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The influence of variation of gastric pH on the gelation and release characteristics of in situ gelling pectin formulations

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Abstract

The aim of this study was to examine the influence of variation of gastric pH over the range 1–3 on the gelation of liquid formulations of pectin and on the in vitro and in vivo release of paracetamol and ambroxol from the resultant gels. The formulations were dilute solutions of pectin containing complexed calcium ions that form gels when these ions are released in the acidic environment of the stomach. Gels suitable as vehicles for sustained delivery of these drugs were formed in vitro at pH < 3 from pectin solutions of concentrations 1.0–2.0% (w/v). Very weak gels were formed at pH 3.0 resulting in poor sustained release characteristics compared with those at pH 1.2; no significant in vitro gelation was observed at pH 3.5. The bioavailabilities of paracetamol and ambroxol from gels formed in the stomach following oral administration of the liquid formulations were investigated using gastric-acidity controlled rabbits. Visual observations showed in situ gelation of 1.5% (w/v) pectin formulations under conditions of both high (pH 1.0–1.6) and low gastric acidity (pH 3.3–3.6). The bioavailabilities of these drugs were not significantly different when released from gels formed at the two pH limits suggesting that normal variations of gastric acidity in the fasting state will have no effect on the bioavailability of these drugs when delivered using this vehicle.

Keywords: In situ gelation; Oral drug delivery; Sustained release; Pectin gels; Gastric acidity; Paracetamol; Ambroxol

1. Introduction

We have recently explored the possibility of using in situ gelling pectin formulations to achieve sustained release of drugs following oral administration (Kubo et al., 2004a,b, 2005; Miyazaki et al., 2005). Pectins are a family of polysaccharides in which the polymer backbone mainly comprises α-(1 → 4)-D-galacturonic acid residues. They are widely used in the food industry and because of their nontoxicity and biocompatibility have found many pharmaceutical and cosmetic applications. Dilute aqueous solutions (1.0–2.0%, w/v) of low methoxy pectins (degree of esterification <50%) readily form gels in aqueous solution in the presence of free Ca^{2+} ions, which cross-link the galacturonic acid chains. The gelation model originally proposed to describe chain-chain association in pectins was the 'egg-box' model (Grant et al., 1973), which envisaged the packing of the helical galacturonic acid chains with calcium ions located between them. In a recent re-examination of this model using molecular modelling procedures Braccini and Pérez (2001) proposed an alternative model of calcium gelation involving a two-stage process where the formation of strongly linked dimer associations between galacturonic acid chains is followed by the formation of weak inter-dimer associations mainly governed by electrostatic interactions.

A soluble complex of calcium ions was included in our formulation that was designed to break down to release free calcium ions on encountering the acidic environment of the stomach, so ensuring gelation of the orally administered pectin solution. A similar procedure was employed previously in the design of in-situ gelling formulations of the polysaccharides gellan

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(Miyazaki et al., 1999, 2001; Kubo et al., 2003) and sodium alginate (Miyazaki et al., 2000, 2001; Kubo et al., 2003), aqueous solutions of which also readily form gels in the presence of Ca^{++} ions. A perceived problem with such formulations is that the gastric hydrogen ion concentration may not be sufficiently high in some circumstances, even in the fasted state, to ensure adequate release of complexed calcium. It is known that in healthy young Caucasians the gastric pH is less than pH 3 during 90% of the fasted state, although on a minute-to-minute basis may reach as high as pH 7 (Dressman et al., 1990). After ingestion of a meal, the gastric acidity can vary over a wide range depending on the composition of the meal but is typically in the range pH 3–7. This article examines the in vitro gelation and release characteristics of the pectin formulations over the pH range 1–3, and the influence of gastric pH on the bioavailabilities of paracetamol and ambroxol delivered using the in situ gelling pectin formulation in rabbits.

2. Materials and methods

2.1. Materials

Pectin (LM-104AS, DE = 31%, Lot 23001-7) was supplied by SANSHO Co. Ltd., Osaka, Japan. Ambroxol hydrochloride (Lot YT-13) was supplied by YIA Co. Ltd., Shiga, Japan, and paracetamol (acetaminophen) was obtained from Astellas Pharma Inc., Tokyo, Japan. All other reagents were of analytical grade.

2.2. Preparation of sols

Pectin solutions (50 ml) of concentrations 1.0, 1.5 and 2.0% (w/v) were prepared by adding the pectin to ultrapure water containing 0.5% (w/v) (19.37 mmol l^{-1}) sodium citrate and 0.1% (w/v) (9.01 mmol l^{-1}) calcium chloride and heating to 40–50 °C while stirring. Appropriate amounts of paracetamol (1.0%, w/v) or ambroxol hydrochloride (0.6%, w/v) were then dissolved in the resulting solution. Solutions of paracetamol (1.0%, w/v) and ambroxol hydrochloride (0.6%, w/v) were prepared in ultrapure water.

2.3. Gelation of pectin sols

The influence of pH on the gelation characteristics of 1.0, 1.5 and 2.0% (w/v) pectin sols was determined by immersion of 30 ml sol enclosed in dialysis tubing (Viskase Sales Co., Chicago, USA, size 36/32) into dilute solutions of HCl (150 ml) with pH values over the range 1.0–3.5. After equilibration for 24 h at room temperature, the contents of the tube were passed through a sieve (JP XIV, No. 6.5, 2.80 mm) over a period of 30 s and the weight of the gel remaining in the sieve was determined (electronic balance, BL-220H, Shimadzu Ltd., Kyoto, Japan).

2.4. Measurement of in vitro drug release

The release rates of paracetamol and ambroxol were measured at 37 °C using plastic dialysis cells similar to that described previously (Miyazaki et al., 1984). The capacity of each half-cell was 4 ml and the surface area of the membranes was 2.67 cm^2. Sols of pectin (1.0, 1.5 and 2%, w/v) loaded with 1.0% (w/v) of paracetamol or 0.6% (w/v) of ambroxol, were placed in the donor compartment. An equal volume of simulated gastric (pH 1.2 and pH 3.0) or intestinal (pH 6.8) fluid (as specified for the JP XIV disintegration test) was placed in the receptor compartment. The donor phase and the aqueous receptor phase were separated by a cellulose membrane (Viskase Sales Co., Chicago, USA, size 36/32). The assembled cell was shaken horizontally at the rate of 60 strokes min^{-1} in an incubator. The total volume of the receptor solution was removed at intervals and replaced by fresh release medium. The concentration of paracetamol or ambroxol in the samples was determined by HPLC as described below.

2.5. Animal experiments

2.5.1. Gastric acidity controlled rabbits

Control of the pH of the gastric contents of white male rabbits weighing 2.5–3.3 kg was achieved by the method of Takahashi et al. (1983). Each rabbit was fed 100 g/day of special solid diet (CR-3 not containing alfalfa, Clea Japan Inc. Tokyo, Japan) for 1 week. At the end of the week, it was fasted for 24 h and then fed 50 g/day of the special soft diet for 2 days. Finally, just before administration of the pectin formulation, it was fed either 20 g of special soft diet containing hydrochloric acid or 20 g of special soft diet containing 2 g of antacid magnesium aluminometasilicate (Spiegel™, Fuso Pharmaceutical Industry Osaka, Japan). Rabbits were classified into high and low acidity groups. The statistical significance of the results was assessed by the Student's *t*-test and results are presented as the mean ± standard error of the mean.

2.5.2. Measurement of intragastric pH

Gastric acidity controlled rabbits were anaesthetised with an i.v. injection of pentobarbital (25 mg/kg). A micro pH glass electrode (CM-181, Chemical Instruments Co., Tokyo, Japan) was inserted in the stomach, a comparative ceramic electrode (CMR-535, Chemical Instruments Co.) was glued to the leg and the intragastric pH was directly measured by pH meter (F-22, Horiba Ltd., Tokyo, Japan).

2.5.3. In vivo drug release

A 1.5% (w/v) pectin sol preparation containing 50 mg/5 ml paracetamol or 24 mg/4 ml ambroxol hydrochloride was administered orally using a stomach sonde needle for rabbits (KN-342 Natume Seisakusho Co. Ltd., Tokyo, Japan). At given intervals, a blood sample was taken from the ear vein and analysed as described below. The protocols for the animal experiments were previously approved by the Animal Ethics and Research Committee of the Health Sciences University of Hokkaido.

2.6. Paracetamol assay

The plasma samples were separated by centrifugation and assayed by HPLC (Shimadzu LC-10A with a Shimadzu SPD-10A detector at a wavelength of 254 nm). The assay of

paracetamol was based on the methods described by Ameer et al. (1981) with minor modifications. To 200 μl of plasma was added 200 μl of water, 100 μl of 2-acetoaminophenol solution (100 μg ml^{-1} in 20% methanol) as internal standard, and 7 ml of ethyl acetate. The sample was vortex-mixed and centrifuged, after which 5 ml of the organic layer was evaporated to dryness under a nitrogen stream. The residue was reconstituted with 200 μl of 50% methanol, and aliquots of 20 μl were injected onto a 150 × 4.6 mm i.d. column, packed with Inertsil-ODS. Elution was carried out with acetonitrile: 0.1 M sodium acetate buffer pH 4.0 (15:85) at a rate of 0.8 ml min^{-1} at 40 °C.

2.7. Ambroxol assay

The plasma samples were separated by centrifugation and assayed by HPLC (Shimadzu LC-10A with a Shimadzu SPD-10A detector at a wavelength of 210 nm) using the method described by Botterblom et al. (1987) with minor modifications. To 0.5 ml of plasma was added 100 μl of propranolol hydrochloride solution (0.2 μg ml^{-1}) as internal standard, 100 μl of 1 M sodium hydroxide and 5 ml of diethyl ether and the sample was vortex-mixed and centrifuged. To supernatant was added 150 μl of 0.01 M hydrochloric acid. After shaking and centrifugation, the diethyl ether layer was discarded and 50 μl of the acid layer were injected onto the analytical column (300 mm × 3.9 mm i.d.), packed with Waters μBondapak C18. A column (20 mm × 3.9 mm i.d.) packed with Waters μBondapak C18 was used as a guard column. Elution was carried out with acetonitrile-methanol-0.05 M phosphate buffer pH 3.5 (0.65:1:3) at a rate of 0.8 ml min^{-1} at 40 °C.

3. Results and discussion

3.1. Effect of pH on gelation of pectin sols

Fig. 1 shows the weight of gel formed from 30 ml solutions of 1.0, 1.5 and 2.0% (w/v) pectin after dialysis in solutions of HCl over the pH range 1.0–3.5. Gels formed from 2% pectin solutions

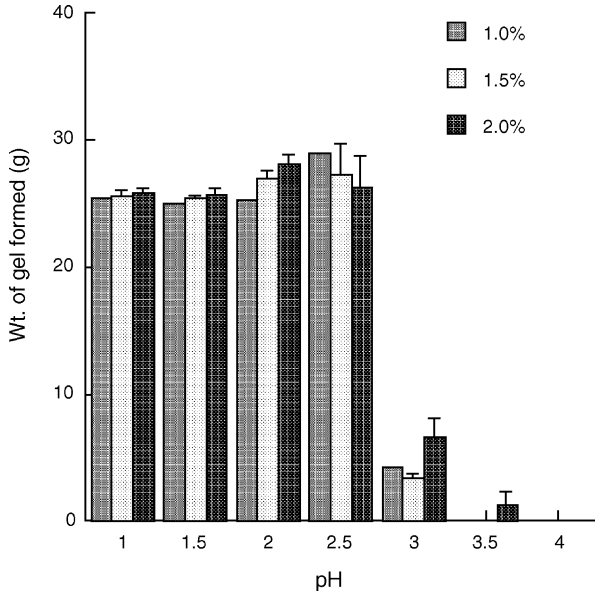

Fig. 1. Weight of gel formed from 30 ml solutions of 1.0, 1.5 and 2.0% (w/v) pectin as a function of pH. Each value is the mean ± S.E. of 3 determinations.

at pH 1.0–2.5 and from 1.0 and 1.5% solutions at pH 1.0–2.0 had a well-defined cylindrical form indicative of effective gelation as a consequence of the release of the complexed calcium ions under these acidic conditions. Although complete gelation of 1.0 and 1.5% solutions was observed at pH 2.5 the resultant gels were not sufficiently strong to maintain their cylindrical form. Fig. 1 shows that the hydrogen ion concentrations at pH 3.0 and 3.5 were not sufficiently high to cause effective breakdown of the calcium complex in the pectin solutions and gelation was poor or non-existent under these conditions.

3.2. Effect of the pH on in vitro drug release

3.2.1. Paracetamol

The release profiles of paracetamol from the 1.5% (w/v) pectin formulations are shown in Fig. 2a; similar plots were

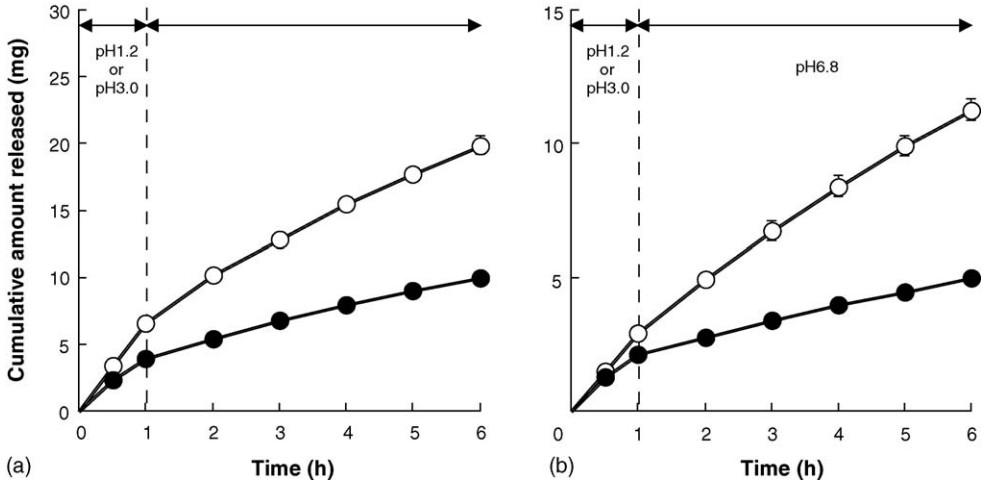

Fig. 2. In vitro release of: (a) paracetamol; and (b) ambroxol from 1.5% (w/v) pectin sols plotted as cumulative release against time. Release was into simulated gastric fluid pH 1.2 (●) or (◯) 3.0 for a period of 1 h and subsequently into simulated intestinal fluid pH 6.8. Each value is the mean ± S.E. of 4 determinations.

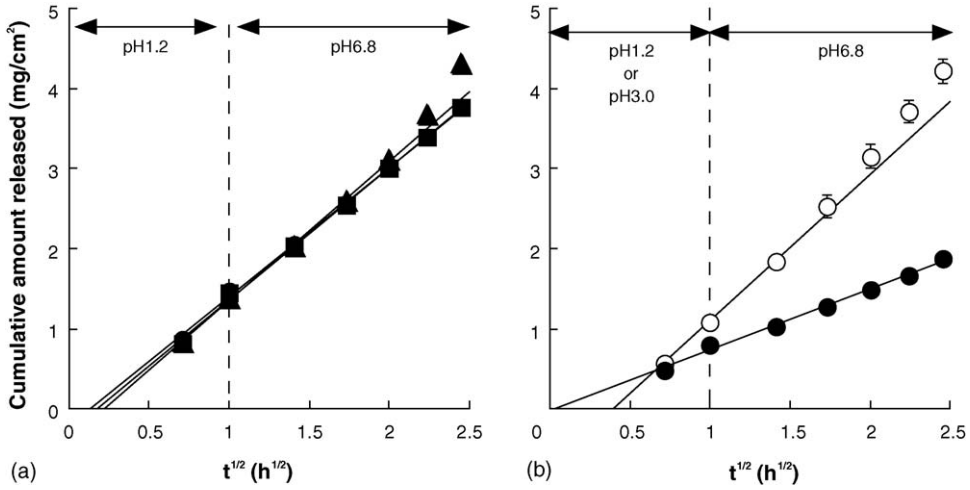

Fig. 3. In vitro release plotted as cumulative release against square root time. (a) Release of paracetamol from (▲) 1.0%, (●) 1.5 and (■) 2.0 (w/v) pectin sols. Release was into simulated gastric fluid pH 1.2 for a period of 1 h and subsequently into simulated intestinal fluid pH 6.8. (b) release of ambroxol from 1.5% (w/v) pectin sols. Release was into simulated gastric fluid pH 1.2 (●) or (◯) 3.0 for a period of 1 h and subsequently into simulated intestinal fluid pH 6.8. Each value is the mean ± S.E. of 4 determinations.

obtained for the release from 1.0 and 2.0% (w/v) formulations. The receptor solutions were changed after 1 h from simulated gastric fluid at pH 1.2 or 3.0 to a simulated intestinal fluid at pH 6.8 to mimic gastro-intestinal transit at two extremes of gastric acidity in the fasting state.

Release of drug from each of the formulations was appreciably faster when the pectin was exposed to a receptor solution at pH 3.0 over the initial release period; observation of the donor cells showed that the formulations were predominantly in sol form throughout the duration of the release period. Diffusion through the dialysis membrane of H^+ ions from the receptor solution at this pH was insufficient to cause the release of complexed calcium ions and consequently gelation of the pectin was incomplete. No further analysis of the data for these formulations was undertaken.

The slower release from formulations initially maintained at pH 1.2 was a consequence of effective gelation under these conditions as confirmed by visual observation of the diffusion cell. The release data over the whole time period of release for these formulations were analysed using the Higuchi equation for drug release from semisolid vehicles containing dissolved drug (Higuchi, 1962),

$$Q = 2C_0\left(D\frac{t}{\pi}\right)^{1/2} \quad (1)$$

where Q is the amount of drug released per unit surface area from gels of initial drug concentration C_0 in time t. Diffusion coefficients, D, of $5.75 \pm 0.36 \times 10^{-6}$ and $5.97 \pm 0.27 \times 10^{-6}$ cm^2 s^{-1} ($n = 4 \pm$ S.E.) were calculated from the linear plots of Q versus $t^{1/2}$ (Fig. 3a) for 1.5 and 2.0% (w/v) gels, respectively; release from 1.0% (w/v) gels was linear for the initial 4 h of release giving $D = 6.59 \pm 0.31 \times 10^{-6}$ cm^2 s^{-1}. The value determined for the 2.0% (w/v) formulation compares with a value of $D = 6.83 \pm 0.38 \times 10^{-6}$ cm^2 s^{-1} ($n = 4 \pm$ S.E.) obtained previously for this formulation (Miyazaki et al., 2005).

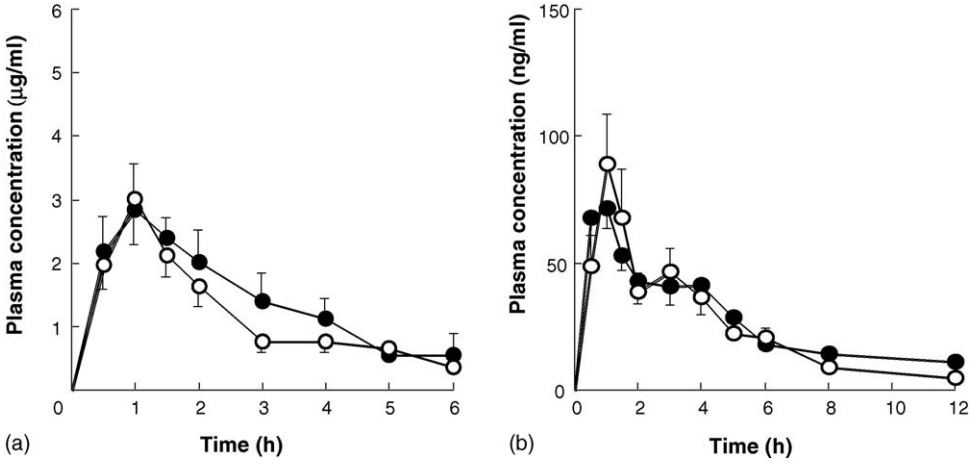

Fig. 4. Plasma concentrations of: (a) paracetamol; and (b) ambroxol after oral administration of 1.5% (w/v) pectin sols to gastric-acidity-controlled rabbits at (●) high and (◯) low acidity (see Table 1 for pH values). Each value is the mean ± S.E. of 4 determinations.

Table 1
Bioavailability parameters of paracetamol and ambroxol from 1.5% (w/v) pectin gels formed in situ in gastric-acidity controlled rabbit stomach

Drug	Gastric pH	C_{max} (μg ml^{-1})	t_{max} (h)	AUC (0–6 h) (μg h ml^{-1})	MRT (h)
Paracetamol	1.30 ± 0.23	3.16 ± 0.64	0.88 ± 0.24	8.68 ± 1.84	2.27 ± 0.18
	3.00 ± 0.12	3.08 ± 0.69	0.88 ± 0.13	7.19 ± 1.16	2.24 ± 0.14
Ambroxol	1.49 ± 0.08	0.080 ± 0.012	0.75 ± 0.14	0.333 ± 0.042	3.98 ± 0.46
	3.37 ± 0.15	0.089 ± 0.020	1.00 ± 0.00	0.306 ± 0.053	3.57 ± 0.04

Each value represents the mean ± S.E. of 4 experiments.

3.2.2. Ambroxol

The release characteristics of ambroxol from 1.5% (w/v) pectin formulations at initial pHs of 1.2 and 3.0 are shown in Fig. 2b. Analysis of the release data for formulations initially maintained at pH 1.2 using Eq. (1) produced linear plots of Q versus $t^{1/2}$ over the total release period (Fig. 3b) from which a value of $D = 3.62 \pm 0.60 \times 10^{-6}$ cm^2 s^{-1} ($n = 4 \pm$ S.E.) was calculated, which compares with $D = 4.56 \pm 0.18 \times 10^{-6}$ cm^2 s^{-1} ($n = 4 \pm$ S.E.) determined previously for this formulation (Miyazaki et al., 2005). Ambroxol release from 1.5% (w/v) gels initially at pH 3.0 was linear for the initial 2 h of release giving $D = 19.98 \pm 1.89 \times 10^{-6}$ cm^2 s^{-1}.

3.3. The effect of gastric acidity on in vivo drug release

The bioavailabilities of paracetamol and ambroxol after oral administration of pectin formulations were determined using gastric-acidity controlled rabbits to ascertain the influence of gastric pH on in vivo drug release from the in situ gelling vehicles. Plasma drug levels following oral administration of paracetamol (50 mg/5 ml) from 1.5% (w/v) pectin sols to rabbits with high (pH 1.30 ± 0.23) and low (pH 3.00 ± 0.12) gastric acidity are compared in Fig. 4a. The area under the plasma concentration-time curve (AUC) and the mean residence time (MRT) obtained from the plasma concentration-time data of each animal using a computer program for model-independent analysis (Yamaoka et al., 1981) are compared in Table 1. No significant difference in bioavailability between the two groups of rabbit could be detected suggesting that gastric acidity had no measurable effect on the bioavailability of paracetamol in the gastric pH range investigated.

Fig. 4b and Table 1 show similar bioavailabilities following the administration of ambroxol (24 mg/4 ml) to the groups of rabbits with gastric pH of 1.49 ± 0.08 and 3.37 ± 0.15.

These findings contrast to the predictions from the in vitro release studies of Section 3.2, which show large differences in release under conditions of high and low gastric acidity as a consequence of the failure of the pectin formulations to form coherent gels when dialysed against solutions at pH > 2.5. Photographs of the contents of rabbit stomachs of high and low gastric acidity groups at time intervals of 1 and 5 h after administration of 5 ml of 1.5% (w/v) pectin sols (containing a marker dye but no drug) show gelation in both groups of rabbits (Fig. 5). Although the gels formed at the higher pH appear less compact than those formed under conditions of low pH, the bioavailability data indicate that these gels are able to function as sustained release vehicles for these drugs. The reason for the difference in gelation characteristics between in vivo and in vitro environments can probably be explained by the presence of calcium in the gastric juice, particularly ionised calcium from gastric interstitial (non-parietal) fluid (Moore and Makhlouf, 1968), which would compensate for the lower amount of complexed calcium released at higher pH so promoting in vivo gelation.

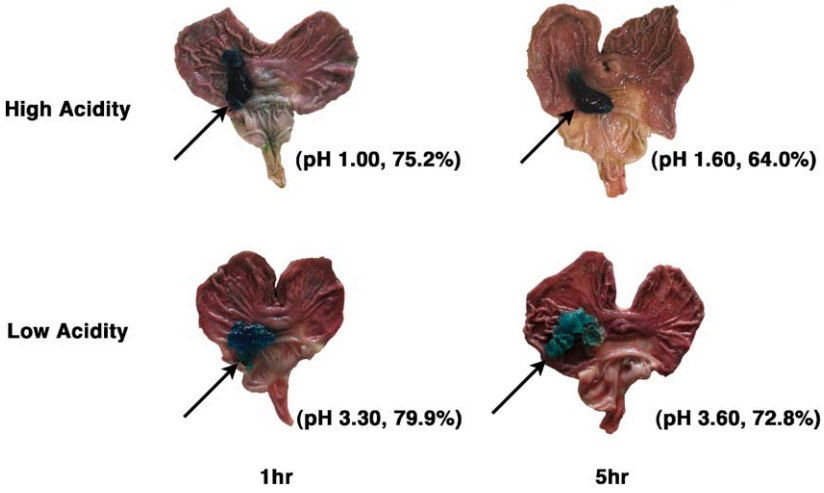

Fig. 5. Photographs showing presence of gels (indicated by arrows) in stomachs of gastric-acidity controlled rabbits 1 and 5 h after oral administration of 1.5% (w/v) pectin sols. Numbers in parenthesis indicate gastric pH and percentage of the gel remaining.

4. Concluding remarks

This study has demonstrated that 1.5% (w/v) pectin sols formulated with a source of calcium in complexed form can form gels in the rabbit stomach under the limits of high and low acidity normally encountered in the fasting state, even though in vitro gelation is not observed at low acidity (pH $\geq$ 3.0). Bioavailability studies have indicated no significant influence of gastric acidity (within the normally expected limits) on the sustained release characteristics of paracetamol and ambroxol from gels formed in situ.

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