and an otherwise identical placebo formulation (devoid of flurbiprofen) were evaluated in an experimental gingivitis model in nine volunteers, in which gingival inflammation was measured using the gingival index. Following seven nightly applications, volunteers who had received the flurbiprofen-containing formulation exhibited decreased gingival inflammation and gingival crevicular fluid volume, whereas those who had received the control formulation displayed increased gingival inflammation and gingival crevicular fluid volume. This preliminary clinical study has therefore shown the clinical improvements associated with the use of topical bioadhesive, flurbiprofen-containing semisolid formulations. The clinical efficacy of this formulation, including quantification of the concentrations of inflammatory mediators, is currently ongoing in multicentered clinical evaluations.

## References and Notes


1. Addy, M. Local delivery of antimicrobial agents to the oral cavity. *Adv. Drug Deliv. Rev.* **1994**, *13*, 123−134.
2. Joyston-Bechal, S. Féderation Dentaire Internationale Technical Report No. 26. Topical and systemic antimicrobial agents in the treatment of chronic gingivitis and periodontitis. *Int. Dental J.* **1987**, *37*, 52−62.
3. Lindhe, J.; Haffajee, A. J.; Sokransky, S. S. Progression of periodontal disease in adult subjects in the absence of periodontal therapy. *J. Clin. Periodontol.* **1993**, *10*, 433−443.
4. Listgarten, M. A. Nature of periodontal diseases: pathogenic mechanisms. *J. Clin. Periodontol.* **1986**, *13*, 418−425.
5. Medlicott, N. J.; Rathbone, M. J.; Tucker, I. G.; Holborow, D. W. Delivery systems for the administration of drugs to the periodontal pocket. *Adv. Drug Deliv. Rev.* **1994**, *13*, 181−203.
6. Fiorellini, J. P.; Paquette, D. M. D. The potential role of controlled-release delivery systems for chemotherapeutic agents in periodontitis. *Periodontol. Restor. Dent.* **1992**, 63−79.
7. Jones, D. S.; Woolfson, A. D.; Djokic, J.; Coulter, W. A. Development and physical characterisation of bioadhesive semisolid, polymeric systems containing tetracycline for the treatment of periodontal diseases. *Pharm. Res.* **1996**, *13*, 1734−1738.
8. Jones, D. S.; Woolfson, A. D.; Brown, A. F. Textural analysis and flow rheometry of bioadhesive, antimicrobial oral gels. *Pharm. Res.* **1997**, *14* (4), 450−457.

9. Gandhi, R. B.; Robinson, J. A. Oral cavity as a site fo bioadhesive drug delivery. *Adv. Drug Deliv. Rev.* **1994**, *13*, 43−74.
10. Collins, A. E.; Deasy, P. B. Bioadhesive lozenge for the improved delivery of cetylpyridinium chloride. *J. Pharm. Sci.* **1990**, *79*, 116−119.
11. Mahdi, A. B.; Coulter, W. A.; Woolfson, A. D.; Lamey, P. J. Efficacy of bioadhesive patches in the treatment of recurrent apthous stomatitis. *Journal of Oral Pathology & Medicine* **1996**, *25* (8), 416−419.
12. Offenbacher, S.; Odle, B. M.; Van Dyke, T. E. Assay of cyclooxygenase products in crevicular fluid in periodontal health and disease. *J. Periodont. Res.* **1986**, *21*, 101−112.
13. Offenbacher, S.; Odle, B. M.; Braswell, L. D.; Johnson, H. G.; Hall, C. M.; McClure, H.; Orkin, J. L.; Strobert, E. A.; Green, M. D. Changes in cyclooxygenase metabolites in experimental periodontitis in macaca-mulatta. *J. Periodont. Res.* **1989**, *24*, 63−74.
14. Williams, R. C.; Offenbacher, S.; Jeffcoat, M. K.; Howell, T. H.; Johnson, H. G.; Hall, C. M.; Wechter, W. J.; Goldhaber, P. Indomethacin or flurbiprofen treatment of periodontitis in beagles − effects of crevicular arachadonic acid metabolites compared with effect on alveolar bone loss. *J. Periodont. Res.* **1988**, *23*, 134−138.
15. Williams, R. C.; Jeffcoat, M. K.; Howell, T. H.; Reddy, M. S.; Johnson, H. G.; Hall, C. M.; Goldhaber, P. Ibuprofen − an inhibitor of alveolar bone resorption in beagles. *J. Periodont. Res.* **1988**, *23*, 166−169.
16. Jeffcoat, M. K.; Williams, R. C.; Reddy, M. S.; English, R.; Goldhaber, P. Flurbiprofen treatment of human periodontitis − effect on alveolar bone height and metabolism. *J. Periodontol. Res.* **1988**, *23* (6), 381−385.
17. Heasman, P. A.; Collins, J. G.; Offenbacher, S. Changes in crevicular fluid levels of interleukin 1$\beta$, leucotriene $B_4$, prostagalndin $E_2$, thromboxane $E_2$, and tumour necrosis factor $\alpha$ in experimental gingivitis in man. *J. Periodont. Res.* **1993**, *28*, 241−247.
18. Heasman, P. A.; Benn, D. K.; Kelly, P. J.; Seymour, R. A.; Aitken, D. The use of topical flurbiprofen as an adjunct to nonsurgical management of periodontal disease. *J. Clin. Periodontol.* **1993**, *20*, 457−464.
19. Heasman, P. A.; Offenbacher, S.; Collins, J. G.; Edwards, G.; Seymour, R. A. Flurbiprofen in the prevention and treatment of experimental gingivitis. *J. Clin. Periodontol.* **1993**, *20*, 732−738.
20. Jones, D. S.; Woolfson, A. D.; Brown, A. F.; O'Neill, M. J. Mucoadhesive, syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: In vitro release kinetics, syringeability, mechanical and mucoadhesive properties. *J. Contolled Release* **1997**, *49*, 71−79.
21. Gurny, R.; Doelker, E.; Peppas, N. A. Modelling of sustained release of water-soluble drugs from porous, hydrophobic polymers. *Biomaterials* **1982**, *3*, 27−32.
22. Medlicott, N. J.; Tucker, I. G.; Rathbone, M. J.; Holborow, D.; Jones, D. S. Chlorhexidine release from Poly($\epsilon$-caprolactone) films prepared by solvent evaporation. *Int. J. Pharm.* **1996**, *143* (1), 25−35.
23. Loe, H.; Silness, P. Periodontal disease in pregnancy (1). Prevealence and severity. *Acta Odont. Scand.* **1963**, *21*, 533−551.
24. Silness, J.; Loe, H. Periodontal disease in pregnancy (2). Correlation between oral hygiene and periodontal condition. *Acta Odont. Scand.* **1964**, *22*, 112−135.
25. Jones, D. S.; Woolfson, A. D.; Brown, A. F. Viscoelastic properties of bioadhesive, chlorhexidine-containing semisolids for topical application to the oropharynx. *Pharm. Res.* **1998**, *15* (7), 1131−1136.
26. Jones, D. S.; Woolfson, A. D.; Brown, A. F. Textural, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers. *Int. J. Pharm.* **1997**, *151*, 223−233.
27. Brown, A. F.; Jones, D. S.; Woolfson, A. D. The effects of emulsifier and drug (chlorhexidine) concentrations on the mechanical and viscoelastic properties of creams. *J. Pharm. Pharmacol.* **1997**, *49* (S4), 127.
28. Schwartz, N. O. Adaptation of the sensory textile profile method to skin care products. *J. Text. Studies* **1975**, *42*, 33−42.
29. Ferrari, F.; Bertoni, M.; Caramella, C.; La Manna, A. Description and validation of an apparatus for gel strength measurements. *Int. J. Pharm.* **1994**, *109*, 115−124.
30. Lucero, M. J.; Vigo, J.; Leon, M. J. The influence of antioxidants on the spreadability of $\alpha$-tocopherol gels. *Drug Deliv. Ind. Pharm.* **1994**, *20*, 2315−2322.
31. Jones, D. S.; Woolfson, A. D.; Djokic, J. Texture profile analysis of bioadhesive polymeric semisolids: mechanical characterisation and investigation of interactions between formulation components. *J. Appl. Polym. Sci.* **1996**, *61* (12), 2229−2234.

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