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

Li-Fang FAN,[a,b] Wei HE,*[,c,d] Min BAI,[d] Qing DU,[c] Bai XIANG,[c] Yong-Zhen CHANG,[e] and De-Ying CAO*[,c]

[a] *Department of Pharmaceutical Analysis, School of Pharmaceutical Science, Hebei Medical University;* [c] *Department of Pharmaceutics, School of Pharmaceutical Science, Hebei Medical University; 361 ZhongShan East Road, ShiJiaZhuang 050017, P. R. China:* [b] *Hebei Yiling Pharmaceutical Group, Medicine Institute; Beijing 100076, P. R. China:* [d] *CSPC Pharmaceutical Technology Co., Ltd.; ShiJiaZhuang 276 ZhongShan West Road, ShiJiaZhuang 050041, P. R. China: and* [e] *Department of Pharmaceutics, XingTai Medical School Facial Feature & Medical Treatment Technic Faculty, XingTai Medical College; 618 GangTie North Road, XingTai 054000, P. R. China.*

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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 $T_{max}$/$C_{max}$ of 14 h/1.6 $\mu$g/ml and 16 h/1.7 $\mu$g/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.[1,3—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[10] as well as reductive enzymes[11] which can potentially be utilized for colon-specific drug delivery. The reductase enzyme releases other polysaccharidases 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 colonic drug 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 $\alpha$-(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.*, ethylcellulose.[15] It restricts the entry of water and consequently swelling of polymer.[16—18]

Pectin/ethylcellulose film coating formulations had been investigated for their potential to obtain colon-specific drug delivery,[15] and physical properties of mixed pectin/ethylcellulose 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.

* To whom correspondence should be addressed. e-mail: hhewwei@126.com; Caody3@163.com

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,[10] 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.[9]

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[9] 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. (Orpington, U.K.). Pectin (with degree of methylation (DM) of approximately 35%) was a gift from Shangyao Fuda Pectin Co., Ltd. (Jingxi, China). 5-FU was obtained as gift sample 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 warmed distilled water. Then, predetermined amounts of Surelease® were added to this solution with stirring. Final dispersion was stirred by using a magnetic stirrer for 5 h and then poured into Teflon plates. The following Surelease® (S) : pectin (P) blend ratios were investigated: 100 : 0, 95 : 5, 90 : 10, 80 : 20, 70 : 30 and 60 : 40 (w/w). The resulted suspension was transferred to Teflon plates. Volume of suspension was 15 ml in each plate and subsequent controlled drying (24 h at 60 °C in an oven). The films obtained were carefully removed from the substrate and macroscopically examined for the presence of air bubbles and cracks, transparency, and flexibility. Then, the films were cut with a scalpel to different special pieces for various tests. The thickness of the films was measured at five different places by using a micrometer (Shanghai Precision Instruments Co., Ltd., China) and the average thickness of 130—150 $\mu$m was selected.

**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[24]:

$$\mathrm{WVT}=g\times 24/t\times A \tag{1}$$

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 cm$^2$ 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[25];

$$I_s\%=(M_s-M_i)/M_i\times 100\% \tag{2}$$

where $I_s$ is the swelling index, $M_s$ is the film mass after a certain swelling period, and $M_i$ 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)[26] 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 cm$^2$) 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 $\mu$g/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 were analyzed by high-performance liquid chromatography (HPLC) assay.[26]

When the stationary state was achieved, the permeability coefficient of the studied drug at was obtained by the following formula[27]:

$$\frac{2PS}{V}t=-\ln\frac{(C_0-2C_a)}{C_0} \tag{3}$$

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 $C$ is the concentration of caffeine in the acceptor compartment. $P$ can be calculated from a plot of $-\ln(C_0-2C_a)/C_0$ *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 $\mu$l aluminum sample pan with a pierced lid to allow evaporation 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 York.[28]

**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.[29] 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.[26] 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 $CO_2$ for another 19 h. At different time intervals, a 1 ml sample was withdrawn from the dissolution media, and the dissolution media was replenished with 1 ml of cecal content (4 or 8%) maintained under anaerobic conditions. The volume of the sample was made up to 10 ml with buffer (pH 6.8), filtered through sintered glass (G-5) filter, and the filtrate was analyzed using HPLC method.

**Drug Administration, Sample Collection and Sample Preparation** The pharmacokinetics of uncoated pellets, 1 : 2.5 pectin : ethylcellulose TWG-22% and 1 : 3.5 pectin : ethylcellulose TWG-18% coated pellets of 5-FU were assessed and compared in beagle dogs in a three formulation, randomized, three-period crossover study. The washout period between administrations was one week. Six male beagle dogs weighing from 8 to 10 kg were used in this study. The dogs were fed standard laboratory chow with water and fasted overnight before the experiments. The animals used in the experiments received care in compliance with the "Principles of Laboratory Animal Care" and "Guide for the Care and Use of Laboratory Animals". Experiments followed an approved protocol from Hebei Medical University Institutional Animal Care and Use Committee.

The uncoated and coated pellets were filled into a hard gelatin capsule, respectively, and orally administered in beagle dogs at a dosage equivalent to 4.5 mg/kg. At time intervals, 2 ml of blood samples were collected from saphenous vein into heparinized tubes and centrifuged at 1000×***g*** for 10 min and stored at −20 °C until assay. Blood sampling time points were 0, 2, 5, 7, 9, 11, 13, 14, 16, 18, 21, 24, 26, 28 and 30 h after administration of coated pellets; for uncoated pellets, blood samples (2.0 ml) was at 0, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 7.0 h after administration. Frozen plasma samples were prepared using the procedure described in our earlier reported[26]; and 5-FU concentration of plasma samples was determined using an HPLC assay.[26]

**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 ($C_{max}$) and time of maximum concentration ($T_{max}$), were obtained directly from the plasma concentration–time plots. The area under the plasma concentration *vs.* time curve up to the last time ($t$) ($AUC_{0-t}$) was determined using the trapezoidal rule. The $AUC_{0-\infty}$ values were calculated by adding the value of $C_t \times K_e^{-1}$ to $AUC_{0-t}$, that was $AUC_{0-\infty}=AUC_{0-t}+C_t \times K_e^{-1}$. The mean residence time (*MRT*) was calculated as *AUMC*/*AUC*. The relative bioavailability (*Fr*) was calculated using the following formula: 100%×($AUC_{\text{coated pellets}}/AUC_{\text{uncoated pellets}}$).

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 vivo* ($F_a$) was examined. The $F_a$ was determined using the Wagner–Nelson method (W.N.) by the following equation: $F_a=(C_t/K_e+AUC_{0-t})/AUC_{0-\infty}$[30] and the Loo–Riegelman method (L.R.) by the following equation: $F_a=[C_t/K_{10}+AUC_{0-t}+(X_p)_t/(V_c \times K_{10})]/AUC_{0-\infty}$.[31] The relationship between percent *in vitro* dissolution in SCF and percent *AUC* ($AUC_{0-t}/AUC_{0-\infty}$) 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.

## 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.

**Swelling Test** The results of swelling experiments are listed in Table 2. Formulations containing Surelease® and pectin had low 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-

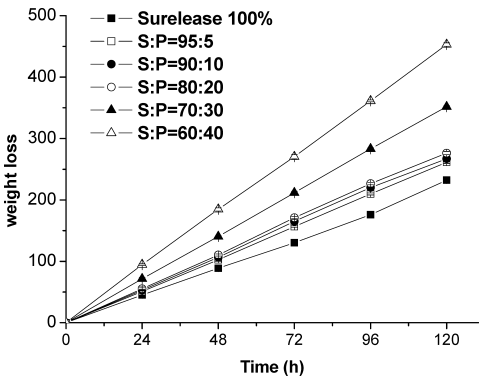


Fig. 1. Profiles of Water Vapor Transmission through Free Films Containing Pectin/Ethylcellulose (Mean±S.D., $n=3$)

Table 1. Water Vapor Transmission of Free Films

| Formulation | Mass change (mg/h) | WVT (g/24 h·m$^2$) |
|---|---|---|
| Surelease® 100% | 1.94±0.16 | 4.64±0.39 |
| S : P=95 : 5 | 2.18±0.05 | 5.23±0.22 |
| S : P=90 : 10 | 2.22±0.12 | 5.34±0.28 |
| S : P=80 : 20 | 2.30±0.10 | 5.53±0.23 |
| S : P=70 : 30 | 2.96±0.06 | 7.03±0.14 |
| S : P=60 : 40 | 3.78±0.09 | 9.06±0.22 |

Data represent mean±S.D., $n=3$.

Table 2. Swelling of Formulation in Different Media

| Formulation | Maximum swelling index, $I_s$ (%) | | |
|---|---|---|---|
| | SGF | SIF | SCF |
| Surelease®=100% | 45.08±3.27 | 45.11±2.36 | 45.04±1.69 |
| S : P=95 : 5 | 50.23±1.33 | 52.18±2.02 | 58.10±2.11 |
| S : P=90 : 10 | 55.36±1.05 | 57.25±2.13 | 63.18±1.08 |
| S : P=80 : 20 | 65.67±2.05 | 68.15±1.00 | 76.27±2.04 |
| S : P=70 : 30 | 73.36±1.32 | 74.23±0.87 | 90.08±0.69 |
| S : P=60 : 40 | 80.38±2.19 | 81.28±0.64 | 93.25±0.64 |

Data represent mean±S.D., $n=3$.

vironments, while at neutral solution pH pectin aggregates tend to dissociate and expand.[32] Compared with the swelling index in SIF and SGF, swelling of free films in SCF significantly increased with addition of pectin up to the ratio of 30% ($p<0.05$). An increase in swelling for polysaccharide materials indicated degradation in SCF.[33] This is due to the presence of bacterial enzymes contained in the cecal contents (The main saccharolytic species are *bacteroides*, *bifidobacteria* and *enterobacteria*, *etc*.) in the media; The bacterial enzymes diffuse into the polymeric chains,[5] hydrolyze the glycosidic linkages within the pectin, reduce the network density and finally increase swelling. Swelling and hydration of the composite film was necessary before enzymatic breakdown occurred. Films containing the highest concentration of the most hydrophilic polysaccharide reach the highest degree of swelling.

**Drug Permeability** The data of permeability of the drug in different media are shown in Table 3. As long as the concentration of the pectin added was low, the permeability of the mixed films remained relatively very low in SGF, as well as in SIF, indicated that the insolubility of the film given by ethylcellulose was maintained. An increase in the amount of pectin induced an increase in diffusion. The results of permeability were agreed with the results of the swelling, which films containing higher concentration of pectin reached higher drug permeability. Similarity to the results of the swelling experiment, permeability in SGF was lower than in SIF for mixed films due to the gelling ability and solubility of pectin depending strongly on the pH of the surrounding media.[32] Lower permeability of mixed films in SGF and in SIF than in SCF ($p<0.05$) demonstrated the susceptibility of pectin in these films to bacterial enzyme and degradation in colonic media. Nesbitt *et al.*[34] reported that the mechanisms for water-soluble drug diffusion through an ethylcellulose membrane were governed mainly by water-filled channels present in the film. 5-FU is a water-soluble drug. The addition of rat cecal contents to the phosphate buffer increased the rate and extent of pectin leaching from ethylcellulose films. The leaching of the large-molecular weight pectin created aqueous channels or water-filled pores that allowed diffusion of drug molecules through the film. Thus, the permeability in SCF was higher than that in SGF and in SIF.

Compared with the values obtained under the SIF conditions, when the composition of pectin in the mixed films was lower than the ratio of 30%, the permeability of ethylcellulose films was not significantly effected by the addition of pectin in SCF, which was agreed with Macleod's report[19]; 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.[19] 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 that one bacterial enzymes were usually involved in its degradation or breakdown.[37]

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

| Formulation | Permeability coefficients ($\times10^{-5}$ cm/s) | | |
|---|---|---|---|
| | SGF | SIF | SCF |
| Surelease® 100% | 1.031±0.051 | 1.022±0.043 | 1.040±0.033 |
| S : P=95 : 5 | 1.684±0.015 | 1.725±0.026 | 1.815±0.048 |
| S : P=90 : 10 | 1.964±0.124 | 2.074±0.221 | 2.152±0.326 |
| S : P=85 : 15 | 2.110±0.421 | 2.242±0.354 | 2.441±0.257 |
| S : P=80 : 20 | 2.302±0.267 | 2.482±0.335 | 2.958±0.254 |
| S : P=70 : 30 | 2.654±0.647 | 2.885±0.541 | 3.725±0.667 |
| S : P=60 : 40 | 2.701±0.254 | 2.952±0.233 | 3.941±0.647 |

Data represent mean±S.D., $n=3$.

***$T_g$*'s** One of the most common polymer properties determined for amorphous polymers is the $T_g$. $T_g$ values for ethylcellulose and pectin films were 82 and 115 °C, respectively (Fig. 2). The addition of pectin to ethylcellulose would increase the $T_g$ of the ethylcellulose films (Fig. 2). However, no significant increasing $T_g$ 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

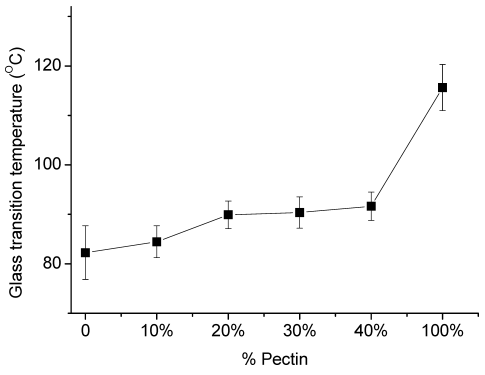


Fig. 2. Glass Transition Temperatures as a Function of Pectin Concentration for Ethylcellulose Films

Error bars indicate S.D. ($n=6$).

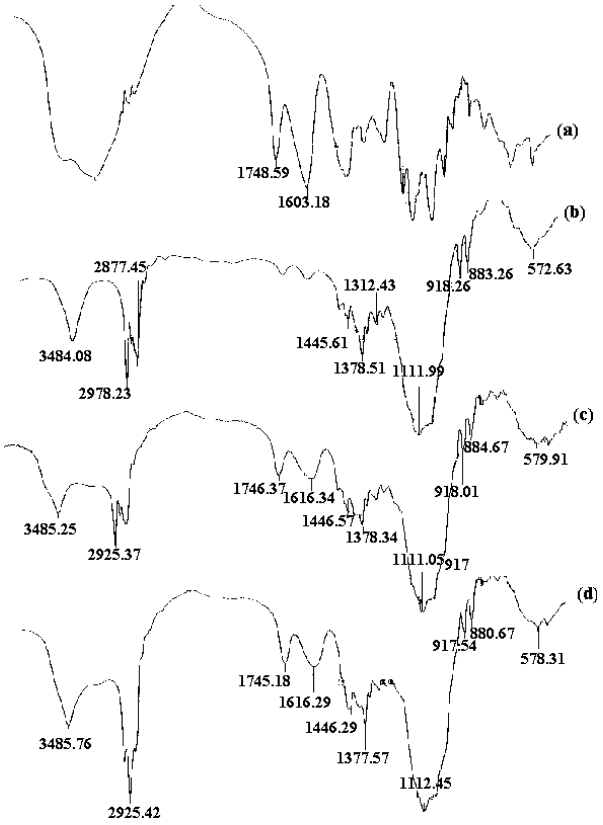


Fig. 3. FT-IR Spectra of (a) Pectin, (b) Ethylcellulose, (c) Physical Mixture of Ethylcellulose and Pectin (7 : 3 ratio) and (d) Ethylcellulose/Pectin Blend (7 : 3 Ratio)

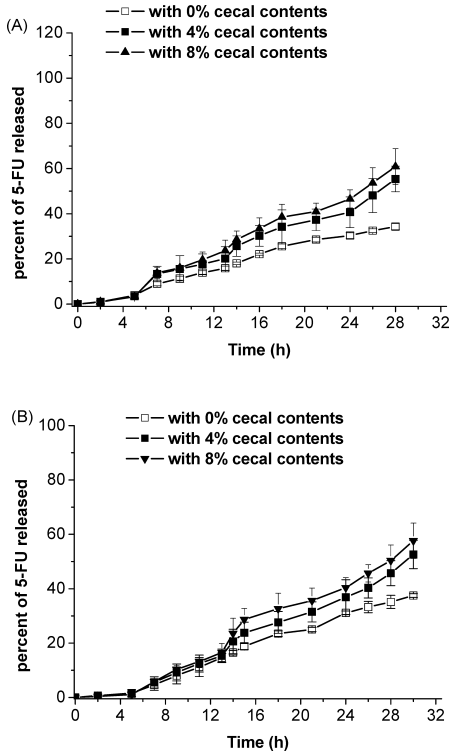


Fig. 4. Mean Percentage Release of 5-FU from 1 : 2.5 Pectin : Ethylcellulose TWG-22% (A) and 1 : 3.5 Pectin : Ethylcellulose TWG-18% (B) Coated Pellets after 2 h in 0.1 M HCl, 3 h in Phosphate Buffer (pH 6.8) and 19 h in Phosphate Buffer (pH 6.8) without and with 4% w/v and 8% w/v of Rat Cecal Contents

(Fig. 3a), bands related to C=O stretching of the ester and carboxyl group could be observed at 1748.59 and 1603.18 $cm^{-1}$, respectively.[40] 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 superimposition of that of ethylcellulose and pectin. Though it was reported that the molecular coalescence of pectin and ethylcellulose was observed in aqueous phase,[19] 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.

Table 4. 5-FU Pharmacokinetic Parameters after Oral Administration of Coated or Uncoated Pellets at a Dose of 4.5 mg/kg of 5-FU Equivalent in Beagle Dogs

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

* Statistically significant difference ($p<0.001$). † No significant difference ($p>0.05$).

**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

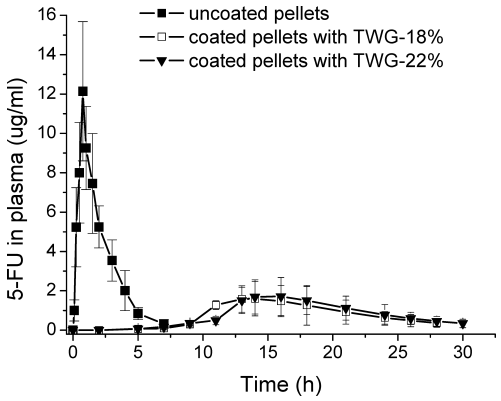


Fig. 5. Plasma 5-FU Concentration *versus* Time Plot after a Single Oral Dose of Uncoated Pellets, 1 : 2.5 Pectin : Ethylcellulose TWG-22% and 1 : 3.5 Pectin : Ethylcellulose TWG-18% Coated Pellets of 5-FU (Mean± S.D., $n=6$)

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 $T_{max}$ 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 $T_{max}/C_{max}$ of 5-FU from TWG-22% and 18% coated pellets were $14.00\pm1.13$ h/$1.60\pm0.87$ $\mu$g/ml and $16.00\pm2.11$ h/$1.71\pm0.97$ $\mu$g/ml, respectively. In the case of uncoated pellets of 5-FU, $C_{max}$ was $12.14\pm1.02$ $\mu$g/ml and $T_{max}$ was $0.75\pm0.06$ 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/ml.[41] 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.[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, bioinactivation 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.[26] 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 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]

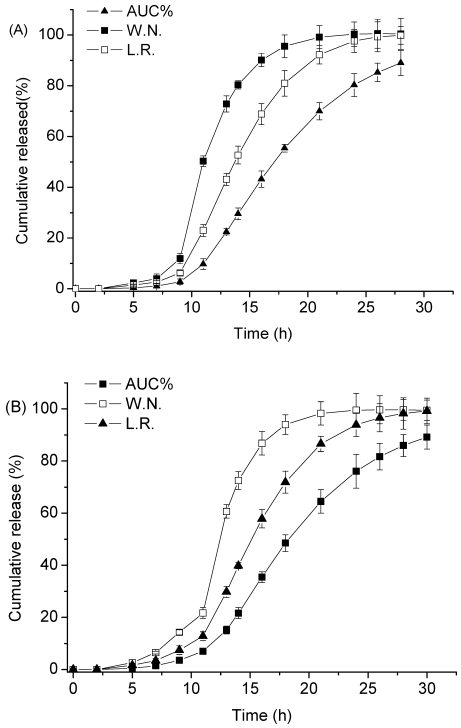


Fig. 6. *In Vitro* Releases in SCF (8% w/w) and *in Vivo* Cumulative Releases Using the Wagner–Nelson (W.N.) and Loo–Riegelman (L.R.) Methods and Percent *AUC* of 5-FU in Dogs from 1 : 2.5 Pectin : Ethylcellulose TWG-22% (A) and 1 : 3.5 Pectin : Ethylcellulose TWG-18% (B) Coated Pellets

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 $AUC_{0—\infty}$ 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 $AUC_{0—\infty}$ 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

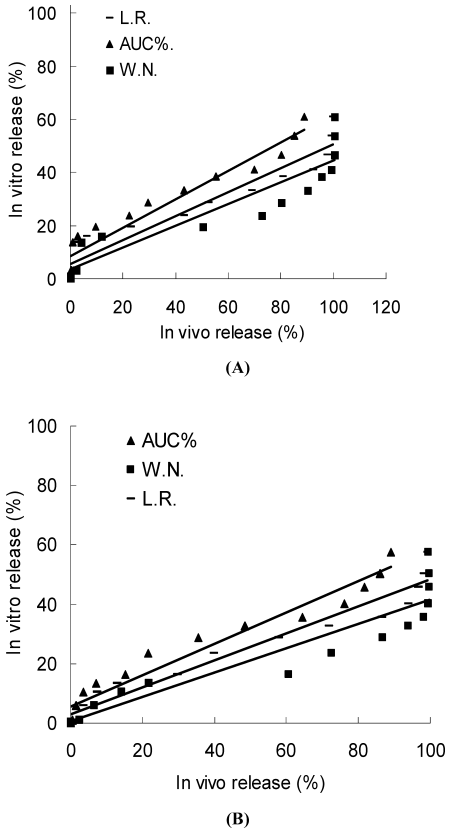


Fig. 7. IVIVC Model Linear Regression Plots of Cumulative Absorption and Percent *AUC vs.* Percent Dissolution of 5-FU from 1 : 2.5 Pectin : Ethylcellulose TWG-22% (A) and 1 : 3.5 Pectin : Ethylcellulose TWG-18% (B) Coated Pellets

Table 5. The Values of Correlation Coefficient ($r$) Obtained in the IVIVC Model Using Linear Regression Analysis

| Formulation | *In vivo* absorption model | $r$ |
|---|---|---|
| 1 : 3.5 pectin: | W.N. | 0.962 |
| Ethylcellulose | L.R. | 0.921 |
| TWG-18% | *AUC*% | 0.963 |
| 1 : 2.5 pectin: | W.N. | 0.976 |
| Ethylcellulose | L.R. | 0.934 |
| TWG-22% | *AUC*% | 0.972 |

W.N.: Wagner–Nelson method, L.R.: Loo–Riegelman method, *AUC*%: ($AUC_{0-t}$/$AUC_{0-\infty}$)×100%.

*in vivo* were much higher than the SCF (8%). The percent drug absorbed and percent $AUC_{0-\infty}$ *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 $AUC_{0-\infty}$ ($r$=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,[42—44] 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,[50] 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.[51] 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.[52] In this study, though no parameters were lacked, to further estimate IVIVC by percent $AUC_{0-\infty}$, 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*, suggesting that *in vitro* dissolution testing in SCF could be used to establish IVIVC for the colon-specific drug delivery systems activated by microflora.

### References

1) Basit A. W., *Drugs*, **65**, 1991—2007 (2005).
2) Chourasia M. K., Jain S. K., *J. Pharm. Pharm. Sci.*, **6**, 33—66 (2003).
3) Rubinstein A., *Crit. Rev. Ther. Drug Carrier Syst.*, **12**, 101—149 (1995).
4) Friend D. R., *Adv. Drug Deliv. Rev.*, **57**, 247—265 (2005).
5) Sinha V. R., Kumria R., *Eur. J. Pharm. Sci.*, **18**, 3—18 (2003).
6) Vandamme T. F., Lenourry A., Charrueau C., Chaumeil J. C., *Carbohydr. Polym.* (2002).
7) Minko T., *Adv. Drug Deliv. Rev.*, **56**, 491—509 (2004).
8) Kosaraju S. L., *Crit. Rev. Food Sci. Nutr.*, **45**, 251—258 (2005).
9) Yang L., *J. Controlled Release*, **125**, 77—86 (2008).
10) Hawksworth G., Drasar B. S., Hill M. J., *J. Med. Microbiol.*, **4**, 451 (1971).
11) Rowland I. R., *Drug Metab. Rev.*, **19**, 243—261 (1988).
12) Larsen C., Harboe E., Johansen M., Olesen H. P., *Pharm. Res.*, **6**, 995—999 (1989).

13) McLeod A. D., Friend D. R., Tozer T. N., *Int. J. Pharm.*, **92**, 105 (1993).
14) Sinha V. R., Kumria R., *Pharm. Res.*, **18**, 557—564 (2001).
15) Wakerly Z., Fell J. T., Attwood D., Parkins D., *Pharm. Res.*, **13**, 1210—1212 (1996a).
16) Sinha V. R., Kumria R., *Int. J. Pharm.*, **224**, 19—38 (2001).
17) Jain A., Gupta Y., Jain S. K., *J. Pharm. Pharm. Sci.*, **10**, 86—128 (2007).
18) Chourasia M. K., Jain S. K., *Drug Deliv.*, **11**, 129—148 (2004).
19) Macleod G. S., Fell J. T., Collett J. H., *Int. J. Pharm.*, **157**, 53—60 (1997).
20) Munjeri O., Collett J. H., Fell J. T., *J. Controlled Release*, **46**, 273—278 (1997).
21) Fernandez-Hervas M. J., Fell J. T., *Int. J. Pharm.*, **169**, 115—119 (1998).
22) Chang K. L. B., Lin J., *Carbohydr. Polym.*, **43**, 163—169 (2000).
23) Chambin O., Dupuis G., Champion D., Voilley A., Pourcelot Y., *Int. J. Pharm.*, **321**, 86—93 (2006).
24) Van den Mooter G., Samyn C., Kinget R., *Int. J. Pharm.*, **111**, 127—136 (1994).
25) Blanchon S., Couarraze G., Rieg-Falson F., Cohen G., Puisieux F., *Int. J. Pharm.*, **72**, 1—10 (1991).
26) He W., Du Q., Cao D. Y., Xiang B., Fan L. F., *Int. J. Pharm.*, **348**, 35—45 (2008).
27) Flynn G. L., Yalkowsky S. H., Roseman T. J., *J. Pharm. Sci.*, **63**, 479—509 (1974).
28) Okhamafe A. O., York P., *J. Pharm. Sci.*, **77**, 438—443 (1988).
29) He W., Du Q., Cao D. Y., Xiang B., Fan L. F., *PDA J. Pharm. Sci. Technol.*, **61**, 121—130 (2007).
30) Wagner J. G., Nelson E., *J. Pharm. Sci.*, **52**, (1963).
31) Loo J. C. K., Riegelman S., *J. Pharm. Sci.*, **57**, 918—928 (1968).
32) Liu L. S., Fishman M. L., Kost J., Hicks K. B., *Biomaterials*, **24**, 3333—3343 (2003).
33) Langer R. S., Peppas N. A., *Biomaterials*, **2**, 201—214 (1981).
34) Nesbitt R. U., Mahjour M., Mills N. L., Fawzi M. B., *J. Controlled Release*, **32**, 71—77 (1994).
35) Moore W. E. C., Holdeman L. V., *Appl. Microbiol.*, **27**, 961—979 (1974).
36) Finegold S. M., Sutter V. L., Sugihara P. T., Elder H. A., Lehmann S. M., Phillips R. L., *Am. J. Clin. Nutr.*, **30**, 1781—1792 (1977).
37) Salyers A. A., Leedle J. A., "Human Intestinal Microflora in Health and Disease," ed. by Hentge D. J., Academic Press, New York, 1983, pp. 129—146.
38) Tarvainen M., Sutinen R., Peltonen S., Tiihonen P., Paronen P., *J. Pharm. Sci.*, **91**, 282—289 (2002).
39) Ravindra R., Rao A. K., Khan A. A., *J. Appl. Polym. Sci.*, **72**, 689—700 (1999).
40) Doner L. W., ACS Symposium Series 310, Washington, 1986, pp. 13—21.
41) Gamelin E., Boisdron-Celle M., Delva R., Regimbeau C., Cailleux P. E., Alleaume C., *J. Clin. Oncol.*, **16**, 1470—1478 (1998).
42) Buur A., Bundgaard H., *Int. J. Pharm.*, **203**, 209—222 (1985).
43) Buur A., Bundgaard H., *Int. J. Pharm.*, **36**, 41—49 (1987).
44) Lee V. H. L., Yamamoto A., Buur A., Bundgaard H., *Proc. Int. Syrup. Control. Rel. Bioact. Mater.*, **16**, 56—57 (1989).
45) Queener S. F., Morris H. P., Weber G., *Cancer Res.*, **31**, 1004—1009 (1971).
46) Peters G. J., "Fluoropyrimidines in Cancer Therapy," ed. by Rustum Y. M. E., Humana Press Inc., Totowa, 2002, pp. NJ1—NJ27.
47) Sobrero A. F., Aschele C., Bertino J. R., *J. Clin. Oncol.*, **15**, 368—381 (1997).
48) Mukherjee K. L., Curreri A. R., Javid M., Heidelberger C., *Cancer Res.*, **23**, 68—77 (1963).
49) He W., Du Q., Cao D. Y., Xiang B., Fan L. F., *J. Pharm. Pharmacol.*, **60**, 35—44 (2008).
50) Hwang S. S., Bayne W., Theeuwes F., *J. Pharm. Sci.*, **11**, 1145—1150 (1993).
51) Schliecker G., Schmidt C., Fuchs S., Ehinger A., Sandow J., Kissel T., *J. Controlled Release*, **94**, 25—37 (2004).
52) Woo B. H., Kostanski J. W., Gebrekidan S., Dani B. A., Thanoo B. C., DeLuca P. P., *J. Controlled Release*, **75**, 307—315 (2004).