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Multifunctional polyvinylpyrrolidinone-polyacrylic acid copolymer hydrogels for biomedical applications

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Abstract

Copolymers of *N*-vinylpyrrolidinone and acrylic acid, crosslinked with ethylene glycol dimethacrylate and polyethylene glycol 600 dimethacrylate were prepared by UV-polymerisation. These polymers were analysed for their extractable content by Soxhlet extraction of the samples at 100 °C for 72 h. Aspirin and paracetamol were incorporated into the polymer structure at 25 wt.% during the curing process and their presence confirmed by Fourier transform infrared spectroscopy. It was found that the release rate of the drug from the polymer matrix was dependent on intermolecular bonding between the polymer and active agent with aspirin being released slower than paracetamol in all cases. Results showed that paracetamol was completely released after 24 h whereas complete release of aspirin took up to 70 h. Finally preliminary *in vitro* biocompatibility testing was performed for crosslinked polyvinylpyrrolidinone, by determining human hepatoma HepG2 cell viability in the MTT assay and DNA damage in the comet assay following direct contact with various concentrations of polyvinylpyrrolidinone-containing media. Cytotoxicity data suggests a dose-dependent effect for both crosslinkers, with concentrations in the range 0.025–2.5 mg ml^{-1} showing a marginal decrease in viability to, at most, 70% that of untreated cells. Again DNA migration in the comet assay following short-term exposure to EGDMA crosslinked hydrogels correlates with MTT data.

Keywords: Acetylsalicyclic acid; Paracetamol; Controlled release; Hydrogels; pH-responsive; Biocompatibility

1. Introduction

Hydrogels are becoming increasingly important materials for pharmaceutical applications. They are used in a variety of applications including diagnostic, therapeutic, and implantable devices such as catheters (Whitbourne, 1994) biosensors, artificial skin, controlled release drug delivery systems (Graham, 1990; Ravichandran et al., 1997; Risbud et al., 2000; Varshosaz and Koopaie, 2002; Akhgari et al., 2005) and contact lenses (Shoji et al., 1997). Hydrogels have been widely used in such applications because of their biocompatibility with the human body. In addition to this, hydrogels resemble natural living tissue more than any other class of synthetic biomaterial due to their high water content and soft consistency which makes them similar to natural tissue (Ratner and Hoffman, 1976).

The selection of hydrogels used in such pharmaceutical processes depends on the characteristics of the gel and on the application of the drug or protein. Hydrogels have several important characteristics that play an important role in drug diffusion including ionisation of the gel, swelling ratio, and specific mesh or pore size. Functional groups along the polymer chain can also react to the external environment for example temperature (Aikawa et al., 1998; Kono et al., 1999a,b), ionic strength (Peppas and Wright, 1998; Bales et al., 2000; Rodríguez et al., 2003) of the swelling agent (Ende and Peppas, 1996; Yaung and Kwei, 1997; Peppas et al., 2000; Bures and Peppas, 2000) or a combination of two or more factors (Jones, 1999; Alvarez-

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Lorenzo and Concheiro, 2002). Aikawa et al. (1998) investigated the effects of pH and temperature on hydrogel formation of polyvinylacetal diethylaminoacetate (AEA) and drug release from these polymers. Scanning electron microscope (SEM) observations suggested that AEA in solution becomes a hydrogel when the pH changes from 4 to 7.4, and that temperature change also accelerates network formation in the hydrogel.

The swelling ratio is also a very important parameter as it describes the amount of water that is contained within the hydrogel at equilibrium and is a function of the network structure, crosslinking ratio, hydrophilicity, and ionisation of the functional groups. Swelling ratio may be calculated from swelling studies and can be used to determine the molecular weight between crosslinks and the mesh size of the hydrogel. The mesh or pore size is the space available for drug transport (Peppas and Wright, 1998).

Many pharmaceutical systems are essentially made up of a polymeric carrier hosting the active agent inside a three dimensional network. They are often prepared as particulate systems, especially in the case of oral administration, since these forms present remarkable advantages over the single unit devices. The easier dispersion inside the stomach results in an appreciable reduction of local drug concentration that is usually responsible for gastric irritation (Grass et al., 2000). The use of hydrogels as carriers for these active agents has been studied, as well as methods for controlling their release. Ravichandran et al. (1997) studied a polyvinylpyrrolidinone-acrylic acid-polyethylene glycol copolymer and performed drug release experiments in simulated gastric fluids. It was found that the drug was released in an ordered fashion, and that modification of the crosslink density of the polymeric matrix could be used to achieve desirable drug release profiles. Lyons et al. (2006) compared the use of fillers within the polymer matrix to slow drug dissolution and reduce the cost of the overall drug delivery system. It was found that agar significantly reduced the release rate of the active agent, and as agar is biocompatible and relatively cheap it would have potential in commercial products. Friend (2005) puts forward a review of several methods of controlling the release of active agents in the gastro intestinal tract. Some of these methods include time-based delivery systems, pH based systems using enteric coatings, and combinations of both amongst others. Akhgari et al. (2005) also discusses the use of polymer coatings over the pellets that contained drug. It was also found that these coatings delayed the release of the active agent, thus allowing site specific drug release. Varshosaz and Koopaie (2002) analysed the release of an active agent from a crosslinked PVOH polymer and found that the crosslink density of the hydrogel affected the release of the drug used, i.e. there was a significant decrease in drug release as the percentage of crosslinking agent was increased. However, Devine et al. (2005) combined the use of a hydrogel for use as a lubricious coating with its ability to carry drug, and analysed the drug release from a lubricious coating with potential as a medical device coating. It was found that by varying the length of the crosslink chains the release of the active agent could by varied.

This work is a continuation of the work on the development of a novel co-polymer for use in biomedical applications. Here a series of PVP-PAA copolymers were analysed for their potential as a multifunctional hydrogel. Soxhlet extraction was carried out to determine the extractable content of the hydrogels and to ascertain which co-polymer composition may prove useful as a drug delivery device. The encapsulation of both aspirin and paracetamol within the polymer matrix was shown using Fourier transform infrared spectroscopy and the release of these active agents was determined using the USP XXV Basket method for drug dissolution. Selected hydrogels were subjected to preliminary cytotoxicity and genotoxicity testing to establish their suitability for use in the body.

2. Experimental

2.1. Preparation of samples

The hydrogels investigated in this work were prepared by free-radical polymerisation. The monomers used were *N*-vinylpyrrolidinone (NVP, Lancaster synthesis) and acrylic acid (AA, Merck-Schuchardt, Germany). The polymers tested had monomeric feed ratios of 100 wt.% NVP, 90 wt.% NVP/10 wt.% AA, 80 wt.% NVP/20 wt.% AA and 70 wt.% NVP/30 wt.% AA. These polymers were analysed both with and without the incorporation of crosslinking agents. The crosslinking agents used in this work were using ethylene glycol dimethacrylate (EGDMA) and polyethylene glycol dimethacrylate with a molecular weight of 600 (PEG600DMA, Sigma–Aldrich) at various weight percentages of the total monomer content. Both monomers and crosslinking agents were used as received. To initiate the reactions, 1-hydroxycyclohexylphenylketone (Irgacure® 184, Ciba speciality chemicals) was used as a UV-light sensitive initiator at 3 wt.% of the total monomer weight. This was added to the NVP/AA monomeric mixture and stirred continuously until completely dissolved. The solution was pipetted into a silicone mould (W.P. Notcutt, Middlesex) that contained disk impressions and rectangular impressions for use in Fourier transform infrared spectroscopy (FTIR). The mould was then positioned horizontally to the gravity direction under two UVA 340 UV lamps (Q-panel products) and the solution was cured for approximately 1 h in an enclosed environment. The films were dried in a vacuum oven at 40 °C, 500 mmHg for 24 h prior to use.

Prior to biocompatibility testing individual hydrogel discs were weighed and dissolved in an appropriate volume of complete culture medium to give a final concentration of 25 mg ml^{-1}. Following incubation overnight at 37 °C, and brief vortexing, hydrogel suspensions were filter sterilised (0.2 μm pore size) and subject to serial dilution in complete culture medium to give the concentration range 25–0.025 mg ml^{-1}.

2.2. Soxhlet extractions

Soxhlet extractions were carried out on UV cured samples crosslinked with varying amounts of either EGDMA or PEG600DMA. The test was carried out by placing a preweighed circular disk with an average weight of 0.99 g into a 25 mm × 80 mm Soxhlet thimble. The thimble was then placed into a 100 ml capacity Soxhlet extraction apparatus, and the test

was carried out in distilled water at 100 °C for 72 h. After the 72 h test period had elapsed the samples were removed from the thimbles and dried in an oven at 80 °C for a minimum of 48 h. The samples were then re-weighed so as to calculate the extractable content of the hydrogel.

2.3. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy was carried out on UV polymerised rectangular samples with and without 25 wt.% of aspirin or paracetamol. The samples were exposed to atmospheric conditions for a minimum of 7 days prior to testing. The tests were carried out using a Nicolet Avator 360 FTIR, with a 32 scan per sample cycle.

2.4. Drug dissolution

Dissolution studies were conducted on various polymers, in 900 ml of 0.2 M buffered solutions at various pH values, i.e. pH 2, 6.8 and 9, using a Sotax® on-line dissolution apparatus. Potassium chloride, monobasic potassium phosphate, and boric acid/potassium chloride were used to prepare pH buffers pH 2, pH 6.8, and pH 9, respectively. The test was carried out in triplicate using the Basket method (USP XXV) at 37 ± 0.5 °C at 100 rpm. At predetermined time intervals, samples were withdrawn automatically, filtered and passed through a Perkin-Elmer Lambda 20 UV/vis spectrometer, before being returned to the bowl. The wavelength and absorption of a 100% drug concentration for each drug and pH value were determined in triplicate using a Perkin-Elmer Lambda 40 UV/vis spectrometer. The average value was entered into software calculations prior to commencement of testing. The drugs used in this experiment were aspirin and paracetamol. In previous work (Devine and Higginbotham, 2005) it was found that the crosslinked polymers containing 20–30 wt.% acrylic acid had water uptake values greater then 1000% when swelled in buffered solutions of pH 7 and 9.2. Therefore drug release of crosslinked samples that contained acrylic acid was carried out in pH 2 only.

2.5. Cytotoxicity and genotoxicity testing

2.5.1. MTT assay

HepG2 cells were seeded at 1×10^4 cells per 100 μl per well and microtitre plates incubated overnight at 37 °C. Following subsequent exposure for 3 or 24 h, hydrogel-containing culture medium was replaced with fresh culture medium supplemented with MTT at a final concentration of 0.05 mg ml^{-1} for 4 h at 37 °C. After careful removal of MTT medium, blue formazan crystals were solubilised in 100 μl per well acidified isopropanol and microtitre plates agitated for 10 s at medium intensity prior to recording optical densities at 560 nm using an Anthos (htIII) microplate reader (Mosmann, 1983; Vistica et al., 1991).

2.5.2. Single cell gel electrophoresis (SCGE) or comet assay

Duplicate wells of a 24-well culture plate were seeded at 2×10^4 cells ml^{-1} in 2 ml aliquots and following overnight incubation at 37 °C culture medium was replaced with various concentrations of hydrogel-containing culture medium for a further 3 or 24 h. Subsequently the comet assay procedure of Klaude et al. (1996) was employed, with slight modifications, to detect DNA strand breakage in individual cells. Cells were embedded in 1% LMP agarose on gel bond electrophoresis film and subject to lysis, alkaline unwinding and electrophoresis at high pH. Following ethidium bromide staining (10 μg ml^{-1}) DNA migration was measured using image analysis software (Comet II, Perceptive Instruments, UK) and expressed as a function of the 'tail moment' parameter, a product of the fluorescence intensity of the tail and the extent of migration.

3. Results and discussion

3.1. Preparation of samples

Copolymers of both NVP and NVP/AA were photopolymerised using Irgacure® 184 as a photoinitiator. These samples were cured on a silicone moulding, and prior to use dried for 24 h in a vacuum oven. Visual inspection of the samples prepared in this study showed no significant differences to those prepared in previous work (Devine and Higginbotham, 2003).

The addition of aspirin did not affect the curing rate of the polymer; however, it was found that paracetamol slowed the curing process. Therefore longer curing times and longer drying periods in the vacuum oven were used for samples containing paracetamol. It was also observed that in samples containing 30 wt.% acrylic acid, paracetamol did not appear to fully dissolve prior to curing, but was instead dispersed within the monomeric solution.

3.2. Soxhlet extractions

Soxhlet extractions were carried out using distilled water as the solvent for 72 h, in order to quantify the effect of crosslinking agent incorporation on the extractable content of the co-polymer.

From the results illustrated in Figs. 1 and 2 it is observed that the samples which contained a monomeric concentration of both 100 wt.% NVP had lost at least 80 wt.% of their total weight and samples that contained 90 wt.% NVP/10 wt.% AA had lost at least 60 wt.% of their total weight during extraction, irrespective of the crosslinking agent content. Thus, indicating that these samples did not warrant further study, as the weight loss was too great. This weight loss was due to a lack of intermolecular bonding (Devine and Higginbotham, 2003), as the AA content in the samples was 0 and 10 wt.%, respectively. However, it can be seen from the data obtained that as the crosslinking agent content increased there was a corresponding decrease in the extractable content of the polymer. Hong et al. (1996) using a similar polymer system also found that as the crosslinking agent content increased, the polymer changed from that of a 'homogeneous solution' at 0.1 wt.% to a 'transparent material, with minimal fragmentation' at 1 wt.% crosslinking content.

From analysis of the samples that contained both 80 wt.% NVP/20 wt.% AA and 70 wt.% NVP/30 wt.% AA it was found that only a slight difference in solubility was observed with com-

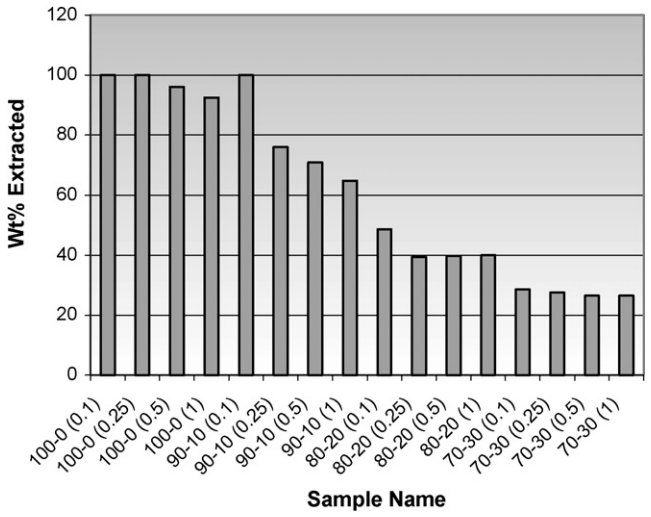

Fig. 1. Extractable content of hydrogels crosslinked with varying concentration of EGDMA, where 100–0 (0.1) represents samples whose composition was 100 wt.% NVP, 0 wt.% AA crosslinked with 0.1 wt.% EGDMA.

parison to the crosslinking agent content. It was also found that the 70 wt.% NVP/30 wt.% AA polymers exhibited greater stability over the duration of the test as the recorded weight loss was approximately 28 wt.% compared with approximately 40 wt.% for the 80 wt.% NVP/20 wt.% AA samples. These results show good correlation with results described in pervious work (Devine and Higginbotham, 2003, 2005), where it was found that an increase in AA content yielded an increase in intermolecular bonding, which in turn decreased solubility of the polymer, even without crosslinking content. The results achieved in this work exceed the values illustrated by Nho and Park (2002), who performed a similar test at 70 °C for approximately 48 h on PVOH/PVP–chitosan copolymers. From results stated it was found that the polymers tested for wound dressing applications had weight losses in excess of 40%.

When the hydrogels produced in this work are compared with reference to the molecular weight of the crosslinking agent it was found that the samples crosslinked with EGDMA exhibited a slightly higher solubility profile in comparison to samples crosslinked with PEG600DMA. This also correlates with results described in previous work (Devine and Higginbotham, 2005), where it was found that the comparative gel strength of the copolymers increased with an increase in the molecular weight of the crosslinking agent.

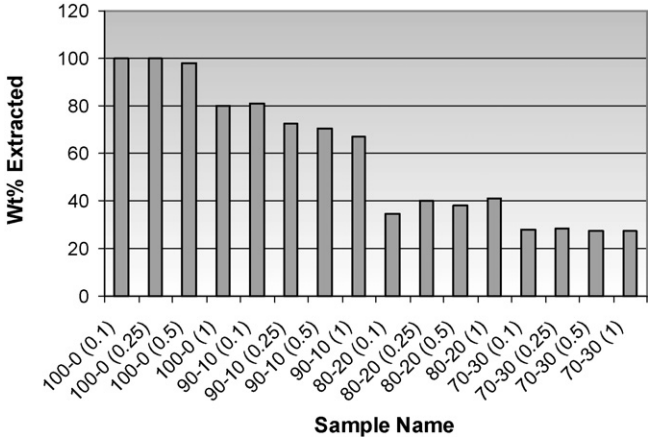

Fig. 2. Extractable content of hydrogels crosslinked with varying concentration of PEG600DMA, where 100–0 (0.1) represents samples whose composition was 100 wt.% NVP, 0 wt.% AA crosslinked with 0.1 wt.% PEG600DMA.

3.3. Fourier transform infrared spectroscopy

FTIR experiments were carried out in order to determine the effect that the addition of an active agent had on the UV cured polymers. The polymers tested consisted of a monomeric mixture of NVP, AA and in some cases a crosslinking agent.

The FTIR spectra of the polymers examined herein have been studied extensively (Devine and Higginbotham, 2003, 2005; Devine et al., 2005), and therefore will not be discussed here. However from comparison of the IR spectra of the polymers (Fig. 3), and of the polymers containing an active agent, it was found that the presence of paracetamol within the polymer structure was confirmed by the presence of characteristic peaks in the region of 1512 cm^{-1} attributed to C–H symmetric bonds. More importantly a peak attributed to N–H in plane deformation was observed in the region of 1554 cm^{-1}. The significance of this peak is attributed to the fact that no other compound used in this work contains an N–H bond (Moynihan and O'Hare, 2002).

The IR spectra of the samples that contains aspirin, gave evidence that the carboxylic acid group of aspirin formed a dimer similar to that observed where the carboxylic acid group of acrylic acid formed a dimer by hydrogen bonding to an adjacent carboxylic acid group (Devine and Higginbotham, 2005). This is observed as a shoulder/peak on the main PVP carbonyl peak in the region of 1708 cm^{-1}. This peak was not evident in the samples containing 100 wt.% PVP with or without paracetamol added. With the addition of acrylic acid a shoulder did appear in the spectrum of the polymer without aspirin as was found previously. However the magnitude of this peak increased with the addition of aspirin, signifying that hydrogen bonding occurred between the carboxylic groups of both aspirin and acrylic acid.

3.4. Drug dissolution

Dissolution studies and the release of aspirin and paracetamol were done in triplicate at pH values of pH 2, pH 6.8 and pH 9, using a Sotax® on-line dissolution apparatus. The wavelength and absorption of 100% drug concentration for each drug and pH value were determined using a Perkin-Elmer Lambda 40 UV/vis spectrometer. These values were entered into software calculations prior to commencement of testing, and an E11 value was calculated. The drugs used in this experiment were aspirin and paracetamol.

From the dissolution results obtained, it was found that for all polymers analysed at each pH value aspirin was released slower than paracetamol. In this work it was found that at pH 2, 100 wt.% PVP had released its paracetamol content after approximately 1 h 20 min. However, total aspirin release was not observed until approximately 48 h. With the incorporation of 30 wt.% AA, the release of both active agents had slowed

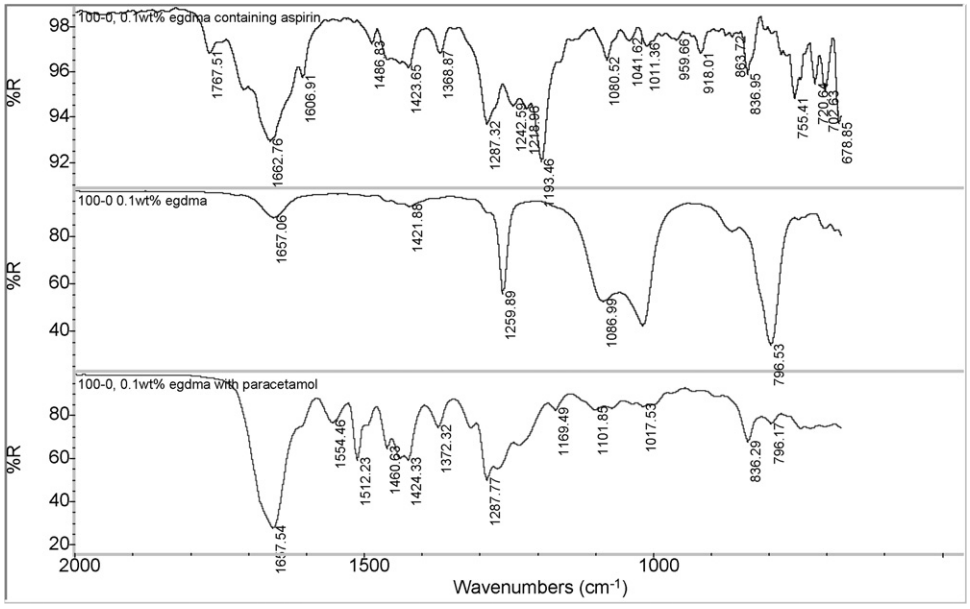

Fig. 3. FTIR spectrums of samples containing 100 wt.% PVP polymer crosslinked with 0.1 wt.% EGDMA crosslinking agent. From top to bottom: polymer containing 25 wt.% aspirin, polymer with no active agent and polymer containing 25 wt.% paracetamol.

with paracetamol release reaching a maximum after approximately 24 h; however the release of aspirin was also prolonged, up to 70 h. The release of the active agents at pH 6.8 and 9 followed a similar trend to the release of the active agents at pH 2. 100 wt.% PVP had released its paracetamol content after approximately 1 h 10 min and 1 h 40 min and total aspirin release was observed after approximately 11 and 14 h for pH 6.8 and 9, respectively. The addition of 30 wt.% AA again prolonged the controlled release of both active agents. In this instance paracetamol was released after approximately 5 h at both pH 6.8 and 9, and aspirin was released after approximately 17 and 22 h at pH 6.8 and 9, respectively. Hydrogen bonding between the carboxylic acid group of aspirin and the carboxylic acid group of acrylic acid in the copolymer is believed to be the primary cause of the retardation of the aspirin release in comparison to paracetamol.

Fig. 4 illustrates the release profile obtained by both aspirin and paracetamol, above and below the $pK_{initial}$ of acrylic acid (4.07–4.49) (Devine and Higginbotham, 2003). It can be seen that at pH 2, samples containing aspirin, which has a pK_a of 3.49, released the active agent in a slow and sustained manner after an initial burst release of the drug. The incorporation of acrylic acid further slows the release of the active agent, as a higher degree of physical crosslinking is achievable via the carboxylic acid groups of both acrylic acid and aspirin. At pH 9, it was also found that the release of aspirin was faster than that achieved at pH 2. This was expected as above the $pK_{initial}$, the carboxylic acid groups are ionized, and therefore hydrogen

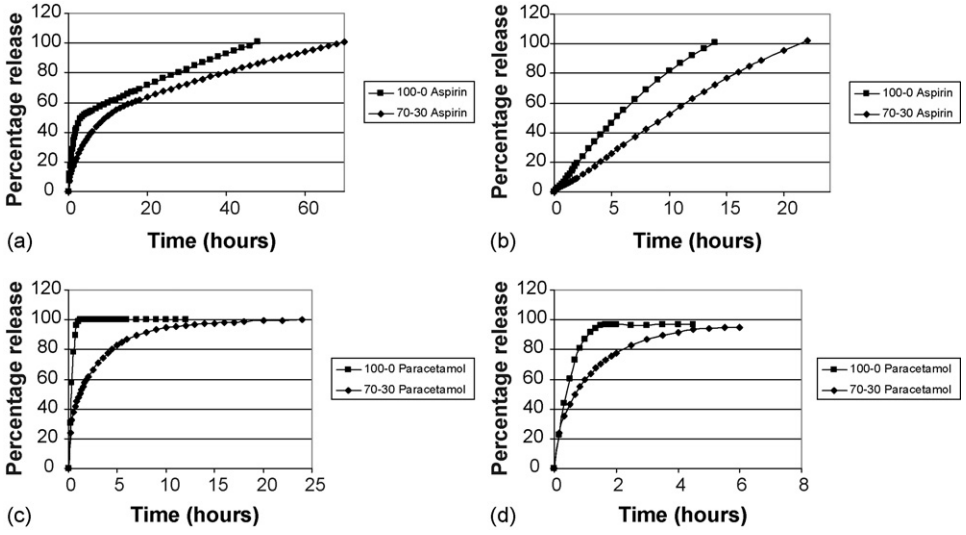

Fig. 4. Dissolution curves illustrating the effect of acrylic acid on the release rate of both active agents: (a) aspirin release at pH 2; (b) aspirin release at pH 9; (c) paracetamol release at pH 2; (d) paracetamol release at pH 9.

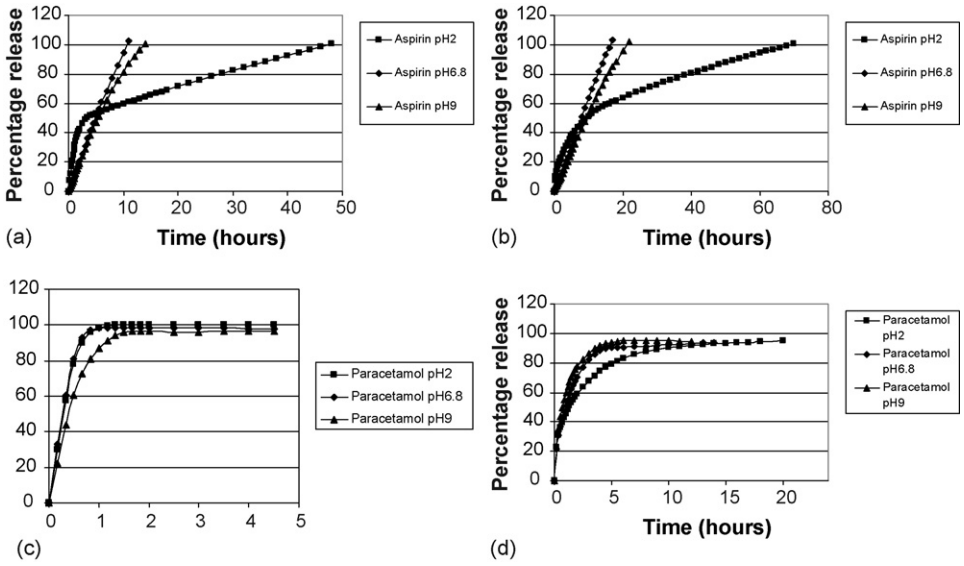

Fig. 5. The release profile of aspirin and paracetamol at various pH values: (a) 100–0 aspirin; (b) 70–30 aspirin; (c)100–0 paracetamol; (d) 70–30 paracetamol.

bonding is not readily achievable. The release profile observed for samples containing paracetamol, which has a pK_a of 9.35, shows that without the incorporation of acrylic acid, the active agent is released relatively quickly at both pH values. With the incorporation of acrylic acid, paracetamol release slows down at both pH values; however at pH 2 the difference in release rate is far more pronounced. These release profiles can be explained by virtue of the fact that paracetamol is not as likely to form hydrogen bonds as aspirin, either to itself or to the monomers used. Therefore without acrylic acid the release of the active agent is dependent on the dissolution rate of the polymer and is not affected by the pH of the dissolution media. With the incorporation of acrylic acid a more physically crosslinked structure is achieved below the p$K_{initial}$ and therefore a slower release rate is observed. The release profiles obtained for paracetamol also showed a reduction in the release rate at all pH values as the acrylic acid content was increased, however in this case the slowed release rate was due to increased bonding in the polymer only and not polymer/drug intermolecular bonding.

Fig. 5 illustrates the release profile of both active agents in relation to change in the pH value of the dissolution media. On examination of the release profiles observed for aspirin (Fig. 5a and b), it was found that below the p$K_{initial}$ of acrylic acid and the pK_a of aspirin, i.e. pH 2, where a relatively high level of hydrogen bonding is obtainable, the release rate of aspirin is relatively slow. However samples containing acrylic acid yielded a slower release profile due to increased hydrogen bonding. It was found that the release profile at both pH 6.8 and 9 were similar. Above the p$K_{initial}$ of AA and the pK_a of aspirin, respectively, the carboxylic acid groups are ionized, and therefore hydrogen bonding between these groups is not readily possible. Therefore, the polymers tested would not have a high level of physical crosslinks at either pH value, thus the release profiles achieved would be similar.

Fig. 5c and d also illustrates the effect that the pH of the dissolution media has on the release profile of paracetamol. It was found that the polymers synthesised from a monomeric feed ratio of 100 wt.% NVP, the release profile observed at pH 2 and 6.8 are similar, however the profile obtained at pH 9 is slower. The pK_a of paracetamol is 9.35, therefore ionisation occurs at pH values higher than 9.35 and ionic bonds are formed, thus slowing the release of the active agent. With the incorporation of acrylic acid the overall pH of the system would be acidic. Therefore above the p$K_{initial}$ of AA the release profile observed was similar, while below the p$K_{initial}$ of AA the release rate of the active agent slowed down due to the physical crosslinks within the polymer.

Chemical crosslinking agents were incorporated into the monomeric feed ratio to determine their effect on the release rate of the active agent. Samples having a monomeric feed ratio of 70 wt.% NVP and 30 wt.% AA were used in this experiment. Drug release from these crosslinked samples were carried out in

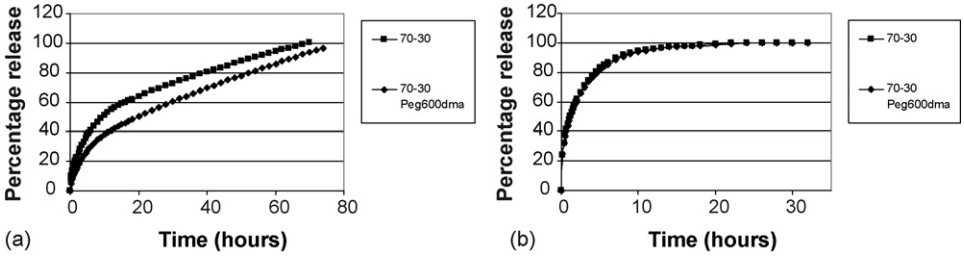

Fig. 6. The effect of crosslinking agent on the release rate of the active agents: (a) 70–30 aspirin at pH 2; (b) 70–30 paracetamol at pH 2.

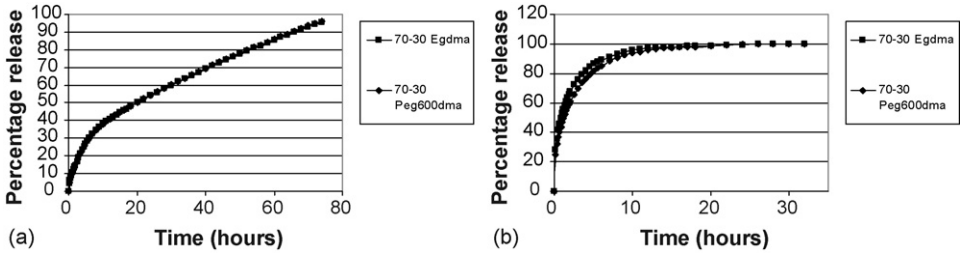

Fig. 7. The effect of varying the molecular weight of the crosslinking agent on the release rate of the active agents: (a) 70–30 aspirin at pH 2 crosslinked; (b)70–30 paracetamol at pH 2 crosslinked.

pH 2 media only as these samples could swell to over 1000% in a pH above the $pK_{initial}$ of acrylic acid. From Fig. 6 it can be seen that with the incorporation of PEG600DMA, the release rate of aspirin is slowed down. This may be due to the crosslinks holding the molecular chains more tightly together, thus allowing more hydrogen bonding to occur. The release from paracetamol was similar to that obtained without the incorporation of crosslinking agent.

Fig. 7 illustrates the release profiles obtained when the molecular weight of the crosslinking agent is altered. It was found that the molecular weight of the crosslinking agent did not have an effect on the release profile as observed at pH 2. It is believed that at pH 2 the molecular chains of the hydrogel were held tightly together with physical crosslinks, therefore the molecular weight of the crosslinks had little overall effect.

3.5. Cytotoxicity and genotoxicity testing

A dose-dependent decrease in HepG2 cell viability was observed in the MTT assay for 100 wt.% PVP hydrogels crosslinked with PEG600DMA (PVP × PEG600DMA). This effect was most pronounced at concentrations >2.5 mg ml^{-1} with a PVP concentration of 25 mg ml^{-1} reducing cell viability to approximately 10% of untreated control cells, independent of exposure time (Fig. 8). While PVP × PEG600DMA hydrogel concentrations in the range 0.025–2.5 mg ml^{-1} also decreased cell viability, such reductions in viability were no greater than 70% of untreated cell values after either 3 or 24 h exposure (Fig. 8). Conversely 100 wt.% PVP hydrogels crosslinked with EGDMA (PVP × EGDMA) produced rather conflicting results at concentrations ≥2.5 mg ml^{-1} (Fig. 8). Essentially, following 24 h exposure a reduction in cell viability was observed at all PVP × EGDMA hydrogel concentrations in the range 0.025–25 mg ml^{-1}, again in keeping with the results obtained for PVP crosslinked with PEG600DMA. This decrease was most pronounced at 25 mg ml^{-1}, representing a reduction in cell viability to 30% of untreated cell values. In contrast, short-term 3 h exposure seemed to exert a slight proliferative effect on HepG2 cell growth at NVP × EGDMA hydrogel concentrations ≥2.5 mg ml^{-1} (Fig. 8).

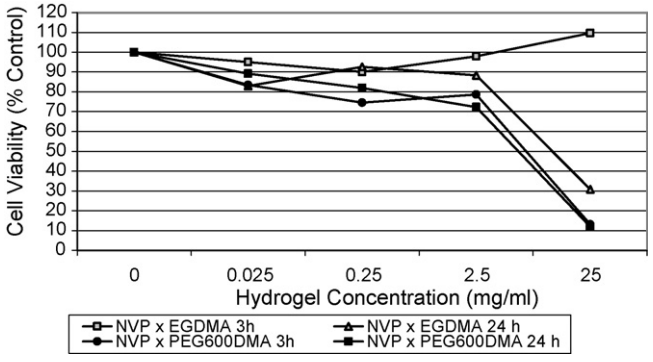

Fig. 8. Effect of NVP-based hydrogels crosslinked with either ethylene glycol dimethacrylate (EGDMA) or polyethylene glycol 600 dimethacrylate (PEG600DMA) on HepG2 cell viability in the MTT assay after 3 and 24 h exposures at 37 °C. Each data point represents the mean of three separate experiments ($n = 18$).

Overall such effects on HepG2 cell viability, in response to direct contact or incubation with PVP-containing media, did not correspond to alterations in HepG2 cell epithelial morphology as observed by light microscopy (Fig. 9).

Interestingly such results are somewhat similar to those obtained by Smith et al. (2006), whereby PVP hydrogels crosslinked with EGDMA reduced the viability of human dermal fibroblasts in direct contact studies while promoting the growth of such cells when there is indirect contact. Indeed the growth promoting effects of NVP × EGDMA has also been reported by others, albeit during indirect contact but in a polymer concentration-dependent manner independent of serum (Hong et al., 1997).

Despite the fact that hydrogels, including those synthesised from NVP, are considered biocompatible it is possible that decreased viability observed for HepG2 cells may be the result of high concentrations of unreacted monomer, oligomers, initiator or indeed methacrylates (Peppas et al., 2000). Long-term animal toxicity studies have previously shown the liver and upper respiratory tract as the main targets for NVP toxicity (Klimisch et al., 1997a,b). Furthermore, methacrylates tend to be less cytotoxic than acrylates, although this appears to be structure-related and dependent upon the length of oxyethylene chains. The mutagenic effects of acrylate esters have also been documented (Yoshii, 1997; Dearfield et al., 1989; Schweikl and Schmalz, 1999). However it is inappropriate at such an early stage in the biocompatibility assessment of NVP-based hydrogels crosslinked with either PEG600DMA or EGDMA to comment on the exact mechanisms underlying the decreased cell viability and proliferative effects observed. Suffice to say that future *in vitro* biocompatibility studies on PVP hydrogels will focus on the independent effects of NVP monomer and the

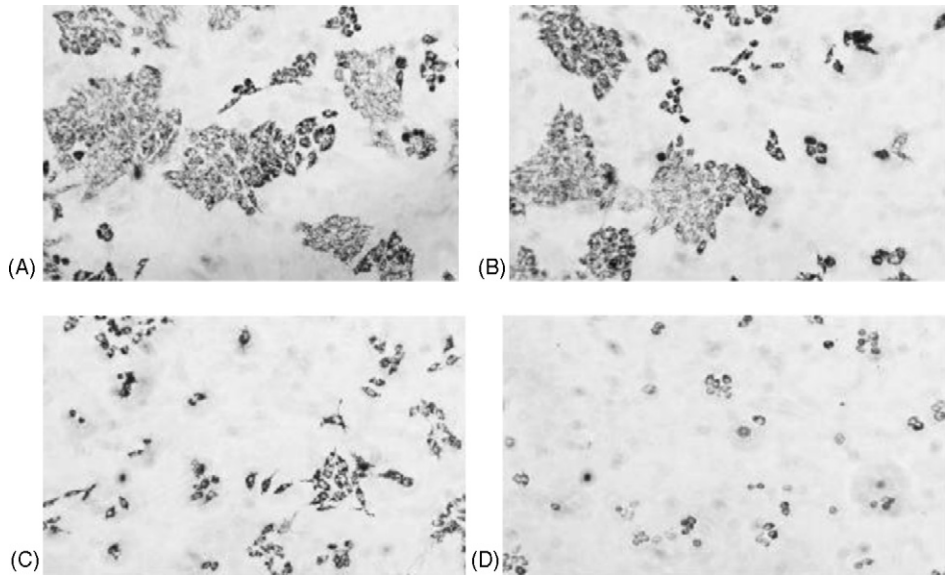

Fig. 9. HepG2 cell reduction of MTT tetrazolium salt following culture for: (A) 24 h in Hepes modified DMEM + Hams F-12 (1:1) complete culture medium (negative control); (B) 3 h in the presence of 0.025 mg ml^{-1} poly(NVP) crosslinked with EGDMA; (C) 24 h in the presence of 25 mg ml^{-1} poly(NVP) crosslinked with EGDMA; (D) 24 h in the presence of 25 mg ml^{-1} poly(NVP) crosslinked with PEG600DMA (magnification 200×).

different crosslinkers using extract dilution, direct and indirect contact assays as recommended by ISO guidelines (ISO10993-5: 1999). Moreover, the exact nature underlying any cytotoxic or growth promoting effects can be determined by specific apoptosis/necrosis and proliferation assays where appropriate.

Preliminary results for PVP crosslinked with EGDMA in the alkaline comet assay suggest a four- to six-fold increase in HepG2 cell DNA single strand breaks in the range 0.25–25 mg ml^{-1} compared to untreated cells following 3 h exposure, as outlined in Table 1 and illustrated in Figs. 10 and 11. Indeed such increases in DNA migration correlate with the cytotoxic and proliferative effects observed for PVP crosslinked with EGDMA following short-term exposure in the MTT assay (Fig. 8). In effect, the lowest levels of DNA migration correspond with the slight decreases in cell viability observed over a similar concentration range of 0.025–0.25 mg ml^{-1} (Figs. 10 and 8, respectively). Moreover the slightly higher levels of DNA migration at concentrations ≥2.5 mg ml^{-1}, as illustrated in Fig. 10, are compatible with the proliferative effects also observed at such concentrations in the MTT assay (Fig. 8). This may be attributed to the fact that sites of active DNA replication can also lead to increased DNA migration and tail moment values in the alkaline comet assay (Salagovic et al., 1997).

The comet assay has been previously demonstrated as a fast, sensitive technique for determining the genotoxic potential of biomaterials (Chauvel-Lebret et al., 2001; Kleinsasser et al., 2006). However future, more robust, application of the comet assay to investigations of the genotoxic potential of PVP-PAA hydrogels and crosslinking agent effects will employ both positive and negative control agents in addition to further optimisation of the most important parameters that affect assay outcome such as lysis, alkaline unwinding and electrophoresis times to ensure maximum sensitivity and specificity (Tice et al., 2000).

Table 1
Sample comet assay data for HepG2 cells exposed to poly(NVP) crosslinked with EGDMA for 3 h at 37 °C

Poly(NVP) crosslinked with EGDMA (mg ml^{-1})	Mean tail moment[a] (±S.E.M.)
0	0.768 ± 0.165 (1.0)
0.025	1.106 ± 0.268 (1.4)
0.250	3.270 ± 0.815 (4.3)
2.500	3.570 ± 0.678 (4.7)
25.000	4.61 ± 1.762 (6.0)

[a] Values are the mean ± the standard error of the mean from a single experiment in which 50 single cells were analysed per concentration. Values in parentheses represent tail moment values expressed as a multiple of the untreated control.

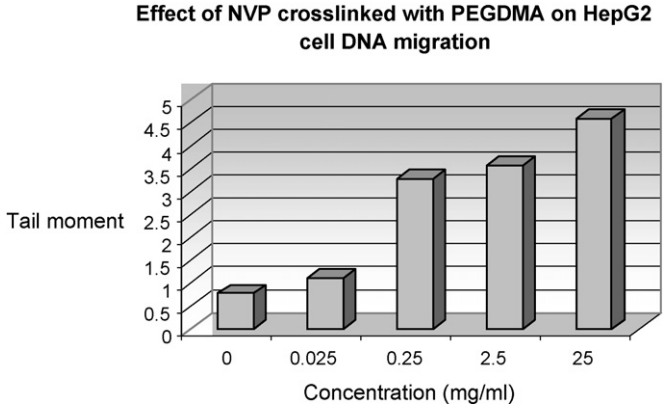

Fig. 10. DNA strand breakage expressed as tail moment in HepG2 cells following exposure for 3 h at 37 °C to PVP crosslinked with EGDMA. Results represent the mean of a single experiment, with 50 comets scored per concentration.

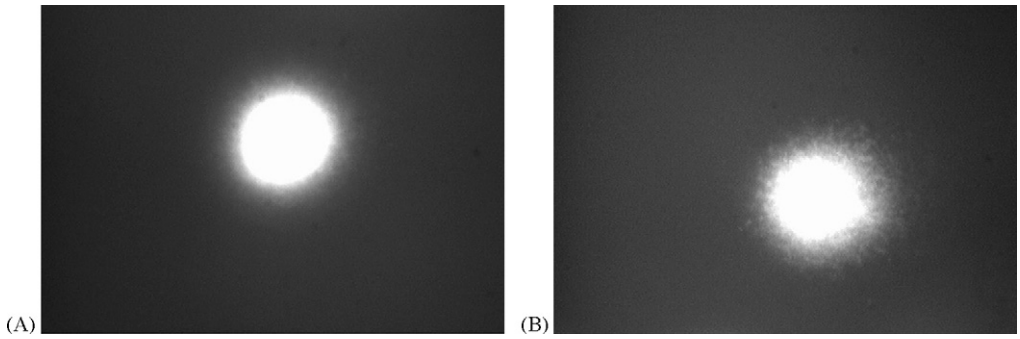

Fig. 11. Typical comet images captured by image analysis following alkaline SCGE and ethidium bromide staining. Panel A: untreated HepG2 control cell; panel B: HepG2 cell exposed for 3 h at 37 °C to 25 mg ml^{-1} PVP crosslinked with EGDMA (magnification 1000×).

4. Conclusions

In this work we have evaluated the potential of polyvinylpyrrolidinone-polyacrylic acid copolymers developed in our laboratory for use as multifunctional hydrogels in biomedical applications. We have shown that the extractable content of these hydrogels is relatively low, allowing these hydrogels to be used in applications where this is advantageous. We have demonstrated that the dissolution profile of active agents from these polymers vary depending upon the dissolution media used. Finally preliminary *in vitro* biocompatibility results obtained for polyvinylpyrrolidinone crosslinked with either EGDMA or PEG600DMA are encouraging; with only slight increases in cytotoxicity and genotoxicity observed for metabolically competent hepatoma HepG2 cells when in direct contact with low concentrations of hydrogel-containing media.

In conclusion these hydrogels are capable of releasing various active agents in a predictable and controlled fashion depending on the pH of the dissolution media, and therefore have potential for use in oral drug delivery applications. Due to their relatively low extractable content, and the favourable cytotoxicity and genotoxicity results, they also have potential for use in other biomedical applications such as medical device coatings and wound healing dressings.

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