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An in vitro model for investigating the gastric mucosal retention of ^{14}C-labelled poly(acrylic acid) dispersions

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Abstract

Polymers that bind from solution onto gastric mucosae can be used as a means of facilitating localised drug delivery, or act as therapeutic agents in their own right (e.g. by forming a protective layer or by inhibiting enzymes). Previous workers have used semi-quantitative methods to identify the ability of commercially available poly(acrylic acid)s to bind to gastric mucosa. In this study, the binding and retention of labelled poly(acrylic acid)s to sections of gastric mucosa from the pyloric region of pigs stomach were evaluated using 'static' and 'dynamic flow' test systems. Dispersions (3%) of 'low', 'high' and 'ultra high' (cross-linked) polymers were seen to adhere to porcine pyloric mucosa after exposure and rinsing in the 'static' system. The high molecular weight polymer showed the greatest retention in the 'dynamic' test system when washing continuously with simulated gastric acid. Changing the pH of the dispersions from 4.3 to 6.2 had little effect on polymer retention. It was concluded that polymers that were sufficiently mobile in solution to spread on, and interact with, the mucosal surface, but had a sufficiently high molecular weight to form viscous solutions and/or bioadhere to the mucosa, may be retained on the mucosal surface for the longest periods.

Keywords: Mucoadhesion; Bioadhesion; Poly(acrylic acid)s; Gastric mucosa

1. Introduction

Interest in mucoadhesive materials is based on their potential use as vehicles in site-specific drug delivery. For example, because of their affinity for gastric mucosa, such materials may be employed in the treatment of peptic ulcers to render antibiotics active against *Helicobacter pylori* (Allen et al., 1997; Nagahara et al., 1998). Furthermore, mucoadhesive polymers may possess therapeutic qualities by forming a protective physical barrier around inflamed gastrointestinal tissue (Copeman

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et al., 1994; Dettmar et al., 1986) or by the inhibition of proteolytic enzymes (Nakanishi et al., 1998; Leußen et al., 1995).

In our previous work (Riley et al., 2001a), the synthesis and characterisation of various poly(-carboxylic acid)s containing a ^{14}C label in the polymer backbone was described. In this study, the in vitro retention of dispersions of these polymers on gastric mucosa will be investigated, for the purpose of assessing their potential use as drug delivery or mucosal protective agents.

Two similar techniques have been described to evaluate semisolid/liquid retention on mucosal surfaces (Young and Smart, 1998; Batchelor, 2000). These involved monitoring the retention of a marker molecule incorporated into a liquid or semisolid formulation when applied to a model mucosal surface and challenged with a flow of simulated intestinal liquid. An issue with both of these studies is the possible loss of the marker molecule as it diffuses out of the dispersion into the tissue or surrounding medium. The incorporation of the label as part of the polymer backbone means that the distribution of the polymer can be determined with confidence without having to significantly alter its physicochemical properties. This study used two methods, the first 'preliminary' investigation being a simple 'static binding' study to look at quantitative polymer adhesion on a unit area of mucosa. The technique of Young and Smart (1998) was then used to investigate labelled polymer binding to, and retention on, gastric mucosal surfaces. The pH and polymer concentration used in this work are based on those identified as optimal for mucus polymer interaction in a previous rheological synergism study (Riley et al., 2001b).

2. Materials and methods

2.1. Materials

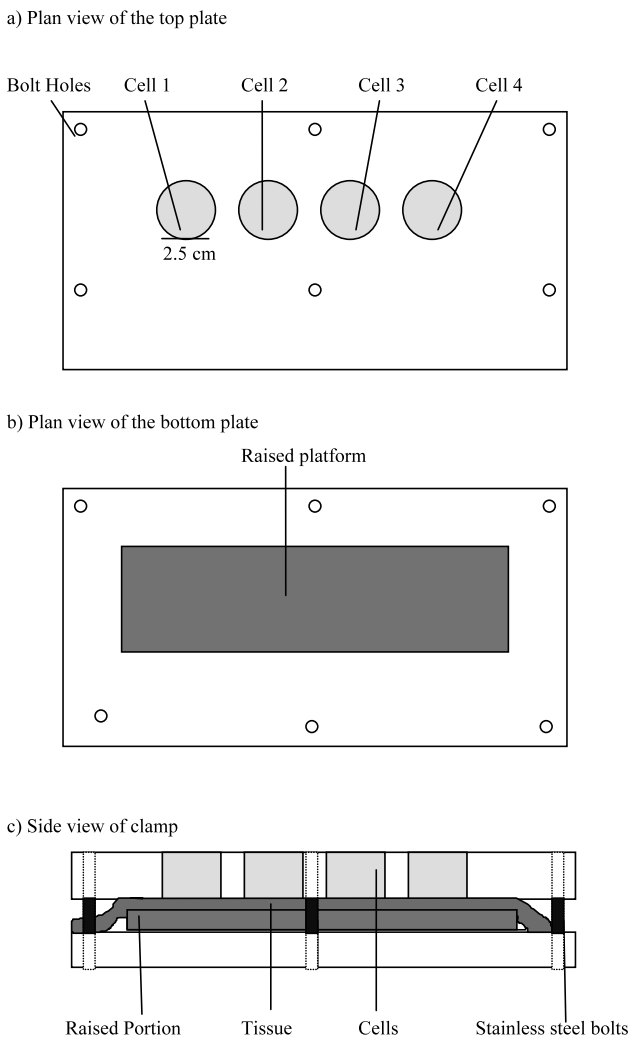
All reagents and solvents (analytical-grade) were purchased from Aldrich and, unless otherwise stated, were used as supplied. Scintillation fluid (Hionic Fluor) was purchased from Canberra Packard Ltd. The synthesis and characterisation of polymers used in this study are described elsewhere (Riley et al., 2001a), and some details of their properties are given in Table 1.

Pig stomachs, obtained fresh from a local abattoir, were gently rinsed with water to remove foodstuff. Strips, 4 cm by 15 cm, were cut from the lower pyloric region and the underlying muscle carefully removed. The strips were gently rinsed with a non-ionic isotonic aqueous solution (0.25 M sucrose), then flash-frozen in liquid nitrogen and stored at -20 °C. Preliminary studies revealed that freezing was essential for providing the flat surface essential for this study and to minimise the effects of enzymatic and bacterial degradation. Flash-freezing has been shown in

Table 1
Synthesised low, high and ultra-high poly(acrylic acid)s used in this study (from Riley et al., 2001a)

Polymer	Reaction details	Radioactivity (MBq/g)	Average molecular weight	[c]Tan δ at 1 Hz (1% aqueous solutions pH 6.2)
Low M_w	Aqueous (no cross-linker)	0.0193	[a]140 000	6.1
High M_w	Aqueous (no cross-linker)	0.0184	[a]2 960 000	0.7
Ultra high M_w	Toluene (cross-linker)	0.0252	[b]$10^6 < x < 10^9$	0.3

[a] Determined by gel permeation chromatography.
[b] Estimated from solution rheology.
[c] Tan δ (the loss modulus divided by the storage modulus) is an indicator of the viscoelastic nature of the material on oscillating at 1 Hz. The smaller the value of Tan δ, the more 'solid-like' the material is.



Cell area 4.91 cm^2; maximum cell volume 8.8 cm^3.

Fig. 1. Diagram of the perspex clamp system for assessing 'static' in vitro binding of polymer solutions.

previous work to minimise tissue damage (Young and Smart, 1998). Prior to use the tissue was defrosted by placing into an isotonic aqueous solution (0.25 M sucrose).

2.1.1. Polymer dispersion preparation

^{14}C-Poly(acrylic acid) (3.00 g) was dispersed overnight in distilled water (60 g), the pH was adjusted to 4.3 or 6.2 with sodium hydroxide solution (0.1–1 M), and the total weight made to 100.00 g with water. The dispersion was allowed to stand at 4 °C for 1 week before use to allow full hydration of the polymer to occur. The dispersions were carefully examined to confirm that there was no evidence of microbiological or other degradation prior to testing.

2.2. Preliminary study: ^{14}C-poly(acrylic acid) binding to gastric mucosa

A section of mucosal tissue from the pyloric region of the pig's stomach was washed gently with water from a running tap, then gently rinsed with aqueous sucrose solution (0.25 M). The tissue was clamped between two sheets of perspex, the upper containing a series of 4 wells, with just enough pressure to produce an efficient seal (Fig. 1). Polymer dispersions (4.0 g) were weighed and placed onto the clamped tissue. After 20 min at 37 °C the excess polymer was carefully removed using a syringe, the tissue rinsed thoroughly with sucrose solution (3 × 5 ml, 0.25 M) and the washings combined. The tissue was placed into sodium hydroxide solution (9 ml, 4 M) and left to dissolve (1 week) prior to counting. The combined washings were diluted to 20.0 g, a sample (0.6 ml) was placed into scintillation fluid (15 ml), mixed and left for 4-h before counting. The tissue samples were also made to 20.00 g with sodium hydroxide solution (4 M) and a sample (0.6 ml) taken and mixed with scintillation fluid for liquid-scintillation counting. A liquid scintillation analyser (Packard 2000CA Tri-CARB®) with quench curve correction was used to count the samples. For the presentation of results the dilution factors were multiplied by the sample count after subtraction of the background.

2.3. ^{14}C-Poly(acrylic acid) retention

The apparatus and procedure used were based on those previously described by Young and Smart (1998, 2000). Strips of porcine mucosa from the pyloric region were mounted onto the apparatus, mucosal side up and inclined at 30° from horizontal to expose a 15 mm by 120 mm section of mucosa (Fig. 2). An angle of inclination was selected in preliminary studies to allow opti-

mum discrimination between the retention of the applied solutions (although a range of inclination angles would be expected within the pyloric region of the stomach). Once clamped, the underside of the tissue was held in place by a gentle vacuum ($\approx$ 10 Torr). The tissue was allowed to equilibrate for 1-h at 37 °C under 100% humidity conditions and a constant flow of hydrochloric acid solution (0.01 M, 1 ml/min, 37 °C) a rate sufficient to continually wash the surface of the

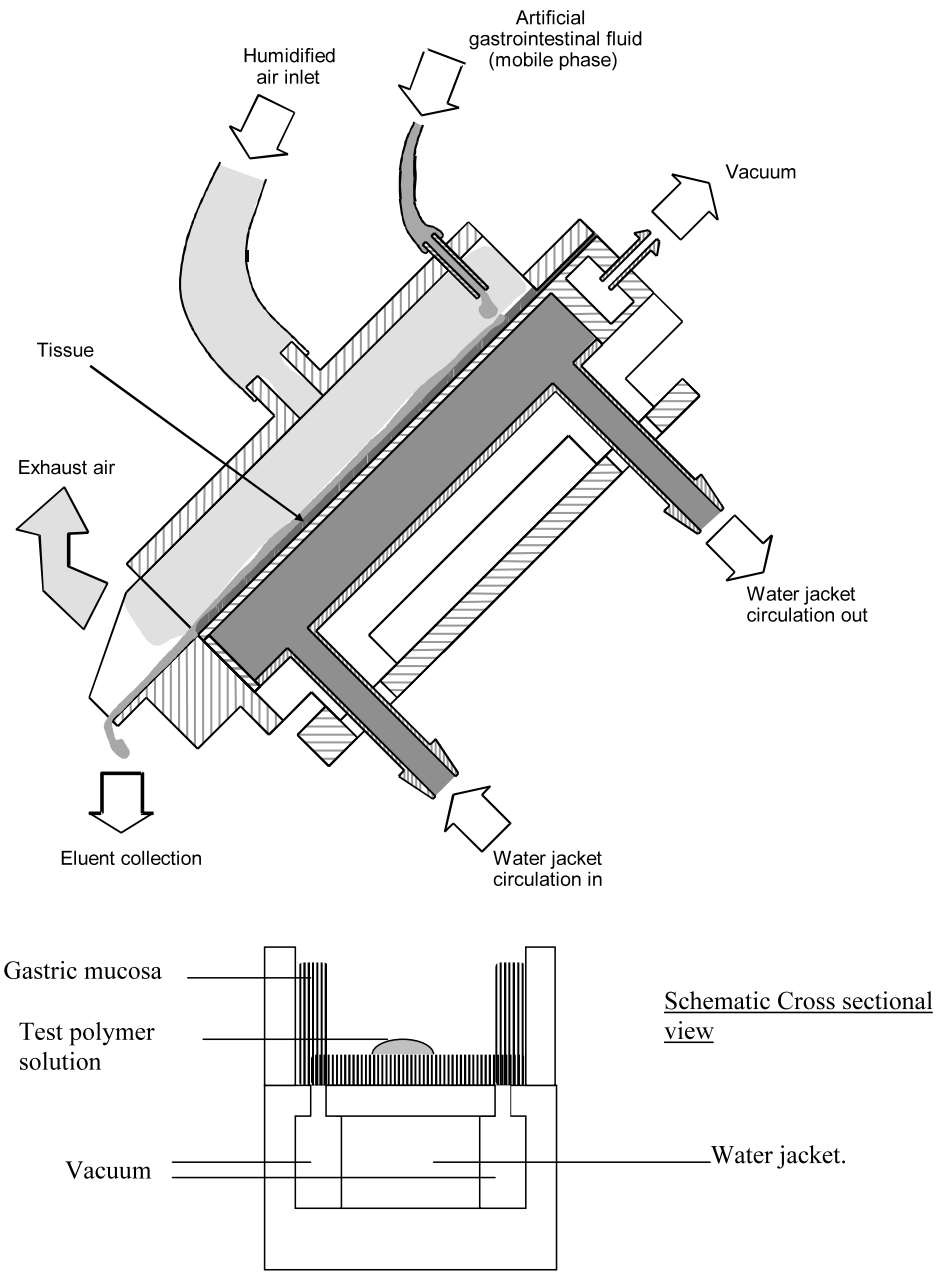


Fig. 2. Diagram of the 'dynamic' test apparatus for assessing in vitro binding and retention of polymer solutions.

Table 2
The binding of labelled poly(acrylic acid)s to gastric mucosa ($n = 8$)

Polymer	% Polymer bound	Weight polymer bound per area (mg/cm^2) (s.d.)
Low M_w	2.69	0.70 (0.04)
High M_w	4.64	1.1 (0.2)
Ultra high M_w	8.18	2.4 (0.4)

mucosa, mimicking the flow of gastric contents in the stomach. ^{14}C-Polymer dispersions (0.5 g, 3% w/w, pH 4.3) were applied (via a syringe) to the centre of the top 2 cm section of the tissue and fractions collected at 30 s intervals. The aqueous hydrochloric acid solution (0.01 M, 1 ml/min, 37 °C) was applied just behind the point of application of the test sample. Fractions eluted off the end of the platform were collected over a 1-h period. After this time the tissue was removed from the apparatus and cut horizontally (top to bottom) into six equal (2 cm) strips, which were dissolved separately in sodium hydroxide solution (4 M, 9 ml). All samples were analysed by scintillation counting. Scintillation fluid (15 ml) was added to each eluted fraction, mixed and left for 4-h before counting. The dissolved tissue samples were also made to 9.00 g with sodium hydroxide solution (4 M) and a sample (0.6 ml) was taken and mixed with scintillation fluid (15 ml) prior to liquid-scintillation counting. The dilution factors were multiplied by the sample count after subtraction of the background count to give the actual sample count, and the results expressed as a % of the total applied activity.

All statistics were completed using Minitab 13, the tests employed included one way analysis of variance and Tukey's multiple comparison tests.

3. Results

The initial 'static' polymer binding experiment revealed significant differences between the test and background controls for the three polymers tested ($P < 0.05$, one way analysis of variance) (Table 2), with the ultra high molecular weight polymer giving the greatest (8.18%), and the low molecular weight polymer showing the least, retention (2.69%).

The retention results for the 'dynamic' test system, in terms of the % activity remaining on the tissue (i.e. unrecovered in the eluent) at set time intervals are shown in Figs. 3 and 4.

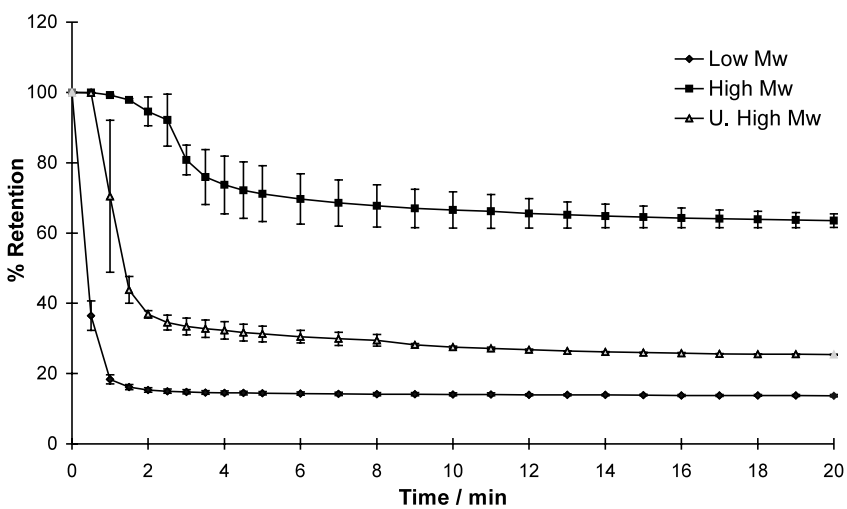


Fig. 3. Retention of low, high and ultra-high molecular weight radiolabelled polymer on pig pyloric gastric tissue (pH 4.3, 3% w/w, $n = 3$, bars = s.d.).

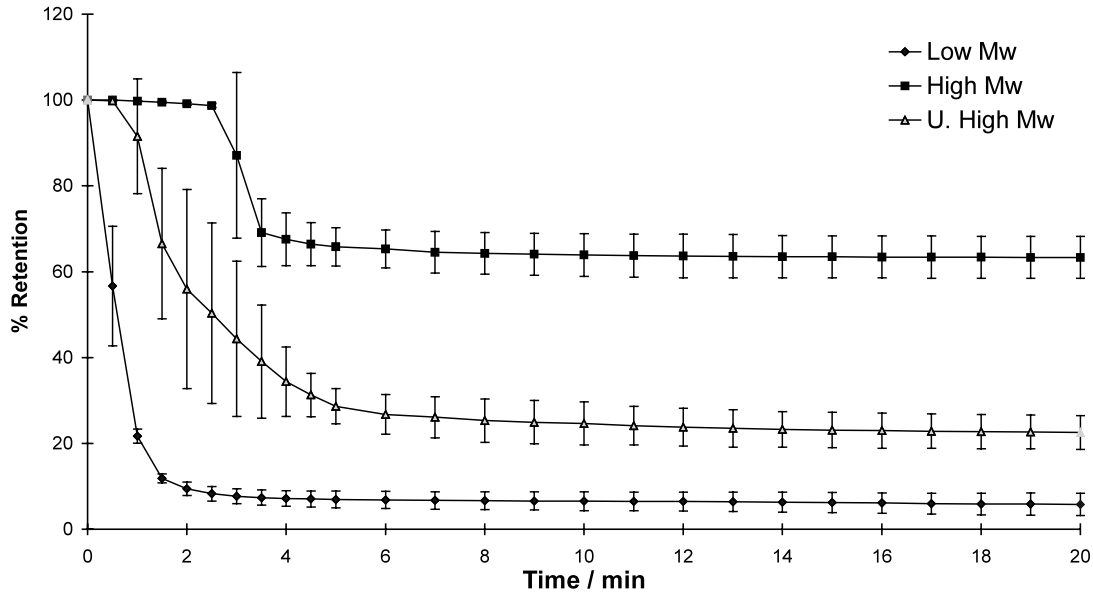


Fig. 4. Retention of low, high and ultra-high molecular weight radiolabelled polymer on pig pyloric gastric tissue (pH 6.2, 3% w/w, $n = 3$, bars = s.d.).

The low molecular weight polymer dispersion showed very low retention (Fig. 3), with less than 20% of the activity unrecovered after 2 min. The high molecular weight polymer dispersion exhibited a prolonged lag time (2.5 min) with little being recovered after 2 min, and only $\approx 30\%$ being recovered after 20 min. The ultra high molecular weight polymer exhibited a much shorter 'lag' phase (30 s) with over 60% of this material being recovered after 5 min.

Changing the pH of the three polymer dispersions resulted in only small changes in the retention profiles (Fig. 4).

The retention curves did not fit any single mathematical model for the data. Therefore, two component parts of the retention curves were identified, with an exponential decay mathematical model ($A_t = A_0 \cdot e^{-kt}$), (where A = retained% at time t, A_0 is % retained at time 0 (100%) and t = time (min)) being applied to each. The initial stages (first component of the retention profile) was considered to indicate 'fast' kinetics, which may be attributed to unbound polymer being lost from the tissue. Later stages (10–20 min, the second component) give a logarithmic straight-line relationship and are consistent with a slow 'decay' (polymer loss) mechanism. This may considered to represent elimination of 'bound' polymer from the surface. If both decay curves are added and plotted together, the original data points fit this new decay model, giving two separate half-lives (Fig. 5).

The two component retention model does not fit the very start of the curve, during the lag phase, for the high molecular weight polymer. From these model curves the half-lives and proportion of polymer remaining can be calculated. Table 3 shows the lag time, the half-lives (50% polymer), calculated percentage retained at 20 min and the counted percentage retained at 20 min. Extrapolated second decay curve half-lives are very long (≈ 110 min) and are not shown in the table.

3.1. Polymer distribution on the tissue surface after 1 h

Figs. 6 and 7 summarise the distribution of the three polymers at both pH's. At pH 4.3 the low molecular weight polymer was present on all sec-

tions but in very low concentrations just above background count levels. The high molecular weight polymer showed the most pronounced differences with the first three segments retaining significantly greater activity relative to the ultra high and low molecular weight polymers ($P < 0.05$, Tukey's Multiple Comparison test). Increasing the pH to 6.2 produced no significant differences ($P > 0.05$ one way analysis of variance) in polymer retention relative to the pH 4.3 experiment.

4. Discussion

The aim of this work was to investigate the in vitro polymer retention on gastric mucosa. The preliminary 'static' method looked at polymer binding to a defined surface area of mucosa, whereas the second dynamic system considered the distribution and retention of polymer dispersions on gastric mucosa when placed on an incline and washed with simulated gastric acid solution. In the preliminary study, all the polymers were

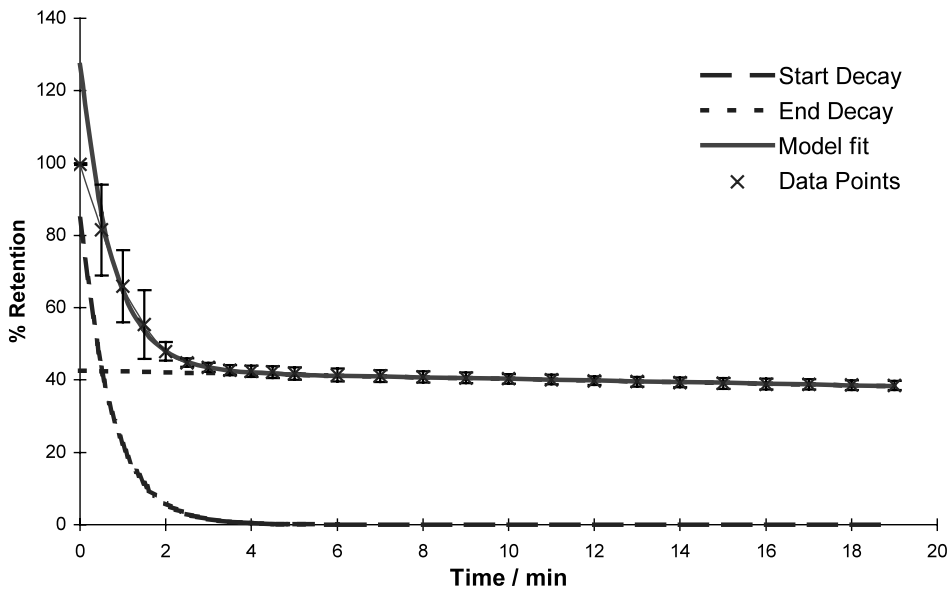


Fig. 5. Example of modelling of the experimental data in terms of two regions and an exponential fit ($n = 3$, bars = s.d.).

Table 3
Summary of the retention parameters calculated from mathematical modelling

Polymer	Concentration % w/w	pH	T_{lag}/min	Half life/min	Modelled % at 20 min	Retained % at 20 min
Pyloric tissue with polymer at pH 4.3						
Low	3	4.3	−0.23	0.21	13.67	13.71
High	3	4.3	1.81	69.88	63.21	63.49
Ultra high	3	4.3	0.28	1.27	24.14	25.39
Pyloric tissue with polymer at pH 6.2						
Low	3	6.2	0.02	0.44	5.81	5.77
High	3	6.2	2.29	122.25	62.61	63.35
Ultra high	3	6.2	0.51	2.329	22.49	22.54

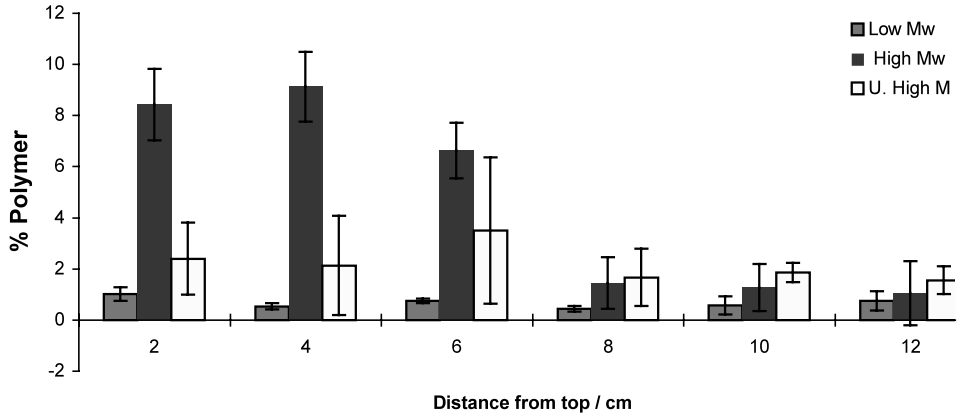


Fig. 6. Distribution of bound low, high and ultra-high molecular weight polymer on pig pyloric gastric tissue at pH 4.3 after 1-h ($n = 3$, bars = s.d.).

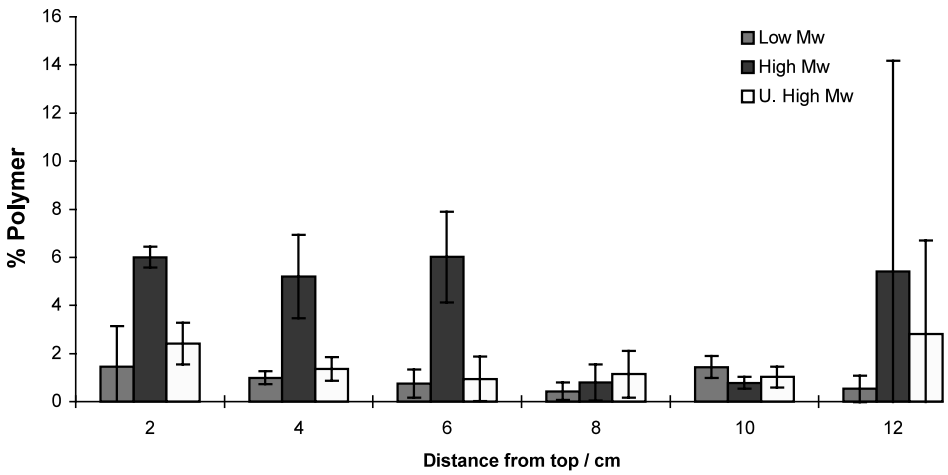


Fig. 7. Distribution of bound low, high and ultra-high molecular weight polymer on pig pyloric gastric tissue at pH 6.2 after 1-h ($n = 3$, bars = s.d.).

seen to bind to some extent, with the ultra-high molecular weight showing the greatest, and the low molecular weight polymer the least, binding. If the polymer and solvent were to bind to the mucosal surface together as a 3% dispersion, then this would give a calculated average thickness of the adhered layer of ≈ 3–4 mm for the high molecular weight polymer. A previous rheological synergism study, using these polymers at the same concentration and pH, showed little evidence of mucus polymer interaction for the low molecular weight polymer, unlike the high and ultra-high molecular weight polymers (Riley et al., 2001b). In other work employing these labelled polymers, the low molecular weight polymer was cleared more rapidly from the stomach of a rat, relative to the higher molecular weight polymers (Riley et al., 2001c). However, in this static system, clearly the efficiency of the 'rinsing off' of the polymer dispersions will be rheology dependent, with the most gel like/viscous polymers being the most difficult to remove. This is reflected in the data observed and may also be indicative of the situation in vivo.

The challenges presented by the second 'dynamic' in vitro test system were designed in part

to mimic those experienced in vivo, with the mucosa being inclined and washed by a model gastric acid solution. However, the presence of digestive enzymes, variable gastric contents, gastric motility, and varying surface inclinations were not considered, and could all be considered in further studies. The model did allow discrimination between the retentive properties of different polymers. The low molecular weight polymer was again readily removed, with little binding being evident. The greatest retention was seen with the high molecular weight polymer, particularly over the first 60 mm of tissue. Extrapolation of the data presented in Fig. 3 would suggest that retention would continue for several hours. However, after 1 h it is interesting to note that retained polymer detected was considerably less than that predicted from the extrapolated data, i.e. actually $\approx 25-30\%$ for the high molecular weight (Figs. 6 and 7) as opposed to 63% from extrapolating the modelling data (Table 3). This suggests that the mechanism of clearance will change between 20 min and 1 h, and this should be investigated in further work. It is not clear from this study if the polymer molecules actually adsorb onto the tissue from dispersion, or whether the retention is largely of the whole dispersion. If the former is the case the affinity of the polymer for the surface relative to the surrounding medium, along with the mobility of the polymer within the medium, will be key parameters for retention. If the latter is the case then surface properties (the interaction with the liquid on the mucosa), the rheological properties of the dispersion and the interaction of the dispersion with the surrounding medium would be key parameters. These factors will need to be considered in further work. The ultra-high molecular weight polymer dispersion was very gel-like, not allowing spreading and interaction of the polymer with the surface, and in fact it was observed to 'roll-up' and wash off the tissue with time. This may explain the differences between the preliminary 'static' and this 'dynamic' study where, in the latter, the retention of the ultra high molecular weight polymer was less relative to the high molecular weight polymer.

The pH of the initial polymer dispersion was seen to have little effect on retention. The pH of 4.3 and concentration of 3% was chosen as one that showed optimum mucus polymer interactions in a previous rheological synergy study (Riley et al., 2001b). Increasing the pH to, or just above, the pK_a of these poly(acrylic acid)s did not have a significant effect on tissue retention. Ionisable polymers are known to be very sensitive to solution pH, which, in turn, is know to profoundly affect both their rheology (Riley et al., 2001b) and mucoadhesion (Park and Robinson, 1985). The observed behaviour may be due to the polymer dispersion pH converging (lowering) in each case on exposure to the simulated gastric acid solution, in a similar manner to that proposed by Jackson et al. (2000) in vivo.

In conclusion, the in vitro test systems used in this study allowed the discrimination between the retentive properties of the different polymer dispersions. The results revealed that a polymer dispersion with sufficient mobility (i.e. not showing gel-like properties) to allow spreading and interaction with the mucosal surface, while having sufficient viscosity and/or bioadhesive properties to prevent dislodgement by the flow of simulated acid and gravitational effects, would give the most retentive formulation.

Acknowledgements

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