Moisture-Activated, Electrically Conducting Bioadhesive Hydrogels as Interfaces for Bioelectrodes: Effect of Film Hydration on Cutaneous Adherence in Wet Environments

A. D. WOOLFSON,[1,*] D. F. McCAFFERTY,[1] C. R. McCALLION,[1] E. T. McADAMS,[2] and J. McC. ANDERSON[2]

[1]School of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, Belfast BT9 7BL, Northern Ireland, U.K.; [2]Northern Ireland Bio-Engineering Centre, University of Ulster at Jordanstown, Newtownabbey BT37 0QB, Northern Ireland U.K.

SYNOPSIS

The water contents of bioadhesive polymeric films of poly(methylvinyl ether–maleic anhydride) copolymer, stored at two humidities and at two temperatures, have been determined by continuous gravimetry. The water contents of the films increased under storage conditions of increasing humidity. The bioadhesion of these films was assessed using an *in vitro* bioadhesive tester previously validated in our laboratory. Water uptake during the bioadhesive test was taken into account and the final water content of films recorded as a "corrected equilibrium water content" (CEWC). A good quality second-order polynomial relationship between bioadhesion and the CEWC of films stored at both 23 and 35°C was developed using standard curve fitting analyses. A 2 × 2 factorial design experiment revealed that there was a synergistic relationship between temperature and percentage relative humidity with regard to their effect on the bioadhesion of polymeric films, presumably due to their combined effect on film water content. These findings have implications for both the storage and packaging of hydrophilic polymeric films. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Moisture-activated bioadhesive hydrogel films develop maximum adhesion at an optimum degree of hydration.[1,2] Excessive hydration of such films results in the formation of a slippery nonadhesive mucilage which eventually becomes displaced by water. Thus, it is important to investigate the relationship between bioadhesion and the water content of such films. An appropriate method for water content measurement is therefore required.

An indirect method for determining the water content of hydrogels, involving the measurement of the refractive index of the cast film, has frequently been used to determine the moisture content of soft content lenses.[3] In this method the refractive index is correlated with water content using the Brix Scale, a calibration procedure based on the weight fraction of sucrose in an aqueous solution at a given temperature. However, films that do not exhibit the same relationship between water content and refractive index as the sucrose/water solution give erroneous values. In addition, surface changes in the films may affect the results since the critical angle of refraction is involved in the measurement.

A more recent indirect method has involved immersing samples in an aqueous Blue Dextran 2000 (BD2000) solution of known concentration and ultraviolet absorbance, and measuring the change in absorbance of the solution after the hydrogel absorbs water, thereby making the solution more concentrated.[4] Since dextran is a large molecule, it is assumed that it is not absorbed. Errors are inherent in this method, however, since the sample must be removed prior to measurement. In addition, the chemical potential of water in a BD2000 solution is not exactly equal to that of pure water.[4]

The most commonly used method of determining the water content of films is by gravimetry, a more

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 58, 1291–1296 (1995)
 CCC 0021-8995/95/081291-06

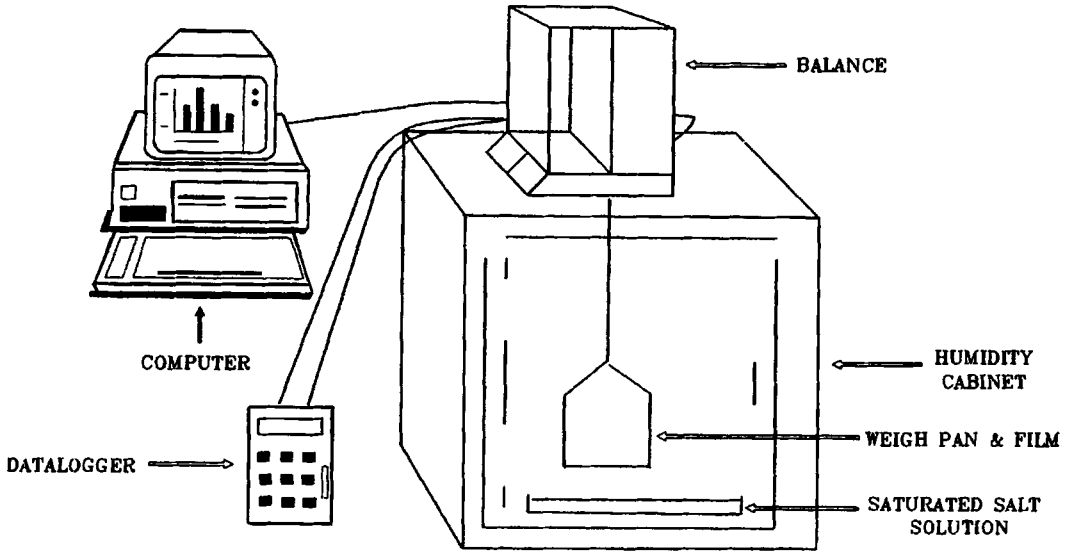

Figure 1 Line drawing of the continuous-humidity gravimetric system.

direct procedure. The equilibrium water content of the film is defined according to eq. (1).

$$EWC = \frac{WWF - DWF}{WWF} \times 100 \qquad (1)$$

where EWC = equilibrium water content of film, WWF = wet weight of film, and DWF = dry weight of film.

Gravimetry usually involves submerging the preweighed, dry sample in water for a defined period, followed by sample removal, drying, and reweighing. EWC is then calculated from eq. (1). Traditionally this method, although direct, has been compromised by the need to remove the sample from the aqueous solution and to blot it dry prior to weighing. Accuracy and precision are thus dependent on the degree of blotting. Thus, if the sample is overdried this leads to dehydration, while insufficient drying may leave excess moisture on the film. The speed with which the sample is weighed will also affect the results obtained.

In the present study a gravimetric system is reported whereby the weights of films may be monitored continuously *in situ* under conditions of variable relative humidity at a particular temperature. With this system, a quantitative relationship has been developed between the equilibrium water content of a hydrophilic polymeric film and its bioadhesion *in vitro* to excised full-thickness hairy porcine skin.

EXPERIMENTAL

Materials

Polyvinylpyrrolidone (PVP, Kollidon® 90, USP grade) was obtained from BASF, Ludwigshafen, Germany. Gantrez AN-139, a copolymer of methyl vinyl ether and maleic anhydride (PMVE/MA), was provided by ISP Co. Ltd., Manchester, U.K. All other chemicals used were of analytical reagent quality.

Water Content Analysis

Films were cast from aqueous blends of copolymer/plasticizer/thickener (with 0.1% w/w NaCl added), as described previously.[5] Clear flexible hydrophilic polymeric films of surface area approximately 600 mm^2 and thickness approximately 0.2 mm were produced on a nonwoven polyester substrate, thickness 50 μm. All films tested were freshly cast and were initially conditioned for a 4-h equilibration period in an atmosphere of 52% relative humidity at ambient temperature before being transferred to storage at the required relative humidity level.

A line drawing of the gravimetric system is shown in Figure 1. A freshly cast film was placed on the weigh-pan extension of an Oertling Model NA264 balance. The balance was interfaced with an IBM XT computer with custom software for continuous weight recording. Temperature and humidity were also continuously monitored on a Psion organiser datalogger (Model LZ64) with a humidity (model

Table I Percentage Relative Humidities (% RH) Obtained with Various Saturated Salt Solutions

Saturated Salt Solution	% RH ± 3 (25°C)	% RH ± 3 (35°C)
Potassium acetate	22	20
Magnesium chloride · $6H_2O$	33	32
Potassium carbonate	43	43
Sodium bromide	58	56
Sodium nitrite	65	62
Potassium chloride	85	84

R5SF) and temperature (Model THSF) probe attached. Relative humidities were altered by using an appropriate saturated salt solution in the tray of the humidity cabinet (LTE, Oldham, UK). Table I shows the salt solutions used and the corresponding relative humidities.

Weight changes were recorded for four replicate film samples for each humidity over a 24-h period at 23 ± 1.5 and at 35 ± 1.5°C. The water contents of the films were determined using eq. (1).

Bioadhesion Testing

A bioadhesion tester, developed in our laboratory,[6] was used to measure the bioadhesion of 1 cm^2 samples of the bioadhesive polymeric films to full-thickness porcine skin previously wetted by immersion in water for 10 sec. The technique used has been detailed in a previous study.[5] Film samples were stored at a particular humidity for 7 days prior to the determination of their bioadhesion to porcine skin.

Weight Changes during Bioadhesion Testing

The weight gains of films, due to hydration, during bioadhesion testing were determined by weighing films before and after bioadhesion testing.

Factorial Design Experiment

A 2 × 2 factorial design experiment was carried out to investigate the combined effect of changing temperature and humidity on the bioadhesion of hydrophilic polymeric films.

Statistical Analyses

ANOVA (2-way with repeated measures) was performed with Minitab® and the Newman–Kuels multiple range test with Kwikstat®, both PC versions. Curve fitting was performed with Minim® software (Apple Macintosh version).

RESULTS AND DISCUSSION

Gravimetric analysis of water content has often been compromised by the need to remove the sample under test from the experimental environment in order to weight it. Use of a continuous gravimetric analytical technique enables monitoring to continue while the sample remains in the experimental environment. A typical weight–time profile obtained using a continuous gravimetric system is shown in Figure 2. At low humidities, the initial rapid weight loss was succeeded by a steady-state weight upon reaching equilibrium. This equilibrium is achieved rapidly, typically occurring between 500 and 1000 min after set-up. At higher humidities, an initial rapid weight gain is succeeded by a steady-state weight at equilibrium (Fig. 2).

The relationship between percentage weight change (%WCH) and percentage relative humidity (%RH) was plotted for films stored at 23 ± 1.5°C for 7 days under various humidities (Fig. 3). Curve fitting of these data yielded a linear relationship (r^2 = 0.986) in the range of data tested. This relationship is expressed in eq. (2).

$$\%WCH = 0.288\,(\%RH) - 12.239 \qquad (2)$$

Adsorption isotherms for textiles such as cotton and wool, and for many hydrophobic hydrogels, have a classic sigmoidal shape.[7,8] This shape has been ex-

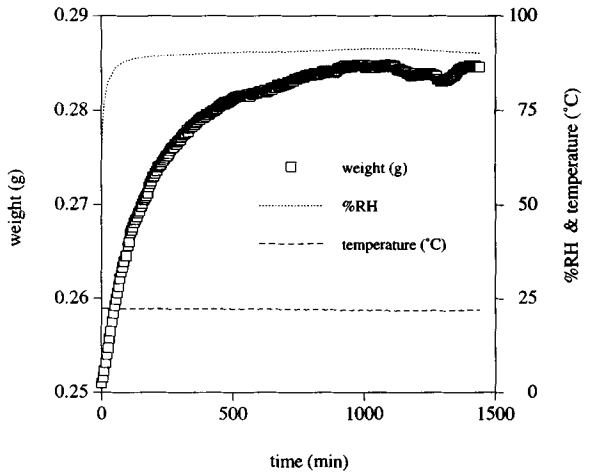

Figure 2 Typical time (min)–weight (g)–%RH profile for a bioadhesive film stored at 88 ± 3% RH and 23 ± 1.5°C.

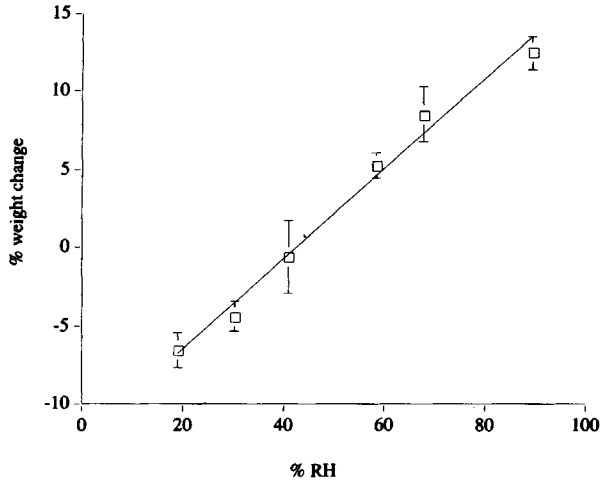

Figure 3 Relationship between %RH and % weight change of bioadhesive films. Error bars represent the standard deviation of the mean of four replicates.

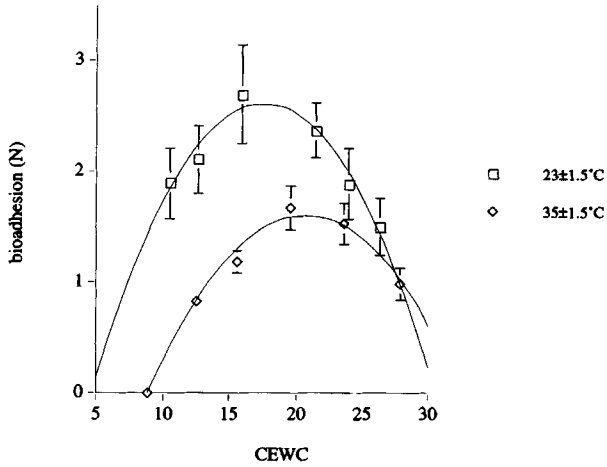

Figure 4 Relationship between bioadhesion and corrected equilibrium water content (CEWC) of bioadhesive films stored at both 23 and 35 ± 1.5°C for 7 days. Error bars represent the standard deviation of the mean of eight replicates.

plained by the presence of three classes of water (bulk, bound, and intermediate) which are thought to be present in hydrogels.[9,10] For hydrophilic polymeric films such as methylcellulose, Kanig and Goodman[11] have shown the relationship between percentage absorbed moisture and %RH to be directly proportional. However, they also showed that for moisture absorption by water-insoluble ethylcellulose films, the relationship is not directly proportional. An earlier study on polyvinylpyrrolidone films revealed that the moisture content of the films is directly related to %RH, with the equilibrium moisture content being about one third of this value.[12] More recently, Mortada and co-workers[13] indicated that the percentage of absorbed moisture in *n*-propyl and *n*-butyl half-esters of PMVE/MA films is directly proportional to percentage relative humidity.

Extrapolation of eq. (2) back to zero percent relative humidity revealed that, at this level, the percentage decrease in film weight was 12.239% of its initial value, assuming that all the films cast initially had the same water content. The theoretical weight of a film at zero percent relative humidity was assumed to represent the theoretical dry weight (DWF) included in eq. (1). From the gravimetric data, the EWF at the various humidities was calculated from eq. (1) using the mean wet weight (WWF) recorded for each film between 1000 and 1400 min. A linear relationship ($r^2 = 0.98$) between these parameters was derived according to eq. (3).

$$EWC = 0.242\,(\%RH) + 1.634 \qquad (3)$$

Figure 4 shows the relationship between bioadhesion and the corrected equilibrium water content (CEWC) of bioadhesive polymeric films following storage at 23 and 35 ± 1.5°C. During the bioadhesion test procedure, the film under test will inevitably hydrate further to some undetermined state due to the presence of moisture on the excised porcine skin substrate. To relate the bioadhesion of polymeric films to their water content at the time of adhesion, an estimate of the degree of hydration during testing is necessary. Table II shows a summary of the weight gain of films (originally stored at various humidities and at 23 ± 1.5°C) during adhesion testing. These values can be used to determine the CEWC for the films, thus allowing for additional hydration during the test procedure.

From Figure 4 it is apparent that there is an optimum film water content for maximum *in vitro* bioadhesion, in agreement with the wet adhesion

Table II Summary of Recorded Percentage Weight Gains during Bioadhesive Testing of Films Stored at Various Percentage Relative Humidities (% RH) and at 23 ± 1.5°C

% RH ± 3	% Weight Gain (n = 6)
20	25.19 ± 4.18
30	20.67 ± 3.32
40	14.71 ± 1.94
57	14.85 ± 1.43
66	13.40 ± 2.38
85	9.39 ± 1.29

Table III Mean *in Vitro* Bioadhesion (N) of Films Stored at 23 ± 1.5 and 35 ± 1.5°C for 7 Days at 30 and 90% RH (2 × 2 Factorial Design)

	Bioadhesion (N) (n = 8)	
% RH ± 3	23 ± 1.5°C	35 ± 1.5°C
30	2.101 ± 0.304	0.832 ± 0.074
90	1.501 ± 0.257	0.982 ± 0.136

theory proposed by Chen and Cry.[1] Thus, during the dynamic process of bioadhesion, polymer chains are thought to be released from their restraining dry lattice forces by the action of hydration. These chains then move and entangle into the matrix of the substrate. Maximum adhesive strength is attained when perfect matching occurs between the active adhesive sites on the polymeric chains and the substrate, increasing the magnitude of van der Waals' forces or even promoting chemical bond formation. The amount of water present at the bond site is decisive in determining the extent of liberation of polymer chains, as the process is hydration dependent. Overhydration results in the formation of a wet slippery mucilage with no adhesive strength.

A second-order polynomial, eq. (4) best described the relationship between CEWC and bioadhesion (r^2 = 0.946) for films stored at 23 ± 1.5°C.

$$\text{Bioadhesion} = -0.015\,(\text{CEWC})^2 + 0.545\,(\text{CEWC}) - 2.189 \quad (4)$$

A second-order polynomial relationship [eq. (5), r^2 = 0.988] also described the relationship between CEWC and bioadhesion for films stored at 35°C for 7 days.

$$\text{Bioadhesion} = -0.011\,(\text{CEWC})^2 + 0.474\,(\text{CEWC}) - 3.318 \quad (5)$$

Films (stored at 35 ± 1.5°C) with CEWC values less than 8% lost all bioadhesion and, in addition, these films were inflexible and cracked easily, possibly due to the dual effects of storage at low humidity and increased temperature. Bioadhesion increased progressively with increasing CEWC up to a maximum adhesion of around 1.5 N at about 20% (w/w) water content. This adhesion is much reduced compared to the adhesion of films stored at room temperature, where the maximum bioadhesion was around 2.5 N (Fig. 4) for films with a CEWC estimated to be around 15% (w/w). Presumably, this is due to the effect of storage at an elevated temperature on the films.

A 2 × 2 factorial experiment was designed to investigate the effect of increasing the storage temperature from 23 to 35°C for two humidity levels (30 and 90% RH) on the bioadhesion of films stored for 7 days under the various conditions listed. A summary of the results is given in Table III. The results of statistical analysis of the data in Table III by 2-way ANOVA with repeated measures (n = 8) are summarized in Table IV. Thus, it is apparent that increasing the ambient storage temperature from 23 ± 1.5 to 35 ± 1.5°C significantly decreased the bioadhesion over the two humidity levels (p < 0.001). Bioadhesions for films stored at 30% RH and at 90% RH at 35°C are not statistically different from each other at the 95% confidence limits (based on Newman–Keuls multiple range test). This would tend to suggest that at higher storage temperatures, humidity does not exert such a critical effect on film bioadhesion. However, the results obtained (Table III) are significantly influenced by the combined effects of temperature and humidity (p < 0.001, Table IV).

An increased storage temperature, when combined with lower humidity, significantly reduced film bioadhesion. This may be due to increased cross-linking of polymer chains under these conditions. During subsequent hydration of the films, the en-

Table IV ANOVA (2-Way with Repeated Measures) of the Effect of Temperature (°C) and Percentage Relative Humidity (% RH) on *in Vitro* Bioadhesion (N) to Neonate Porcine Skin (2 × 2 Factorial Design)[a]

Source	DF	SS	MS	F Value	p
Temperature (°C)	1	651.6454	651.6454	140.7411	< 0.001
% RH	1	41.2342	41.2342	8.9057	0.006
Interaction	1	114.6485	114.6485	24.7616	< 0.001
Error	28	129.6436	4.6301		
Total	31	937.1717			

[a] DF, degrees of freedom; SS, sum of squares; MS, mean square error; p, significance probability.

tangled chains of the polymeric matrix would then no longer be free to uncurl and present their active sites for bioadhesion. This would also explain the complete loss of bioadhesion for films stored at 35°C and 20% RH for 7 days (CEWC < 8%), as shown in Figure 4. At higher humidities, temperature would appear to exert a lesser, although still statistically significant, effect ($p < 0.001$) since the bioadhesion of films stored at 90% RH is similar for films at both storage temperatures (Table III).

The results of this study clearly demonstrate the importance of carefully controlling the storage conditions, both in respect of temperature and humidity, for bioadhesive films. There are also practical implications for the packaging of such films, including the desirability of using low moisture vapor transmission materials.

REFERENCES

1. J. L. Chen and G. N. Cry, *Adhesion in Biological Systems,* Academic Press, New York, 1970.
2. J. D. Smart, I. W. Kellaway, and H. E. Worthington, *J. Pharm. Pharmacol.,* **36,** 295 (1984).
3. S. L. Galas and J. B. Enns, *Optomet. Vision Sci.,* **70,** 577 (1993).
4. M. B. Huglin and D. C. F. Yip, *Makromol. Chem. Rapid Commun.,* **8,** 237 (1987).
5. A. D. Woolfson, D. F. McCafferty, C. R. McCallion, E. T. McAdams, and J. McC. Anderson, *J. Appl. Polym. Sci.,* to appear.
6. A. D. Woolfson, D. F. McCafferty, S. P. Gorman, P. A. McCarron, and J. Price, *Int. J. Pharm.,* **84**(1), 69 (1992).
7. A. J. Hailwood and S. Horrobin, *Trans. Faraday Soc.,* **42**(B), 84 (1946).
8. A. F. El-Shimi and H. M. Princen, *Colloid Polym. Sci.,* **256**(2), 8 (1978).
9. M. S. Jhon, S. M. Ma, S. Hattori, D. E. Gregonis, and J. D. Andrade, *Hydrogels for Medical and Related Applications,* ACS Symposium Series 31, American Chemical Society, Washington, D.C., 1976.
10. M. F. Refojo, *Hydrogels for Medical and Related Applications,* ACS Symposium Series 31, American Chemical Society, Washington, D.C., 1976.
11. J. L. Kanig and H. Goodman, *J. Pharm. Sci.,* **51**(1), 77 (1962).
12. J. M. Wilkinson, G. G. Stoner, E. P. Hay, and D. B. Witwer, *Proc. Chem. Specialties Mfrs. Assoc.,* **May,** 25 (1954).
13. S. M. Mortada, M. A. El Egakey, A. M. Motami, and K. A. El Khodrey, *Pharm. Ind.,* **52,** 107 (1990).

Received December 6, 1994
Accepted April 8, 1995