Biphasic Drug Release: Permeability and Swelling of Pectin/Ethylcellulose Films, and in Vitro and in Vivo Correlation of Film-Coated Pellets in Dogs

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The major objectives of this study were i) to evaluate the permeability and swelling characteristics of isolated films prepared by mixing of pectin with ethylcellulose; and ii) to assess the absorption and in vitro/in vivo correlation (IVIVC) of 5-FU film-coated colon-targeted pellets in dogs. Free films were prepared by casting and solvent evaporation method. These free films were evaluated by swelling experiment and permeability to 5-FU in different media. Pectin/ethylcellulose films had suitable characteristics for colonic delivery; and when the addition of pectin was up to the ratio of 30%, the swelling and permeability of the mixed films was significantly increased in the simulated colonic fluid (SCF). Pharmacokinetic study in dogs gave T1/2/C∞ of 14 h/1.6 μg/ml and 16 h/1.7 μg/ml for total weight gain (TWG)-22% and 18% coated pellets, respectively. The plasma 5-FU levels of the TWG-22% and 18% coated pellets were maintained at a much lower level with a mean residence time (MRT) of 18—20 h, longer than 2.1 h for 5-FU uncoated pellets, confirming delayed absorption. There was no statistically significant difference in the area under the plasma concentration vs. times curve (AUC) values between the uncoated pellets and the coated pellets. Moreover, a good linear regression relationship was observed between the percent in vitro dissolution in SCF and the percent absorption or percent AUC. It was concluded that i) pectin within the mixed films were susceptible to colonic enzymes, and the film-coated pellets are potentially useful for colonic drug delivery; and ii) in vitro dissolution testing in SCF could be used to establish certain IVIVC for the colon-specific drug delivery systems activated by microflora.

Key words  pectin; permeability; swelling; water vapor transmission; colonic delivery; in vitro/in vivo correlation

Targeting drugs and delivery systems to the colonic region of the gastrointestinal (GI) tract has received considerable interest in recent years.1,2) The successful delivery of drugs to the colon via the GI tract requires the protection of a drug from being released in stomach and small intestine. Commonly used pharmaceutical strategies to achieve a colonic drug delivery include timed-release approximating the GI transit time, pH-sensitive polymer coating, prodrug, and colonic microflora activated delivery systems.1—6) Of these systems, microflora activated delivery systems are considered to be preferable and promising since the abrupt increase of the bacteria population and associated enzymatic activities in ascending colon represents a non-continuous event independent of GI transit time and pH.5,7—9) In the colon, an extensive growth of anaerobic microorganisms is observed. In contrast, the microflora of the upper GI tract is not as prominent, and consists mainly of aerobic microorganisms. These colonic microflora produce a large number of hydrolytic enzymes as well as reductive enzymes which can potentially be utilized for colon-specific drug delivery. The reductase enzyme releases other polysaccharides like glucosidases; glycosidases are released by colonic microflora, which are responsible for the degradation of polysaccharides.12,13) Hence, drug delivery systems based on polysaccharide can be used for colondrug delivery. Polysaccharides may be utilized for colon-specific delivery in various forms: fermentable coating of the drug core, embedding of the drug in the biodegradable matrix, formulation of drug–saccharide conjugate (prodrugs).14)

Pectins are non-starch, linear polysaccharides extracted from the plant cell walls. They are predominantly linear polymers of mainly α-(1→4) linked D-galacturonic acid residues interrupted by 1,2-linked L-rhamnose residues. Pectin has a few hundred to about one thousand building blocks per molecule, corresponding to an average molecular weight of about 50000 to about 180000. Pectin tends to produce lower viscosities than other plant gums. It is refractory to host gastric and small intestinal enzymes but is almost completely degraded by the colonic bacterial enzymes to produce a series of soluble oligalactorunates. Being soluble in water, pectin is not able to shield its drug load effectively during its passage through the stomach and small intestine. An ideal approach is to modify the solubility while still retaining its biodegradability. One way to alleviate this problem is by the use of hydrophobic polymers, e.g., ethylcellululose.15) It restricts the entry of water and consequently swelling of polymer.16—18)

Pectin/ethylcellululose film coating formulations had been investigated for their potential to obtain colon-specific drug delivery,15 and physical properties of mixed pectin/ethylcellululose films were initially studied only in presence of commercial enzymes by Macleod and his coworkers.19) However, in contrast with some literature data,20—23) pectinolytic enzymes seemed to be not very effective in promoting the degradation rate of pectinate beads or films containing pectin.
In fact, the ideal model for investigation of pectin digestibility containing in ethylcellulose films in man is human colonic contents. However, the in vivo measurement of carbohydrate fermentation in the human colon is difficult due to the relative inaccessibility of the proximal colon. Since the rat cecum and colon have been shown to have much the same microbial contents as the human colon in terms of predominant bacterial species and their levels, and rats are the most readily available and commonly used laboratory animal species, rat cecal and colonic contents or enzymes would be reliable for use as an in vitro degradation system to mimic the microbial environment in the human colon. Moreover, rat cecal contents has been the popular choice for evaluating the colon-specific drug delivery activated by microflora; and some standardization would be established in the experiment procedure in the future, which regards the volume of testing medium, the concentration and source of rat cecal contents, and the agitation intensity to allow meaningful comparison of different delivery systems.

However, there are few reports for the characteristics of the pectin/ethylcellulose free films in presence of cecal and colonic contents or enzymes until now. Additionally, with the knowledge of the colonic environment being far more complex than the testing conditions using rat cecal contents represented, it remains to be seen whether or not such dissolution testing can be used to establish certain in vitro/in vivo correlation (IVIVC) for the colon-specific drug delivery systems activated by microflora. Yang reviewed the use of biorelevant dissolution testing for microbially triggered drug delivery to the colon, but little is known about the IVIVC for this drug delivery system.

The major objectives of this study were to evaluate the permeability and swelling characteristics of isolated films prepared by mixing of pectin with ethylcellulose in presence of rat cecal contents. Furthermore, the IVIVC investigations of pectin/ethylcellulose-film coated pellets in dogs were also initially performed for colonic drug delivery activated by microflora. 5-FU was used as a model drug.

Experimental

Materials

Ethylcellulose was used in the form of Surelease® (E-7-7050, 25% solids) and was a gift from Colorcon Ltd. (Orrington, U.K.). Pectin (with degree of methylation (DM) of approximately 35%) was a gift from Shijiazhuang No. 4 Pharmaceutical Co., Ltd. (Shijiazhuang, China). All chemicals were of analytical grade.

Preparation of Free Films

Firstly, specific amounts of pectin were dissolved in warm distilled water. Then, predetermined amounts of Surelease® were added to this solution with stirring. Final dispersion was solved in warmed distilled water. Then, predetermined amounts of sample from Shijiazhuang No. 4 Pharmaceutical Co., Ltd. (ShiJiaZhuang, China) (with degree of methylation (DM) of approximately 35%) was a gift from earlier report. The major objectives of this study were to evaluate the permeability and swelling characteristics of isolated films prepared by mixing of pectin with ethylcellulose in presence of rat cecal contents. Furthermore, the IVIVC investigations of pectin/ethylcellulose-film coated pellets in dogs were also initially performed for colonic drug delivery activated by microflora. 5-FU was used as a model drug.

Water Vapor Transmission (WVT) Test

Water vapor permeation of free films was determined gravimetrically in triplicate. The permeability cups were 2.0 cm in diameter. The inside of the cup was filled with 10 ml of distilled water, and the film was subsequently attached to the cup with a cyanoacrylate adhesive Super Glue (Shantou, China). The cup with the film was then weighed and stored in a desiccator filled with silica gel. After 24, 48, 72, 96, and 120 h of storage the cups were reweighed in order to determine the permeated amount of water (mass loss) and the profile of mass change was plotted versus time for each free film. WVT was calculated using following equation

\[ WVT = g \times 24 \times 10^{-4} \times t \]  

where \( g \) represents mass loss, \( t \) is time (measured in hours during which the weight loss occurred), and \( A \) is the exposed area of the film.

Swelling Experiments

Firstly, a piece of 1 cm² of each free film was dried in an oven at 50°C for 24 h. Then, dried film was accurately weighed and immersed in a flask of dissolution test containing 250 ml of different media at 37°C. At specific intervals, the swollen sample was withdrawn from the medium and weighed after removal of excess surface water by light blotting with a filter paper. During the first 10 min, intervals of sampling were 1 min. Sampling time was gradually increased after this period until 3 h. The swelling behavior of the films was calculated as follows

\[ I_s = (M_f - M_0) / M_0 \times 100\% \]  

where \( I_s \) is the swelling index, \( M_f \) is the film mass after a certain swelling period, and \( M_0 \) is the dry film mass. Swelling tests were separately carried out in simulated gastric fluid (SGF) with pH 1.2, simulated intestinal fluid (SIF) with pH 6.8 and also simulated colonic fluid (SCF) with adding rat cecal contents (4%, w/w) to the media with pH 6.8. Three parallel measurements were performed in each case.

Drug Permeability

Permeability of the model drug across the polymeric films was determined in horizontal side-by-side diffusion cell (diffusion area 0.95 cm²) at 37°C. Different experimental conditions were set up to examine the permeability of the drug through polymer films. The initial concentration of drug in the donor compartment was 12 µg/ml (saturated solution). The donor and acceptor compartments were both composed of SGF, SIF and SCF with rat cecal contents (4%, w/w). The drug concentrations in sample volumes were analyzed by high-performance liquid chromatography (HPLC) assay.

When the stationary state was achieved, the permeability coefficient of the studied drug at was obtained by the following formula

\[ \frac{2PS}{V} = -\ln \left( \frac{C_a - C_t}{C_t} \right) / C_0 \]  

where \( P \) is the permeability coefficient, \( S \) is the surface area of the film through which diffusion takes place, \( V \) is the volume of the acceptor or donor compartment, \( t \) denotes time, \( C_0 \) is the initial concentration of 5-FU in the donor compartment, and \( C_t \) is the concentration of caffeine in the acceptor compartment. \( P \) can be calculated from a plot of \( \ln(C_t - 2C_a) / C_0 \) vs. time. Three parallel measurements were performed in each case.

Glass Transition Temperature (\( T_g \))

The \( T_g \)'s of the films pectin/ethylcellulose were measured by a differential scanning calorimeter, Perkin-Elmer 7 DSC (Perkin-Elmer, U.S.A.), with an intracooler and nitrogen purge. Each film sample consisted of 8 mg of small discs piled into a 50 µl aluminum sample pan with a pierced lid to allow vaporization of volatile materials and, at the same time, to avoid sample expansion and warping of the sample pan. The sample was first heated from 0 to 200°C at a rate of 10°C/min, and then cooled back to 0°C at a rate of 20°C/min. This step was designed to remove moderately bound moisture and solvent residue so that the endotherm would not obscure the glass transition. The sample was then reheated at a rate of 10°C/min until the \( T_g \) passed. The \( T_g \) for each film was determined from the midpoint of a small endothermic rise of the pre and post-transition baselines using six parallel thermograms. The method was similar to the one described by Okhamafe and Young.

Fourier Transform Infrared (FT-IR) Spectroscopy

About 2% (w/w) of films of pectin/ethylcellulose blend, with respect to the potassium bromide (KBr) disc, was mixed with dry KBr (FTIR grade, Aldrich, Germany). The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Each disc was scanned at a resolution of 4 cm⁻¹ over a wavenumber region of 400—4000 cm⁻¹ using a FT-IR spectrometer (Spectrum 100 FT-IR System, Perkin-Elmer, U.S.A.) coupled to a personal computer with AssureID software packages. The characteristic peaks of IR transmission spectra were recorded.

Preparation of 5-FU Coated Pellets

The pectin/ethylcellulose-coated colon-targeted pellets were prepared using the procedure described in our earlier report. The model drug 5-FU was formulated into pellets of size 0.8—1.0 mm by the process of extrusion-spheronization. The formulation comprised 40% 5-FU and 60% microcrystalline cellulose (MCC). The coating formulations were prepared by mixing 2% pectin aqueous
dispersion with Surelease® in 1:2.5 and 1:3.5 ratios. The pellets (100 g batch size) were coated in a fluidized bed coater (Jiafa Granulating drying equipment, Changzhou, China). The formulations were coating to different film thicknesses and quantified by the total weight gain (TWG %).

**Effect of Concentration of Rat Cecal Contents on In Vitro Drug Release**

The coated pellets of 5-FU were evaluated for their integrity in the physiological environment of stomach and small intestine under conditions mimicking mouth to colon transit as described earlier.23 These studies were carried out using a USP XXIII dissolution rate test apparatus (Apparatus 1, 100 rpm, 37 °C). The coated pellets were tested for drug release for 2 h in 0.1 M HCl (150 ml) as the average gastric emptying time is about 2 h. After this, 50 ml of 0.2 M trisodium phosphates was added to the dissolution media and the pH adjusted to 6.8, and the study at a pH of 6.8 was continued for 3 h as the average small intestinal transit time is about 3 h. At the end of the time periods, two samples each of 1 ml were taken, after which cecal content equivalent to 8 or 16 g was added to 200 ml of buffer (pH 6.8) to give a final cecal dilution of 4 or 8%. The experiments in cecal content media were carried out in presence of a continuous supply of CO2 for another 19 h. At different concentration of rat cecal contents, there was a significant decrease in drug release up to the ratio of 30%. It is well known that increasing the hydrophilic nature of a polymer membrane induces water vapor tendency and as a result increases water vapor permeation. Pectin has a good water solubility and high hydrophilic character. The incorporation of pectin into an ethylcellulose film would engender an increase in hydrophilicity.

**Swelling Test**

The results of swelling experiments are listed in Table 2. Formulations containing Surelease® and pectin had lower swelling index in SGF and SIF. Swelling index in SGF and SIF increased with the addition of pectin up to the ratio of 30% (p<0.05) which is due to hygroscopic characteristics of pectin and the higher water uptake of polymer. Swelling index was lower in SGF than in SIF, because pectin remained as aggregates of macromolecules in acid en-

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**Table 1. Water Vapor Transmission of Free Films**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mass change (mg/h)</th>
<th>WVT (g/24 h·m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surelease® 100%</td>
<td>S: P=95:5</td>
<td>1.94±0.16</td>
</tr>
<tr>
<td>Surelease® 100%</td>
<td>S: P=90:10</td>
<td>2.18±0.05</td>
</tr>
<tr>
<td>Surelease® 100%</td>
<td>S: P=80:20</td>
<td>2.23±0.12</td>
</tr>
<tr>
<td>Surelease® 100%</td>
<td>S: P=70:30</td>
<td>2.30±0.10</td>
</tr>
<tr>
<td>Surelease® 100%</td>
<td>S: P=60:40</td>
<td>2.96±0.06</td>
</tr>
<tr>
<td>Surelease® 100%</td>
<td>S: P=60:40</td>
<td>3.78±0.09</td>
</tr>
</tbody>
</table>

Data represent mean±S.D., n=3.

**Table 2. Swelling of Formulation in Different Media**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Maximum swelling index, I₁ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S: P=95:5</td>
<td>45.08±5.27</td>
</tr>
<tr>
<td>S: P=90:10</td>
<td>50.23±1.33</td>
</tr>
<tr>
<td>S: P=80:20</td>
<td>55.36±1.05</td>
</tr>
<tr>
<td>S: P=70:30</td>
<td>65.67±2.05</td>
</tr>
<tr>
<td>S: P=60:40</td>
<td>73.36±1.22</td>
</tr>
<tr>
<td>S: P=60:40</td>
<td>80.38±2.19</td>
</tr>
</tbody>
</table>

Data represent mean±S.D., n=3.

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**Results and Discussion**

**WVT Experiments**

According to Fig. 1 the rate of water vapor permeation was constant for free films containing Surelease® and pectin. Table 1 lists the results of WVT experiments for all the formulations. The results (Table 1) demonstrate that WVT was affected by the composition of the film. An increase in polysaccharide concentration significantly influences (p<0.05) WVT with the addition of pectin up to the ratio of 30%. It is well known that increasing the hydrophilic nature of a polymer membrane induces water vapor tendency and as a result increases water vapor permeation. Pectin has a good water solubility and high hydrophilic character. The incorporation of pectin into an ethylcellulose film would engender an increase in hydrophilicity.

**Pharmacokinetic and Data Analysis**

Non compartmental pharmacokinetic analysis of concentration time data was performed using Excel software. The pharmacokinetic parameters, such as maximum plasma concentration (Cmax) and time of maximum concentration (Tmax), were obtained directly from the plasma concentration–time plots. The area under the plasma concentration vs. time curve up to the last time (t) \((AUC_{0\rightarrow\infty})\) was determined using the trapezoidal rule. The \(AUC_{0\rightarrow\infty}\) values were calculated by adding the value of \(C_{\infty}\times K_{e}/K_{e}-1\) to \(AUC_{0\rightarrow\infty}\), that was \(AUC_{0\rightarrow\infty}-AUC_{0\rightarrow t}+C_{\infty}\times K_{e}^{-1}\). The mean residence time (MRT) was calculated as \(\text{AUMC}/AUC\). The relative bioavailability \((F_r)\) was calculated using the following formula: 100%× \((AUC_{\text{coated pellet}}/AUC_{\text{uncoated pellet}})\).

The data generated in the pharmacokinetic study were used to develop the IVIVC. The relationship between percent in vitro dissolution in SCF (8%) and the fraction of drug absorbed in vitro \((F_r)\) was examined. The \(F_r\) was determined using the Wagner–Nelson method (W.N.) by the following equation: \(F_r=(C_{\infty}/K_{e}+AUC_{0\rightarrow\infty})/AUC_{0\rightarrow\infty}\) and the Loo–Riegelman method \((L.R.)\) by the following equation: \(F_r=[C_{\infty}/K_{e}+AUC_{0\rightarrow\infty}+(X)/(X\times K_{e})]/AUC_{0\rightarrow\infty}\). The relationship between percent in vitro dissolution in SCF and percent \(\text{AUC}(C_{\infty}/K_{e}+AUC_{0\rightarrow\infty})\) was also examined. Linear regression analysis was applied to the IVIVC plots.

Variations in PK parameters were tested using analysis of variance (ANOVA). Difference in mean PK parameters of 5-FU between 1:2.5 ratio TWG-22% or 1:3.5 ratio TWG-18% coated pellets and uncoated pellets were subjected to t-test to find the statistical significance. In all the cases, a value of \(p<0.05\) was considered statistically significant.
zymes diffuse into the polymeric chains, 5) hydrolyze the gly-

Table 3. Permeability of Different Free Films to 5-FU

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Permeability coefficients ( \times 10^{-5} ) cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGF</td>
</tr>
<tr>
<td>Surelease® 100%</td>
<td>1.031±0.051</td>
</tr>
<tr>
<td>S : P = 95 : 5</td>
<td>1.684±0.015</td>
</tr>
<tr>
<td>S : P = 90 : 10</td>
<td>1.964±0.124</td>
</tr>
<tr>
<td>S : P = 85 : 15</td>
<td>2.110±0.421</td>
</tr>
<tr>
<td>S : P = 80 : 20</td>
<td>2.302±0.267</td>
</tr>
<tr>
<td>S : P = 70 : 30</td>
<td>2.654±0.647</td>
</tr>
<tr>
<td>S : P = 60 : 40</td>
<td>2.701±0.254</td>
</tr>
</tbody>
</table>

Data represent mean±S.D., \( n = 3 \).

however, when the addition of pectin was up to the ratio of 30%, the permeability of the mixed films was significantly increased. Surelease® is an aqueous ethylcellulose latex dispersion; pectin would interact with latex particles in aqueous phase.\(^{39}\) When the ratio of pectin is up to 30%, the effect of interaction between latex particles and pectin would be reduced and more free pectin was dispersed within the film, which would increase the susceptibility to the bacterial enzyme. Moreover, the rat cecal contents contained more than one bacterial enzyme (include bacteroides, bifidobacteria and enterobacteria, etc.) that could degrade pectin would also increase the permeability of the films of the drug.\(^{9,35,36}\)

In Macleod’s report, there was only one enzyme (pectinolytic enzymes) was contained in the SCF. In fact, even with simple liner one-component polysaccharides, more than one bacterial enzymes were usually involved in its degradation or breakdown.\(^{37}\)

\( T_\alpha \)'s One of the most common polymer properties determined for amorphous polymers is the \( T_\alpha \). \( T_\alpha \) values for ethylcellulose and pectin films were 82 and 115°C, respectively (Fig. 2). The addition of pectin to ethylcellulose would increase the \( T_\alpha \) of the ethylcellulose films (Fig. 2). However, no significant increasing \( T_\alpha \) were observed, which indicating that pectin in ethylcellulose films was poorly miscible.\(^{38}\) Poor miscibility suggested that pectin was distributed as isolated domains in the films and the characteristics of pectin within the mixed films were likely to remain unchanged. Pectin within the mixed films should therefore remain recognizable to cecal and colonic bacterial enzymes as a digestion substrate.

FT-IR FT-IR studies were performed to aid in the evaluation of any possible chemical interactions in the amorphous state. The FT-IR spectrum for the pure drug and ethyl cellulose is presented in Fig. 3. The FT-IR spectrum for ethylcellulose shows a distinct peak at 3484.08 cm\(^{-1}\) which is due to the OH groups present on the closed ring structure of the polymer’s repeating units. The same also represents the intra- and intermolecular hydrogen bonding due to the –OH groups.\(^{39}\) The asymmetric peak seen around 2970—2870 cm\(^{-1}\) may be due to –CH stretching. The peak at 1378.51 cm\(^{-1}\) is due to –CH\(_3\) bending and the small peak near 1445.61 cm\(^{-1}\) is due to –CH\(_2\) bending. The broad distinct peak near 1111.99 cm\(^{-1}\) may be due to the C–O–C stretch in the cyclic ether. In the FT-IR spectrum of pectin
bands related to C=O stretching of the ester and carboxyl group could be observed at 1748.59 and 1603.18 cm$^{-1}$, respectively. In the physical mixer and blend of pectin/ethylcellulose film spectrums, the characteristic peaks of both ethylcellulose and pectin could be observed, and the spectrum could be regarded as a simple superposition of that of ethylcellulose and pectin. Though it was reported that the molecular coalescence of pectin and ethylcellulose was observed in aqueous phase, no interactions between the ethylcellulose and pectin in dry films were observed in our experiment. No interaction between them would less hinder access of the enzyme to the active site; the characteristics of being digested by cecal and colonic bacterial enzymes were not altered since the proposed hydrolysis site (the $\beta[1,4]$link) was not affected.

**Effect of Concentration of Rat Cecal Contents on *in Vitro* Drug Release** After the process of film coating, the size of the coated pellets were 1.0—1.2 mm, and the surfaces of coated pellet were yellowish and glossy and appeared to be spherical and smooth. The results of drug release studies without cecal contents (control) and with 4% or 8% w/w of cecal contents are shown in Fig. 4. The presence of rat cecal contents in the dissolution medium resulted in significantly improved drug release when compared to control. Drug release was found to be positively correlated to cecal contents concentration; however, there was no significant difference for cecal contents concentrations of 4% and 8%.

The results clearly demonstrate that pectin contained in the mixed films was susceptible to enzymatic action of rat cecal contents as the percent drug released in the presence of different levels of cecal matter is better than without cecal matter. The function of the ethylcellulose was to control the swelling of the pectin, and it did so successfully and drug release SGF and SIF was minimal. The percent drug release relied on the action of enzymes provided by the colonic bacterial. Therefore, the pectin/ethylcellulose-film coated pellets are supposed to be susceptible to proximal colon microflora in vivo, thus be potentially colon-specific.

**Pharmacokinetic Analysis** The pharmacokinetics of the coated pellets (TWG-22% and 18% for the 1:2.5 and 1:3.5 pectin:ethylcellulose ratios, respectively) was evaluated in dogs and compared with uncoated pellets of 5-FU. Mean plasma 5-FU concentration vs. time profiles after a single oral dose of the three formulations are shown in Fig. 5. Mean

![FT-IR Spectra](image1)

**Fig. 3.** FT-IR Spectra of (a) Pectin, (b) Ethylcellulose, (c) Physical Mixture of Ethylcellulose and Pectin (7:3 ratio) and (d) Ethylcellulose/Pectin Blend (7:3 Ratio)

![Mean Percentage Release](image2)

**Fig. 4.** Mean Percentage Release of 5-FU from 1:2.5 Pectin:Ethylcellulose TWG-22% (A) and 1:3.5 Pectin:Ethylcellulose TWG-18% (B) Coated Pellets after 2 h in 0.1 M HCl, 3 h in Phosphate Buffer (pH 6.8) and 19 h in Phosphate Buffer (pH 6.8) without and with 4% w/v and 8% w/v of Rat Cecal Contents

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Uncoated pellets</th>
<th>Coated pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TWG-22%</td>
<td>TWG-18%</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>12.14±1.02</td>
<td>1.60±0.87* 1.71±0.97*</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.75±0.06</td>
<td>14.00±1.13* 16.00±2.11*</td>
</tr>
<tr>
<td>Mean residence time, $MRT$ (h)</td>
<td>2.10±0.54</td>
<td>18.92±2.59* 19.81±1.87*</td>
</tr>
<tr>
<td>$AUC_{0→t}$ (µg·h/ml)</td>
<td>24.45±2.12</td>
<td>19.84±3.01* 22.03±4.17*</td>
</tr>
<tr>
<td>$AUC_{0→\infty}$ (µg·h/ml)</td>
<td>24.98±2.31</td>
<td>22.41±3.25* 23.61±4.00*</td>
</tr>
<tr>
<td>Relative bioavailability, $Fr$ (%)</td>
<td>—</td>
<td>88.52±2.35 93.52±3.18</td>
</tr>
</tbody>
</table>

*Statistically significant difference ($p<0.001$). † No significant difference ($p>0.05$).
values of pharmacokinetic parameters are summarized in Table 4.

Two different patterns were displayed in the profiles. There was a significant delay in the absorption time and $T_{\text{max}}$ of 5-FU after oral administration of coated pellets. It was that the bacterial enzymes need time to digest the pectin within the mixed films. 5-FU appeared in the plasma within 0.1 h of oral administration of uncoated pellets but it took about 5 and 7 h for 5-FU to appear in plasma when TWG-22% and 18% coated pellets were administered (Fig. 3.). The $T_{\text{max}}/C_{\text{max}}$ of 5-FU from TWG-22% and 18% coated pellets were $14.00 \pm 1.13 \text{ h}/1.60 \pm 0.87 \mu g/\text{ml}$ and $16.00 \pm 2.11 \text{ h}/1.71 \pm 0.97 \mu g/\text{ml}$, respectively. In the case of uncoated pellets of 5-FU, $C_{\text{max}}$ was $12.14 \pm 1.02 \mu g/\text{ml}$ and $T_{\text{max}}$ was $0.75 \pm 0.06 \text{ h}$, which were both significantly different from values obtained with coated 5-FU pellets ($p<0.001$). 5-FU has a relatively narrow therapeutic index, and the therapeutic range for 5-FU plasma levels was $2 - 3 \mu g/\text{ml}$. After administration of coated pellets, a safe and steady plasma drug level was achieved. However, the plasma levels were relatively low. It was the reason that the permeability coefficient of 5-FU in lower region of GI tract was relatively lower than that of upper GI tract due to a balance between water-solubility and lipophilicity. Secondly, when the coated pellets that of upper GI tract due to a balance between water-solubility and lipophilicity.42—44) Secondly, when the coated pellets were delivered to the colon, the rate of drug release was slow, which also resulted in low plasma drug level. Compared with the upper GI tract, the small surface area available for absorption in the colon would also reduce the plasma drug level. In addition, bioactivation by dihydrouracil dehydrogenase in the mucosal membrane of the GI tract would also reduce the plasma level of 5-FU.45) On the other hand, 5-FU has a very short plasma half-life (10—20 min); and short-term exposure to high concentrations of 5-FU induce RNA-directed 5-FU toxicity which is not thought to contribute to its anti tumor effect but mainly to toxic side effects,46 whereas longer exposures to lower concentrations favors DNA-directed effects which is thought to contribute to its anti tumor effect.47 Furthermore, Mukherjee et al.48) reported that bioactivation of 5-FU to 5-fluoro-2’-deoxyuridine was greater in colon cancer than in normal tissue. Thus, the sustained release/absorption characteristics were beneficial to the local treatment of colorectal cancer.

The $MRT$s for uncoated pellets and TWG-22% and 18% coated pellets of 5-FU were 2.10, 18.92 and 19.87 h, respectively, which in another aspect confirmed the delayed absorption of the in situ coated pellets as a result of retardation on initial drug release. There was a no statistically significant difference ($p>0.05$) in the $AUC$ values between the uncoated pellets and the coated pellets, which was not agreed with our previous report.20 It was the reason that the rate of in vivo drug release from coated pellets (TWG-22% and 18% for the 1:2.5 and 1:3.5 pectin:ethylcellulose, respectively) was lower than that obtained from the formulation of TWG-20% for the 1:2 pectin:ethylcellulose. It was also evident that the bioavailability of TWG-22% for the 1:2.5 pectin:ethylcellulose coated pellets was lower than that obtained from TWG-18% for the 1:3.5 pectin:ethylcellulose coated pellets. In our previous report, it was also shown that the bioavailability was improved by the sustained and low rate of drug release for this colon-specific coated system.49)

The Wagner–Nelson (W.N.) procedure and the Loo–Riegelman (L.R) method with the linear trapezoidal rule were used to obtain an in vivo cumulative release profiles. The relationship between percent in vitro dissolution in SCF (8%) of and percent $AUC_{t=\infty}$ of the 5-FU coated pellets was also established.

The in vivo cumulative release profiles (obtained with Wagner–Nelson procedure or Loo–Riegelman method) and percent $AUC_{t=\infty}$ of the 5-FU coated pellets were shown in Fig. 6. It was indicated that sigmoidal release pattern was achieved, which is therapeutically beneficial for timed release and colonic drug delivery, and is always observed in coated systems. The rate of in vitro release was faster than the rate of in vivo release since the concentrations of cecal contents.
in vivo were much higher than the SCF (8%). The percent drug absorbed and percent $AUC_{\infty}$ versus the amount of drug released in vitro plots were shown in Fig. 7. Table 5 showed a good linear regression relationship between the percent in vitro dissolution in SCF and the percent absorption ($r=0.962-0.976$, $p<0.001$ for the Wagner–Nelson method; $r=0.921-0.934$, $p<0.001$ for the Loo–Riegelman method) and percent $AUC_{\infty}$ ($r=0.963-0.972$, $p<0.001$) after oral administrations of the TWG-18% and 22% coated pellets in dogs.

The permeability coefficient of 5-FU in lower region of GI tract was relatively high, which resulted in a rapid absorption of 5-FU in vivo. For a rapid absorption process of drug, it is appropriate to use the Wagner–Nelson procedure or the Loo–Riegelman method for obtaining an absorption profile, because the difference between the cumulative amount released and the cumulative amount absorbed, i.e. the amount of drug released from the dosage form but not yet absorbed, could be negligible. The Loo–Riegelman method is used usually in the calculation of the cumulative absorption of the drug which is fitted to a two-compartment model. Though the Wagner–Nelson method is mainly applied to the pharmacokinetic study of the drug fitted to a one-compartment model, due to its simplicity, this method is also used for the drugs fitted to a two-compartment model. In this study, a similar IVIVC indicated that for the IVIVC analysis of 5-FU coated pellets, these two methods could be used alternatively. The Wagner–Nelson and Loo–Riegelman methods as well as numerical deconvolution are recommended by FDA to calculate the absorption profile. When these procedures were used to acquire the IVIVC, pharmacokinetic parameters from drug immediate release formulation is necessary. Percent $AUC$ has been used in IVIVC analysis of microsphere formulation without using parameters from immediate release formulation. In this study, though no parameters were lacked, to further estimate IVIVC by percent $AUC_{\infty}$, it was tried to use percent $AUC$ to establish IVIVC of the colon-specific and sustained release formulations of 5-FU and a successful IVIVC was obtained.

The established IVIVC for 5-FU in dogs can be scaled to predict drug concentrations and pharmacokinetic–pharmacodynamic relationships in humans to aid drug development process, and to foster the rational design of drug administration regimens in humans. It is also believed that the establishment of IVIVC in human being for 5-FU film-coated colon-targeted pellets is possible. In order to validate the assumption, the absorption properties and IVIVC of 5-FU film-coated pellets in human beings are in progress now.

### Conclusions

The results of this study revealed that pectin/ethylcellulose films were susceptible to enzymes present in the colon. Pharmacokinetics in dogs indicated that the pectin/ethylcellulose film-coated pellets could provide sufficient time delay, which may be related with more effective delivery of drugs to the colon. A good linear regression relationship was observed between the percent in vitro dissolution in SCF and the percent absorption or percent $AUC$ to establish IVIVC of the colon-specific and sustained release formulations of 5-FU film-coated pellets in human beings are in progress now.

### References